U.PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

HENRIQUE DA SILVA FERRÃO NASCIMENTO

INFLAMAÇÃO E RISCO ATEROSCLERÓTICO EM CRIANÇAS E ADOLESCENTES OBESOS. CONTRIBUIÇÃO GENÉTICA E ESTUDO DE "FOLLOW-UP" BASEADO NA REDUÇÃO DO ÍNDICE DE MASSA CORPORAL

INFLAMMATION AND ATHEROGENIC RISK IN OBESE CHILDREN AND ADOLESCENTS. GENETIC CONTRIBUTION AND FOLLOW UP STUDY BASED ON THE REDUCTION OF THE BODY MASS INDEX

Tese do 3º Ciclo de Estudos Conducente ao Grau de Doutoramento em Ciências Farmacêuticas – Especialização em Bioquímica

Trabalho realizado sob a orientação dos Professores

Doutor Luís Belo
Doutora Alice Santos Silva
Doutor Alexandre Quintanilha

Novembro 2013

DECLARAÇÃO

É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA TESE APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

Este trabalho foi realizado no Serviço de Bioquímica da Faculdade de Farmácia e no Grupo da Biologia da Inflamação e Reprodução do Instituto de Biologia Molecular e Celular (IBMC) da Universidade do Porto, com a colaboração do Hospital de São João, dos Hospitais Maria Pia e Santo António do Centro Hospitalar do Porto (CHP), da Faculdade de Desporto da Universidade do Porto e com o apoio de um subsídio sob a forma de bolsa de doutoramento (SFRH/BD/61407/2009) atribuído pela Fundação para a Ciência e Tecnologia (FCT) e Fundo Social Europeu (FSE). Parte deste trabalho foi também financiado pelo FEDER, através de fundos do COMPETE - Programa Operacional Factores de Competitividade, e por fundos nacionais, através da FCT - projeto FCOMP-01-0124-FEDER-028613 (PTDC/DTP-DES/0393/2012)

“O homem descobre-se quando se confronta com o obstáculo.”

Antoine de Saint-Exupéry

Aos meus pais, minhas irmãs e meus irmãos

DECLARAÇÃO

Ao abrigo do artigo 8º do Decreto-Lei nº388/70, declara-se que fazem parte integrante desta tese os seguintes trabalhos já publicados ou submetidos para publicação:

Artigos em revistas de circulação internacional com arbitragem científica:

Nascimento H, Silva L, Lourenço P, Vieira E, Dos Santos R, Rego C, Ferreira H, Quintanilha A, Santos-Silva A, Belo L. "Lipoprotein (a) levels in obese children and adolescents: contribution of pentanucleotide repeat (TTTTA)n polymorphism in apoliporprotein (a) gene". Archives of Pediatrics & Adolescent Medicine. 2009; 163:393-394.

Nascimento H, Silva L, Lourenço P, Weinfurterová R, Castro E, Rego C, Ferreira H, Guerra A, Quintanilha A, Santos-Silva A, Belo L. "Lipid profile in obese children and adolescents. Interaction of apolipoprotein E polymorphism with adiponectin levels". Archives of Pediatrics & Adolescent Medicine. 2009; 163:1030-1036.

Nascimento H, Rocha S, Rego C, Mansilha HF, Quintanilha A, Santos-Silva A, Belo L. "Leukocyte count versus C-reactive protein levels in obese Portuguese patients aged 6-12 years old". The Open Biochemistry Journal. 2010; 4:72-76.

Nascimento H, Costa E, Rocha-Pereira P, Rego C, Mansilha HF, Quintanilha A, Santos-Silva A, Belo L. “Cardiovascular Risk Factors in Portuguese Obese Children and Adolescents: Impact of Small Reductions in Body Mass Index Imposed by Lifestyle Modifications”. The Open Biochemistry Journal. 2012; 6:43-50.

Belo L, Nascimento H, Kohlova M, Bronze-Rocha E, Fernandes J, Costa E, Catarino C, Aires L, Ferreira Mansilha H, Rocha-Pereira P, Quintanilha A, Rêgo C, Santos-Silva A. "Body fat percentage is a major determinant of total bilirubin levels independently of UGT1A1*28 polymorphism in obese children and adolescents". Submitted.

Nascimento H, Costa E, Rocha S, Lucena C, Rocha-Pereira P, Rêgo C, Ferreira Mansilha H, Quintanilha A, Aires L, Mota J, Santos-Silva A, Belo L. "Adiponectin and markers of metabolic syndrome in obese children and adolescents: Impact of 8-month regular physical exercise program". Submitted.

Nascimento H, Quintanilha A, Santos-Silva A, Belo L. "Adiponectin relation with inflammation and metabolic syndrome features in pediatric obese patients - impact of interventional studies". Submitted.

Nascimento H, Catarino C, Rêgo C, Ferreira Mansilha H, Quintanilha A, Santos-Silva A, Belo L. "CDC BMI z-score is a better predictor of metabolic syndrome than WHO BMI z-score in Portuguese obese adolescents". Submitted.

Comunicações publicadas em revistas de circulação internacional

Nascimento H, Silva L, Lourenço P, Rocha S, Castro E, Rocha-Pereira P, Guerra A, Rêgo C, Ferreira Mansilha F, Quintanilha A, Santos-Silva A and Belo L. "Interaction of apolipoprotein E polymorphism and adiponectin levels with lipid profile in obese children and adolescents – a preliminary study". Obesity Reviews. 2008; 9:s2.

Nascimento H, Fortunato AR, Rocha S, Castro E, Rocha-Pereira P, Guerra A, Rêgo C, Ferreira Mansilha F, Quintanilha A, Santos-Silva A and Belo L. "Red Blood Cell changes in obese children and adolescents – a preliminary study". Obesity Reviews. 2008; 9:s2.

Publicação em acta de encontro científico nacional

Nascimento H, Casal S, Rocha S, Fernandes J, Costa E, Rego C, Mansilha HF, Rocha-Pereira P, Quintanilha A, Belo L, Santos-Silva A. “Changes in erythrocyte membrane’s damage markers and fatty acid profile and their association with markers of cardiovascular disease – study in a pediatric obese population” Comunicação por convite, publicada sob forma de artigo. Boletim da Sociedade Portuguesa de Hemorreologia e Microcirculação. 2012; 27:19-20.

Acknowledgements / Agradecimentos

Agradeço, primeiramente, a Deus.

Agradeço, igualmente, a todos que, de algum modo, contribuiram para tornar possível a realização deste trabalho.

Aos meus orientadores, Professor Doutor Luís Belo, Professora Doutora Alice Santos Silva e Professor Doutor Alexandre Quintanilha agradeço o apoio e disponibilidade que sempre demonstraram e os conhecimentos que me transmitiram. Agradeço, igualmente, a compreensão e prontidão com que aceitaram os projectos paralelos que foram surgindo ao longo do tempo.

Ao Professor Doutor Luís Belo agradeço a oportunidade de trabalhar num projecto tão aliciante, assim como o acompanhamento, parceria e rigor científico na execução do mesmo.

À Professora Doutora Alice Santos Silva agradeço a prontidão com que me abriu as portas do laboratório, ainda era eu aluno de licenciatura, ajudando a fazer crescer meu interesse pela investigação. Agradeço, igualmente, o carinho e atenção que sempre demonstrou por cada elemento do grupo de investigação, criando, deste modo, um espírito quase familiar.

Ao Professor Doutor Alexandre Quintanilha agradeço, primeiramente, a honra de ter aceite ser meu orientador. Para além disso devo salientar a clareza e a objetividade que lhe são tão características e que ajudaram a lançar luz sobre pontos, por vezes, esquecidos e a traçar o rumo deste trabalho.

À Professora Doutora Natércia Teixeira, que como chefe do Departamento de Ciências Biológicas da Faculdade de Farmácia, e do Grupo da Biologia da Inflamação e Reprodução do Instituto de Biologia Molecular e Celular, ambos pertencentes a Universidade do Porto, sempre exerceu sua liderança de forma magnânima, contribuindo, e muito, para o óptimo ambiente que ali se respira.

À Professora Doutora Carla Rêgo e à Doutora Helena Ferreira Mansilha pelo grande suporte e acompanhamento que deram ao projecto.

À Professora Doutora Petronila Rocha Pereira pelo apoio na análise laboratórial.

Ao Professor Doutor Elísio Costa e à Professora Doutora Cristina Catarino pela pronta ajuda sempre que necessária.

Ao Doutor João Fernandes e à Doutora Susana Rocha pela ajuda laboratorial e pela amizade.

A todos os integrante do CIAFEL - Centro de Investigação em Actividade Física, Saúde e Lazer, da Faculdade de Desporto da Universidade do Porto, e colaboradores do projecto

ACORDA, particularmente às Professoras Doutoras Clarice Martins e Luísa Aires pelo desenvolvimento de todo o programa relacionado com a prática de exercíco físico.

À Doutora Rosário Santos e à Dra. Emilia Vieíra pelo apoio na avaliação das variáveis genéticas.

A todos os professores do Serviço de Bioquímica, em particular, às Professoras Doutoras Georgina Correia da Silva e Elsa Bronze da Rocha.

A todos os colegas que passaram pelo Serviço de Bioquímica da Faculdade de Farmácia da Universidade do Porto – Doutora Susana Coimbra, Dra. Sandra Ribeiro, Dra. Michaela Kohlová, Dra. Filomena Silva, Doutor Bruno Fonseca, Doutora Cristina Amaral, Dra. Marta Almada, Dr. David Pereira, Dra. Mariana Costa, Dr. Doutor Eduardo Tejera e Dra. Ana Cláudia Portelinha - gostaria agradecer a amizade e os bons momentos passados, contribuindo para o gosto em ir trabalhar e para que motivação não me faltasse.

Aos funcionários do Serviço de Bioquímica da Faculdade de Farmácia da Universidade do Porto, Dona Casemira Vieira, Ana Paula Ribeiro e Dr. Luís Daniel Vasconcelos pelo profissionalismo e eficiência.

A todos os elementos do Serviço de Análises Clínicas da Faculdade de Farmácia da Universidade do Porto, particularmente à Dra. Bárbara Duarte.

As equipas de enfermagem e análises clínicas do Serviço de Pediatria do Hospital de São João e do Hospital de Crianças Maria Pia e do Hospital de Santo António do Centro Hospitalar do Porto

A todas as instituições e entidades que colaboraram com o estudo - Hospital de São João, Hospital de Crianças Maria Pia e Hospital de Santo António do Centro Hospitalar do Porto, ao Centro de Genética Médica Dr. Jacinto Magalhães- Instituto Nacional de Saúde Ricardo Jorge, à Faculdade de Desporto da Universidade do Porto, ao Agrupamento de Escolas de Santa Bárbara de Fânzeres e à Associação de Promoção para a Saúde – Semear o Amanhã.

À Fundação para a Ciência e Tecnologia e Fundo Social Europeu pela bolsa de doutoramento a mim atribuída.

Agradeço, por fim e de forma especial, aos meus pais, irmãs, irmãos, e à Susana Cruz, minha namorada, pelo apoio incondicional e compreensão pelas muitas horas roubadas.

Resumo

Portugal apresenta uma alta prevalência de sobrepeso e obesidade em idade pediátrica, sendo que um terço das crianças e adolescentes apresentam excesso de peso.

A obesidade está associada a doenças tais como a hipertensão arterial, diabetes *mellitus* tipo 2, dislipidemia e aterosclerose e a um risco aumentado de doença cardiovascular no futuro. O aumento da prevalência da obesidade infantil tem contribuído para o aparecimento dessas patologias em indivíduos cada vez mais novos.

O tecido adiposo secreta inúmeros mediadores com um papel importante no desenvolvimento do estado crónico de inflamação de baixo grau presente nos indivíduos obesos. A adiponectina, uma adipocina anti-inflamatória secretada principalmente pelo tecido adiposo, está relacionada com uma melhoria do perfil lipídico e da sensibilidade à insulina. A adiponectina circula como multímeros de alto, médio e baixo peso molecular (APM, MPM e BPM, respectivamente). O multímero de APM, descrito como sendo o mais biologicamente ativo e responsável pelas ações benéficas atribuídas à adiponectina, está reduzido em pacientes obesos. Pouco se sabe sobre as funções biológicas dos outros multímeros.

Neste projecto estudámos numa população pediátrica obesa Portuguesa diversos fatores de risco cardiovascular (novos e clássicos) e polimorfismos genéticos com eles associados. Posteriormente, avaliámos o impacto da redução do índice de massa corporal (IMC) nesses factores de risco, obtida por dois métodos: um programa de modificação do estilo de vida e um programa de intervenção com exercício físico.

Verificámos que as crianças e adolescentes obesos apresentavam um agravamento do perfil de risco metabólico, quando comparado com os controlos normoponderais, observando-se: 1) um aumento da resistência à insulina, com o aumento da insulinemia e do índice *homeostasis model assessment – insulin resistance* ($HOMA_{IR}$); 2) um perfil lipídico com modificações de risco, nomeadamente um aumento dos triglicerídeos (TG), do colesterol total (CT) e do colesterol das lipoproteínas de baixa densidade (LDLc), e redução do colesterol das lipoproteínas de alta densidade (HDLc); 3) uma redução da proteção antioxidante nos indivíduos mais obesos, com redução da bilirrubina plasmática; 4) um estado inflamatório de baixo grau, com aumento da proteína C-reativa (PCR) plasmática e da neutrofilia e diminuição da adiponectina. O agravamento do perfil metabólico foi particularmente notório com o aumento da obesidade abdominal, um componente da síndrome metabólica (SM). Mais ainda, os adolescentes obesos com SM apresentavam um perfil de maior risco, quando comparados com obesos sem SM. A prevalência da SM na nossa população de obesos foi elevada - 24,8%.

Na nossa população de adolescentes obesos, o critério do *Center for Disease Control and Prevention* para a classificação do z-score do IMC em populações pediátricas foi um melhor preditor da SM do que o critério utilizado pela Organização Mundial da Saúde.

Os níveis de adiponectina circulante correlacionaram-se inversamente com marcadores de adiposidade (IMC, circunferência da cintura e % de gordura corporal), com a

dislipidemia (níveis de TG e razão CT/HDLc), com a resistência à insulina (insulinemia e o $HOMA_{IR}$) e com o *status* inflamatório (níveis de PCR); contrariamente, os níveis de adiponectina apresentaram uma correlação positiva com os de HDLc. As diferentes formas circulantes de adiponectina apresentaram atividades distintas nos indivíduos pré-púberes estudados. Enquanto a forma de APM espelhou os efeitos benéficos associados à adiponectina total, particularmente em relação ao perfil lipídico, o multímero de BPM apresentou resultados opostos.

Alguns dos polimorfismos genéticos estudados evidenciaram uma associação com um perfil de maior risco metabólico; os indivíduos obesos com o alelo E4, do polimorfismo da apolipoproteína (apo) E, apresentaram um perfil lipídico de risco mais aumentado (aumento do CT, do LDLc e da razão CT/HDLc); os indivíduos obesos com menor número de repetições no polimorfismo de repetição pentanucleotídica, (TTTAT)n, da apo (a) apresentaram um aumento da lipoproteína (a) sérica; e os indivíduos homozigotos 6/6 para o polimorfismo UGT1A1*28 da UDP-glucuronosiltransferase apresentaram uma redução da bilirrubina plasmática. No entanto, verificámos que o efeito de um perfil genético menos favorável sobre os marcadores de risco pode ser modulado pela redução da adiposidade e dos mediadores inflamatórios. De facto, identificamos a % de gordura corporal como um preditor dos níveis plasmáticos de bilirrubina, independentemente do polimorfismo UGT1A1*28; os níveis circulantes de adiponectina também se associaram a modificações no perfil lipídico (ex. razão TC/HDLc) independentemente do polimorfismo da apo E.

Relativamente ao estudo longitudinal, os resultados de ambas as abordagens foram semelhantes. Uma pequena redução de adiposidade em indivíduos obesos foi associada a melhorias nos marcadores de risco cardiovascular, nomeadamente no perfil lipídico (redução dos TG, do CT, da LDLc e da razão CT/HDLc), e da sensibilidade à insulina (redução da insulina e do $HOMA_{IR}$). Contudo, as alterações nos mediadores inflamatórios não foram significativas, podendo ser necessárias reduções mais pronunciadas no peso para serem notadas, visto a redução da adiposidade se correlacionar com o aumento da adiponectina sérica (total, de APM e de MPM) e com a redução dos valores plasmáticos da PCR.

Em conclusão, encontram-se alterados vários marcadores de risco cardiovascular em idades precoces, em obesos Portugueses. Abordagens de intervenção voltadas para as escolas e comunidades são boas opções para reduzir a obesidade e o sobrepeso e, assim, o risco de doença cardiovascular na vida futura de crianças e adolescentes. Este trabalho demonstrou que mesmo pequenos progressos no IMC já estão associados a melhorias no perfil lipídico e na resistência à insulina. Demonstrámos, igualmente, que estas mesmas reduções ponderais podem contrabalançar perfis genéticos menos favoráveis.

Palavras-chave: obesidade infantil, síndrome metabólica, inflamação, adiponectina, exercício físico.

Abstract

Portugal has a high prevalence of overweight and obesity in pediatric ages, presenting one third of children and adolescents weight excess.

Obesity is associated with diseases such as hypertension, type 2 diabetes mellitus, dyslipidemia, atherosclerosis and an increased risk of cardiovascular disease later in life. The increase in pediatric obesity has contributed to the appearance of those pathologies in increasingly young individuals.

The adipose tissue secrets many mediators and have an important role in the state of chronic low-grade inflammation described in obese individuals. Adiponectin, an anti-inflammatory adipokine secreted mainly by the adipose tissue in humans, is related to an improvement of insulin sensitivity and lipid profile. Adiponectin circulates as low, medium and high molecular weight multimers (LMW, MMW and HMW, respectively). The HMW multimer, reported to be the most biological active form and responsible by the benefic actions attributed to adiponectin, appears to be reduced in obese patients. Little is known about the biological functions of the other circulating forms.

In this project, we studied in a Portuguese obese pediatric population several cardiovascular risk factors (classical and new), and associated genetic polymorphisms,. Afterwards, we evaluated the impact of changes in body mass index (BMI), obtained by two approaches, in those risk factors: a lifestyle modification program and an interventional program involving physical exercise.

We found that obese children and adolescents presented an increased metabolic risk profile, when compared to their lean counterparts, characterized by: 1) a rise in insulin resistance, with increased homeostasis model assessment – insulin resistance ($HOMA_{IR}$) and insulin levels; 2) a risk change in lipid profile, namely with increased triglyceride (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDLc), and reduced high density lipoprotein cholesterol (HDLc); 3) a reduction in the anti-oxidant protection in the more obese individuals, with the reduction of plasmatic bilirubin; 4) a low grade inflammatory status, with increased plasmatic C-reactive protein (CRP) and neutrophilia and reduced adiponectin. The worsening of the metabolic profile was particularly noticeable with increased abdominal obesity, a feature of metabolic syndrome (MS). Moreover, obese adolescents with MS presented a worst risk profile, when compared to obese individuals without MS. The prevalence of MS in our obese population was high – 24.8%.

In our obese adolescent population, the Center for Disease Control and Prevention criterion for the classification of BMI z-score in pediatric populations was a better predictor of MS, in our obese adolescent population, than the criterion used by the World Health Organization.

Adiponectin plasmatic levels correlated inversely with adiposity markers (BMI, waist circumference and % body fat), with dyslipidemia (TG and TC/HDLc ratio), with insulin

resistance (insulin and $HOMA_{IR}$) and with inflammatory process (CRP); contrarily, the levels of adiponectin presented a positive correlation with circulating HDLc). The different circulating forms of adiponectin demonstrated different actions in pre-pubertal individuals. The HMW form mimicked the beneficial effects of total adiponectin, particularly regarding the lipid profile, while the LMW form presented opposite results.

Some of the studied genetic polymorphisms were associated with increased metabolic derangement: individuals with the E4 allele of the apolipoprotein (apo) E polymorphism presented a worse lipid profile (increased TC, LDLc and TC/HDLc ratio); individuals with lower number of repeats of the apo (a) pentanucleotide (TTTAT)n repeat polymorphism presented increased lipoprotein (a) levels, an independent marker of cardiovascular risk; and homozygous 6/6 individuals for the UGT1A1*28 polymorphism of UDP-glucuronosyltransferase presented a reduction of bilirubin levels. Nevertheless, we verified that the effect of a less favorable genetic profile on risk markers can be modulated by reductions in adiposity and inflammatory mediators. In fact, we identified the % of body fat as a predictor of plasma bilirubin, independently of the polymorphism UGT1A1*28; also, the circulating levels of adiponectin were associated with changes in the lipid profile (e.g. TC/HDLc ratio) independently of apo E polymorphism.

Regarding the longitudinal studies, the result with both approaches were very similar. Small reductions of adiposity in obese individuals were associated to improvements in risk markers, namely in lipid profile (reduction of TG, TC, LDLc, TC/HDLc ratio) and insulin sensitivity (reduction of insulin and $HOMA_{IR}$). However, improvements in inflammatory mediators were not significant and might need greater weight improvements to be noticed, as the reduction of adiposity was correlated with improvements in total, HMW and MMW adiponectin and with a reduction of CRP.

In conclusion, several markers of cardiovascular risk are changed in early ages, in obese Portuguese. Intervention approaches aimed at schools and communities are good options to reduce overweight and obesity and, thus, the risk of cardiovascular disease later in the life of children and adolescents. This study showed that even small improvements in BMI are already associated with improvements in lipid profile and insulin resistance. We also demonstrated that the same reductions in weight can modulate less favorable genetic profiles.

Keywords: pediatric obesity, metabolic syndrome, inflammation, adiponectin, physical exercise.

Contents / Índice

Figure contents / Índice de figuras

Table contents / Índice de tabelas

List of abbreviations

AAP	American Association of Pediatricians
ACRP30	Adipocyte complement-related protein of 30 kDa
ADA	American Diabetes Association
ADHD	Attention deficit hyperactivity disorder
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
AEP	Associacíon Española de Pediatría
AHA	American Heart Association
ALT	Alanine aminotransferase
Apo	Apolipoprotein
ART	Aerobic combined with resistance training
AST	Aspartate aminotransferase
AT	aerobic training (alone)
BMI	Body mass index
BP	Blood pressure
CDC	Centre for Disease Control
CH	Carbon hydrates
CHD	Coronary heart disease
cIMT	Carotid internal media thickness
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEXA	Dual energy x-ray absorptiometry
DsbA-L	Disulfide-bond-A-oxidoreductase-like protein
ECOG	European Childhood Obesity Group
ER	Endoplasmic reticulum
Ero1-Lα	Endoplasmic reticulum oxidoreductin-1-Lα
Erp44	Endoplasmic reticulum resident protein 44
GABA	Gamma-aminobutyric acid
GBP28	Gelatin-binding protein of 28 kDa
GH	Growth hormone
HDLc	High density lipoprotein cholesterol
HGF	Hepatocyte growth factor
HMW	High molecular weight
$HOMA_{IR}$	Homeostasis model assessment insulin resistance
IDF	International Diabetes Federation
IL	Interleukin
IOTF	International Obesity Task Force
IR	Insulin resistance
LDLc	Low density lipoprotein cholesterol
LMW	Low molecular weight
LpL	Lipoprotein lipase
MIRACLE	Metabolic individual risk factor and clustering estimation
MMW	Medium molecular weight
NAFLD	Non-alcoholic fatty liver disease
NCAHP	National Child and Adolescent Health Program
NCEP	National Cholesterol Education Program
NCHS	National Center for Health Statistics
NHBPEP	National High Blood Pressure Education Program
NHLBI	National Heart, Lung and Blood Institute
OECD	Organization for Economic Co-operation and Development
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor 1
PMH	Portuguese Ministry of Health
PNR	Pentanucleotide repeat polymorphism
PPAR-γ	Peroxisome proliferator-activated receptor-γ
PWS	Prader-Willi syndrome
RBP-4	Retinol binding protein 4
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
sICAM	Soluble intercellular adhesion molecule
SSRI	Selective Serotonin Re-uptake inhibitor
sVCAM	Soluble vascular cell adhesion molecule
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor α
UGT1A1	Uridinediphosphate glucuronosyltransferase 1A1
USA	United States of America
VAT	Visceral adipose tissue
VEGF	Vascular endothelial growth factor
VLDLc	Very low density lipoprotein cholesterol
WAT	White adipose tissue
WC	Waist circumference
WC/H	Waist circumference / height ratio
WHO	World Health Organization

1. Introduction

Obesity is increasing worldwide in recent years, both in developed and in developing countries, leading many authors to refer to obesity as the pandemia of the 21st century. In fact, obesity is now considered as a disease with its own complexity, and has been deeply studied in the last years. The modern lifestyle, characterized by increased energy intake and reduced energy expenditure, is at the basis of the obesity phenomena (1).

Pediatric obesity is accompanying the adult trend and has reached high rates in many countries. In Portugal around one third of the children are overweight or obese (2, 3).

The weight excess in young ages is a growing concern nowadays. Besides the related comorbidities, both at metabolic and psychological levels, it is associated with an increased risk for the appearance of a number of pathologies later in life, as type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) (4-6). In fact, obesity, namely central obesity, is part of the metabolic syndrome (MS), a cluster of risk factors for CVD, which encloses dyslipidemia, hypertension, insulin resistance (IR) and impaired glucose metabolism (4, 5).

The mechanisms underlying the associations between obesity and other diseases are not completely understood. Nevertheless, inflammatory mediators are likely to be important contributors, as inflammation plays a vital role in the initiation and progression of atherosclerosis, IR and dyslipidemia (4-6). Actually, an increase in circulating inflammatory mediators is often observed in obesity, contributing to the development of a state of chronic low grade inflammation (7, 8).

The white adipose tissue (WAT) is responsible for the secretion of many molecules, most of them related to inflammation and immunity. With the enlargement of the WAT, as occurs in obese individuals, the type and proportions of the secreted substances turns to pro-inflammatory profile. These mediators will exert their actions not only within the WAT, but all over the organism, contributing to the appearance of obese-related comorbidities (7, 8).

Different approaches have been used to overwhelm adiposity. Diet and exercise based programs are the traditional strategies, aiming at a more favorable caloric balance. The success rates of these programs are widely variable and suffer the influence of many factors, such as the studied population, type of exercise used, and program duration.

2. Obesity in Portugal

Overweight and obesity have increased in Portugal, and all over the world, in the last decades, accompanying the rise in sedentarism and unbalanced dietary habits (2, 9).

Portuguese adult population has a high prevalence of obesity. In fact, an increase of obesity prevalence between 1999 and 2006 was observed, from 12% to 15.2%. The prevalence was higher in the age group between 45 and 74 years, presenting a prevalence above 20% (10). Similar trends were found in a previous study that reviewed available data regarding nutritional status of the adult Portuguese population from 1995-2005, which reported that the prevalence of overweight and obesity at 50 years of age were of 40.5% and 22.6% for women, and 52.7% and 19.0% for men, respectively. In the same study men presented a higher prevalence of overweight than women for all ages; men also presented an increased prevalence of obesity until 40 years and, after this age, higher obesity rates were observed for females (11).

Childhood overweight and obesity are accompanying this global trend and has increased markedly not only in developed but also in developing countries. In Europe, this reality is particularly notorious in the Southern countries, including Portugal (1, 2, 9, 12). In fact, Portugal, Greece, Italy, Slovenia and Spain, present an increased prevalence of overweight and obesity in 6-9 years old children, when compared to the Northern countries (2, 9, 12). The adolescents from the Southern European countries also presented a higher prevalence of overweight and obesity than those from the Northern countries, in a study involving 15 years old adolescents, from countries that are members of the Organization for Economic Co-operation and Development (OECD). Actually, the prevalence of ponderal excess in Portuguese adolescents was only lower than that of the United States of America (USA) and Canada, for girls, and than that of the USA, Canada, Greece and Italy, for boys (1).

In a national survey in 2002-2003 Padez *et al.* (2) found that Portugal presented a high rate of overweight and obesity in children from 7 to 9 years old, with 31.6% of the children having weight excess, of whom 11.3% were obese. In Europe, this prevalence of weight excess was only second to Italy (36%). A higher percentage of obesity and overweight was observed in girls, as compared to boys, in the Portuguese population (2, 12).

In Évora, Alentejo, pre-school children (2-6 years) presented a high prevalence of weight excess and obesity (25.4% and 11.6%, respectively) (13), similarly to what was previously

described by Padez *at al* (2). A peak at 3 years of age was present, with 33.3% of the children of this age group being overweight or obese. The prevalence of overweight in girls was also higher than in boys. It is interesting to notice that a high prevalence of parental obesity was present for these children; about 2/3 of the children had at least one of the parents with overweight, although no significant difference was seen between the parents of normal and overweight children (13).

Other studies in cities from different regions of Portugal [Porto (14), Coimbra (3) and Famalicão (15)], involving children with similar ages to the study of Padez *at al* (2), also found a high prevalence of overweight and obesity, although some fluctuations were present, partially caused by actual geographic differences and by the use of different criteria to diagnose obesity.

A recent study in the central region of Portugal involving children from 6-12 years of age, also found alarming results. There was a prevalence of combined overweight/obesity of 33,0%, being 10,7% of the studied children obese. Contrarily to the previous studies, boys presented increased prevalence of overweight (25.9 vs 19.0%) than girls, while no difference was present for obesity prevalence. Boys also presented a worse body fat distribution, as measured by the waist circumference / height (WC/H) ratio; 28.1% of the boys (vs 19.4% of the girls) presented a ratio higher than 0.5, a cut-off used to identify individuals with increased metabolic risk (16). In this study, a trend towards an increase of weight excess in the oldest studied children was noted, when comparing to the previous values reported by Padez *et al*. Indeed, when analyzing only the children between 7-9 years, an increase of about 10% in the prevalence of overweight/obesity, between the two studies, was noticed (2, 16).

An increase in the prevalence of obesity in older ages (15 years) was also found for almost all OECD countries, including Portugal, although for some (few) countries the prevalence of obesity in girls appears to be diminishing (1).

When anthropometric data of Portuguese children through the last 3 decades is analyzed, a clear increase in height and weight is notorious (1970-2002). This increase was associated with the progress of the living conditions and health system during that period. However, although generally positive, this improvement led to the secondary problem of obesity as the secular evolution of weight was more marked than that of height, causing an increased prevalence of children and adolescents with unbalanced body mass index (BMI) (2).

The most recent Portuguese national survey was conducted by Rito *et al.* in 2007-2008 and involved children from 6 to 8 years old. This survey used 3 of the available references to define childhood overweight and obesity: the International Obesity Task Force (IOTF) reference; the 2000 Centre for Disease Control and Prevention (CDC) reference (recommended by the Portuguese Ministry of Health until 2012); and the World Health Organization (WHO) reference, 2007 (the currently accepted reference). The use of the different cut-offs lead to different outputs: the IOTF criterion resulted in lower prevalence of overweight and obesity and increased rates of thinness; the WHO reference led to lower prevalence of thinness and higher prevalence of obesity and overweight; and the CDC criterion resulted in an intermediate prevalence, between the other two references. In fact, using the IOTF reference, the prevalence of thinness, overweight and obesity in Portugal were of 4.8%, 28.1% and 8.9%, respectively; using the CDC reference the prevalence values were of 2.1%, 32.2% and 14.6%, respectively; and according to the WHO reference, the values were of 1.0%, 37.9% and 15.3%. The complexity of using different classifications is not only a matter of moving the distribution curve to the right or to the left; indeed, the prevalence is different in absolute values and, sometimes, the use of different references can give opposite results as, in this study, using IOTF criterion, the girls had increased prevalence of thinness, when compared to boys, while opposite results were found when applying the other 2 references. For all references the boys presented a higher percentage of obesity and combined overweight and obesity than girls, however, gender was not a predictor of the nutritional status (17).

Rito *et al.* studied the nutritional status of children across the different Portuguese regions. Algarve was the region with the lowest prevalence of overweight/obese, while the islands of Açores and Madeira presented the highest prevalence (Table 1, using WHO as reference). This study showed that the risk of childhood obesity was related to the geographic region, increasing for children living in urban areas, and that obesity was not influenced by gender or by age (17).

The regional distribution of obesity prevalence in Portugal for 6-8 years old children was similar to the distribution reported for adults, as presented in Table 1.

Table 1. Prevalence of obesity and overweight in children and adults from different Portuguese regions

	Adults	Children[1]	
	Obesity (%)	Obesity (%)	Overweight[2] (%)
Norte	**14.9**	**14.4**	**38.6**
Centro	**13.1**	**16.0**	**38.1**
Lisboa e Vale do Tejo	**16.8**	**16.0**	**38.3**
Alentejo	**15.5**	**13.0**	**31.6**
Algarve	**12.0**	**9.7**	**21.4**
Açores	**NA**	**16.5**	**39.4**
Madeira	**NA**	**15.3**	**37.9**
Portugal	**15.2[3]**	**15.3**	**37.9**

NA, not available. 1, 6 to 8 years; 2, combined overweight and obesity; 3, values from continental Portugal only. Adapted from (17, 18).

The results of revision studies accessing the prevalence of overweight and obesity in pre and primary school children in different Portuguese regions and in two European countries (Latvia and Italy) are presented in Table 2. The age range (3-12 years old) was chosen because the majority of the prevalence pediatric studies in Portugal has been performed within this range. The option of researchers to work in this age range is probably due to the fact that it is in this development period that the adiposity rebound takes place; therefore, this in an important period for health professionals to intervene and prevent the development of obesity; furthermore, the confounding effects of puberty are still not present (2, 17). Prevalence from Italy and Latvia are highlighted due to their opposite rates: Italy present a high prevalence of childhood overweight and obesity (similar to Portugal), while Latvia present one of the lowest prevalence in Europe (9).

The differences in the prevalence of obesity highlight the importance of the environmental factors, namely, socio-economic realities, which might lead to significant variations, even in a relatively small country as Portugal.

It is also important to emphasize that the use of different criteria for the classification of the children nutritional status makes difficult the comparison between studies. This has also occurred in Portuguese studies.

In the Netherlands there is also a wide variance in the diagnosis criteria and in the treatment strategies followed by different pediatricians. Pediatric specific cut-off values, adapted for age and sex, were only used by 60% of the pediatricians (32% used the IOTF criterion, while 28% used Netherland population specific cut-off). Surprisingly, around 40% of the pediatricians used adult cut-offs for BMI, causing an underestimation of the prevalence of overweight and obesity in children and adolescents (19).

Table 2. Prevalence of overweight and obesity in pre and primary school children, in different Portuguese regions and European countries

Autor	Country	Region	Period	Participants	Female (n)	Age (years)	Criteria	OW+OB (%)	OB (%)
Rito 2006 (3)	**Portugal**	**Coimbra – Central**	**2001**	**2350**	**1168**	**3-6**	**CDC**	**23.6**	**6.7**
Gomes 2010 (13)	**Portugal**	**Évora – Central**	**2007**	**313**	**137**	**2.2-6.8**	**CDC**	**25.4**	**11.6**
Branco 2011 (15)	**Portugal**	**Famalicão – North**	**2009**	**207**	**102**	**6-7**	**CDC**	**46.9**	**28.5**
Ferrão 2013 (14)	**Portugal**	**Porto – North**	**2009**	**2690**	**1377**	**6.7±2.2**	**IOTF**	**31.8**	**10.3**
Albuquerque 2012 (16)	**Portugal**	**Central**	**2011**	**1433**	**747**	**9.3±1.8**	**IOTF**	**33.0**	**10.7**
Padez 2005 (2, 12)	**Portugal**	**National Survey**	**2002-2003**	**4511**	**2274**	**7–9**	**CDC**	**31.6**	**11.3**
Rito 2008 (17)	**Portugal**	**National Survey**	**2007-2008**	**3765**	**1871**	**7.0±0.7**	**IOTF**	**28.1**	**8.9**
							CDC	**32.2**	**14.6**
							WHO	**37.9**	**15.3**
Wijnhoven 2012 (9)[1]	**Europe**	**Portugal**	**2008**	**1815**	**904**	**7**	**IOTF**	**26.8/28.5**[2]	**7.9/9.3**[2]
							WHO	**40.5/35.5**[2]	**16.7/12.6**[2]
		Italy	**2008**	**5144**	**2515**	**8**	**IOTF**	**37.2/34.7**[2]	**13.6/11.8**[2]
							WHO	**49.0/42.5**[2]	**26.6/17.3**[2]
		Latvia	**2008**	**3249**	**1598**	**7**	**IOTF**	**15.3/15.1**[2]	**4.5/3.1**[2]
							WHO	**24.0/18.9**[2]	**8.6/4.6**[2]

CDC, Centre for Disease Control; F, female; IOTF, International Obesity Task Force; M, male; OB, obese; OW, overweight; WHO, World Health Organization; Y, year. 1, European survey of 12 Countries: Belgium, Bulgaria, Czech Republic, Ireland, Italy, Latvia, Lithuania, Malta, Norway, Portugal, Slovenia and Sweden; 2, percentages for males/females.

Obesity has been an important point of discussion in the last years, what might have contributed to an increased awareness of the general public to this problem. Actually, the increase in the obesity and overweight prevalence, as determined by self-reported measurements, in the period of 1995-2005, accompanying the changes in objectively measured anthropometric data, might corroborate that awareness. Nevertheless, the increasing obesity and overweight rates in young adults in the same period highlights that this nutritional problem is far from solved. This fact can be related to an obesogenic environment, present since early in life, and that makes unhealthy choices the easier ones. Thus, a growth of obesity-related comorbidities can be expected in the next years in Portugal, particularly if objective actions to fight it are not taken (11).

3. Risk factors for obesity

The development of overweight or obesity depends on a variety of risk factors, hereditary and environmental. The interaction between specific genes and the environment may trigger the obese phenotype to emerge. Different factors are known to increase the risk for childhood obesity, such as: genetic factors, family habits, socio-economic environment, sedentarism and diet (20, 21). The risk factors are, usually, associated with either an increase in energy intake or with reduced energy expenditure. Some of the most important risk factors for obesity are presented in Table 3.

Intra-uterine life and early childhood appear to be crucial periods for the development of obesity later in life. Differences in growth patterns during intra-uterine life and young ages have been associated with increased risk for developing obesity both in childhood and in adulthood. Early life development must be linked to structural and physiological changes that will modulate the metabolism and influence the development of obesity and other diseases later in life (20, 21).

Although some obesity risk factors are common in different societies due to globalization, others are very cultural-specific, such as particular types of food and socio-economic development (20, 21).

Obesity is closely linked to many cardiovascular disease (CVD) and metabolic risk factors, each one with particular environmental and genetic determinants. The genetic correlation between anthropometric and metabolic risk factors (e.g. lipid profile and glucose metabolism) suggests the existence of pleiotropic genes influencing more than one risk factor and, thus, leading to their clustering (21).

Premature children usually experience what is called a catch-up growth, and are referred to fully achieve their genetic growth potential; however, the risk of obesity according to birth weight seems to follow a U-shaped curve, with increased risk for extreme values (22, 23).

In agreement, premature children (2-6 years old) from Évora, presented the greatest odds for overweight. Nevertheless, no relation was found between obesity and the absolute birth weight or the maternal weight gain during pregnancy. Also, no ponderal differences were present between parents of overweight/obese and lean individuals (13).

Table 3. Risk factors for the development of pediatric overweight and obesity

Group of risk factors	Risk factors
Physical (in)activity	• Increased hours of screen time (TV watching, video games, computers) • Decreased hours of extra-curricular activities • Use of passive transport • Reduced physical activity habits
Energy intake	• General unbalanced diet • Snacking and consume of fast food and sweetened drinks • Consume of processed caloric dense foods • Binge eating and bulimia • Skipping breakfast • Low fiber diet • Low number of meals per day and irregular meal schedule • Increased meal portion size
Personal history	• Maternal gestational diabetes • Prematurity • Low / high birth weight (adjusted for gestational age) • Short duration of breast feeding (< 3 months) • Unbalanced diet in first childhood (e.g. protein excess)
Family factors	• Parental obesity • Family history of obesity and overweight • Small families (low number of siblings) • Lower parental education • Lower socio-economic status
Environmental factors	• Geographic region (e.g. living in islands increase the risk of obesity) • Living in urban areas* • Developing countries* • Ethnic minorities • Exercise and physical activity unfriendly environment

* increased risk is not well established.

Padez *et al.* studied the risk factors associated to ponderal excess in a Portuguese children population (12). Girls presented a higher risk of overweight or obesity than boys. The risk of obesity also increased with parental obesity (more significantly with maternal obesity), lower parental education, smaller families (lower number of siblings), lower number of sleeping hours, increased hours of television watching, increased birth weight and breastfeeding duration shorter than 3 months (12). In agreement, a study in

Famalicão (6 and 7 years old children) also found trends towards increased risk of obesity in girls, for children born with increased birth weight, decreased parental education, increased hours of television watching and for children living in rural areas (15). Padez *et al.* found no influence of physical activity and energy ingestion on the risk of becoming obese (12). The lack of association of these last two variables, commonly associated with obesity, might be caused by the fact that they were obtained through a questionnaire, and not objectively measured (12). In a similar study in Japanese adolescents, an increased risk of obesity was observed for individuals that did less exercise, expended more hours playing video games or did not have breakfast (24).

The importance of the environment on increased risk of childhood obesity is focused in a study by Ferrão *et al.* (14). This study, involving children between 3 and 10 years of age, in Porto, found that children were at increased risk of obesity if their parents had a bad perception of neighborhood safeness for children to walk or ride a bike during day or night, of the local traffic or of the quality of sidewalks (14). Parents that perceive their neighborhood as unsafe are less likely to allow their children to engage in outdoors activities. Interestingly, Branco *et al.* found a trend to an increased risk of obesity in children leaving in rural areas, which are generally accepted as safer to outdoor activities than urban areas (15). Thus, there are different aspects influencing the development of childhood obesity.

Contrarily to the findings of Branco in Famalicão (15), the study by Rito *et al.* found an increased risk of obesity for children living in cities, when compared to children in the rural areas (17). Gomes *et al.*, on the other hand, found no difference in the prevalence of overweight/obese children from rural and urban areas of Évora (13). The definition used to validate a risk factor is crucial for the statistical outcome. The definition used by Rito and Gomes was that of the National Institute of Statistic, based on which Alentejo is the only rural region in Portugal, while the definition used by Branco is not clear. The fact that Évora is a city in Alentejo might have included a bias in the analysis (13).

The risk factors for the development of obesity described in the Portuguese population are similar to other populations. Some differences may, actually, result from socio-cultural factors and others might be influenced by study designs and analytical bias (e.g. use of questionnaires instead of objective measures).

4. Diagnosis and evaluation in pediatrics

The procedures recommended for the diagnosis of overweight and obesity in pediatric patients are standardized, although some differences are seen between pediatricians and centers. These differences involve, mostly, the type of measurements and determinations made and their valorization. Besides these differences within the same country, the differences between different countries are more marked and make it difficult to compare different studies (6, 25).

The Portuguese National Child and Adolescent Health Program (NCAHP) includes several medical appointments, at specific time points, to evaluate pediatric development at physical, psychological and social levels. In each visit, starting at birth, different check points are screened, corrective measures are taken, if necessary, and reevaluated in the next appointment. Anthropometric measurements are taken at all medical visits and nutritional status is evaluated according to growth charts (18).

The use of gender and age adjusted growth charts is crucial to evaluate and follow the child/adolescent development. Portugal used until 2012 the CDC 2000 growth charts, which were built based in the American population. Since 2012, it is recommended the use of the WHO "Child Growth Standards", from birth until 5 years of age, and the "WHO Reference 2007" growth charts, from 5-19 years of age. The WHO criterion is reported to be more sensitive to identify groups of lean, overweight and obese subjects, each presenting different biochemical and vascular characteristics, gradually worsening from lean to obese children (6).

The development of WHO Child Growth Standards (0-5 years) resulted from an initiative involving 5 countries (Brazil, USA, Norway, Oman and India). To develop these new charts only children in their maximum growth potential were included and followed. These children come from normal term pregnancies, were breast-fed for at least 3 months and had a correct nutritional diversification. Country specific charts are constructed using, usually, a single population without the best development conditions; thus, these charts might have limited applicability. As the WHO growth charts were constructed using a multicenter initiative, they can be internationally applied, and used for comparisons between different countries. In fact, these charts were first tested in four countries in pilot studies and similar results were obtained. Furthermore, a good relation between the measured anthropometric variables and other developmental and biochemical markers were confirmed. The construction of the WHO reference 2007 (5-19 years) involved the

use of the 1977 National Centre for Health Statistics (NCHS, USA) data that included children who had reached their full height potential without being overweight, after removing the outliers. The NCHS data were merged with the records of the 18-71 year-olds of the WHO standards sample and used to built the new reference. An objective was to obtain a smooth transition from the Child Growth Standards (0-5 years) to the WHO Reference 2007 (5-19 years), and from this to the older age group (18, 26).

The following WHO curves are available and should be used in every pediatric appointment in Portugal (18, 26):

- Head circumference – birth to 2 years;
- Height/length – birth to 19 years;
- Weight – birth to 10 years;
- BMI – birth to 19 years.

BMI growth charts, adapted to age and sex, are used to evaluate the nutritional status of children and adolescents. The cut-offs used for that classification are presented in Table 4 (26).

Table 4. Cut-offs for nutritional status, according to WHO BMI growth charts

Nutritional Status	Lower limit	Upper limit
Extreme thinness	-	**< -3 SD**
Thinness	**≥ -3 SD**	**< -2 SD (P3)**
Underweight	**≥ -2 SD (P3)**	**< -1 SD (P15)**
Normal	**≥ -1 SD (P15)**	**≤ 1 SD (P85)**
Overweight	**> 1 SD (P85)**	**≤ 2 SD (P97)**
Obesity	**> 2 SD (P97)**	**≤ 3 SD**
Extreme obesity	**> 3 SD**	-

Adapted from (18). SD, standard deviation; P, percentile.

Other criteria are used to evaluate nutritional status in pediatric patients, leading to different classifications. The IOTF criterion, for example, has been used in a considerable number of studies (9, 14, 16, 17). This criterion has some disadvantages, as compared to WHO or CDC criteria. In fact, the WHO criterion has an increased sensitivity and specificity to identify obese children, when compared to IOTF reference. Besides, the

IOTF criterion uses references ranges of BMI instead of providing specific BMI z-score (as occurs with CDC and WHO references). The use of BMI ranges leads to the inclusion in the same classification of children with different BMI percentiles (although the variation is not very wide), losing some of the information of the gradual relation between BMI and other variables, and the power to detect effects of short-term interventions. Besides, IOTF do not have a reference for height measurements (6, 27).

After the initial diagnosis of obesity it is important to identify the underlying causes. Although the majority of obesity cases are secondary to a positive energy balance, other causes, such as punctual mutations, syndromic forms of obesity, metabolic diseases and iatrogenic causes can underlie obesity and should be excluded. The use of an organized anamnesis, focused on the family and individual history, and on the risk factors for the development of obesity, might help to clear some doubts (5).

The NCAHP present a list of check points that should be focused in each visit and that, if correctly followed, might provide important information about the cause of obesity, either genetic or environmental, and unveil some comorbidities that might already be present, such as dyslipidemia, hypertension, IR and nephropathy (5, 18). Some of the points that should be addressed at an obesity appointment are listed in Table 5 (5, 18, 25).

Obese children and adolescents have, usually, a faster development and often present increased height and sexual development, particularly in girls. The presence of obesity with slower development rates should be further studied, as a hormonal (e.g. lack of growth hormone (GH), hypothyroidism) or genetic problem might be present (5, 25). Wrist X-ray and dual energy x-ray absorptiometry (DEXA) can evaluate the bone biological age, which comparison to the chronological age might help to evaluate changes in the development rates and unveil different obesity etiologies (5).

Obese pediatric patients frequently present altered blood analysis. Thus, several variables are routinely assessed during clinical appointments, such as plasmatic triglycerides (TG), high density lipoprotein cholesterol (HDLc), glucose and insulin. Adiponectin, an adipokine secreted by the white adipose tissue (WAT), with anti-inflammatory and anti-atherogenic properties, is reduced in obese individuals. Adiponectin has also been suggested as an indicator of metabolic risk in obesity (5).

Table 5. Points to address in an obesity pediatric appointment

Dimension studied	Factors
Family history	• Birth country and ethnic background; • Family structure – who lives with the children; • Socio-economical context; • Parents and sibling basic anthropometric measurements; • Relatives with T2DM, dyslipidemia, hypertension and other risk factors for CVD; • Family history of early CVD (< 55 years in man, < 65 years in women);
Personal history	• Maternal pregnancy history (maternal weight gain, pre-eclampsia, smoking habits, gestational diabetes, term/pre-term pregnancy…); • Birth weight, length and head circumference; • Period of breast-feeding; • Nutritional diversification following breast-feeding; • Weight and length/height development;

Lifestyle habits	• Sleeping – hours and quality;
	• Dietary habits
	  o Types of food eaten, frequency and size of portions;
	  o Skipping breakfast;
	  o Frequency of meals outside from home;
	  o Consume of sweetened drinks and snacks;
	  o Meals in family;
	  o Binge eating and bulimia;
	• Physical activity habits
	  o Sedentary habits (hours of TV watching, video-game playing, ...)
	  o Sports playing, other active hobbies;
	  o Active transport to school;

Clinical evaluation	• Length/height and weight measurement – comparison to growth charts adjusted for sex;
	• Waist circumference;
	• Sexual development;
	• Body fat distribution – peripheral (gynoid) or central (android);
	• Blood pressure;
	• Presence of striae, acanthosis nigricans;
	• Blood analysis
	  o Lipid profile;
	  o Fasting insulin and glucose;
	  o Other analysis
	  ▪ Thyroid hormones;
	  ▪ Growth hormone;
	  ▪ Glycated hemoglobin;
	  ▪ Hepatic transaminases;
	  ▪ Ionogram;
	  ▪ Creatinine and urea;
	  ▪ Oral glucose tolerance test;
	  ▪ Genetic analysis;
	  ▪ …
	• Urine analysis
	  o Microalbuminuria
	  o Glucose
	• Complementary anthropometric measurements
	  o Body composition (fat mass, lean mass);
	  o Dual energy X-ray absorptiometry, skin folds, …
	  o Wrist X-ray

CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus

Besides the diagnosis of total obesity, it is important to assess the body composition and the fat distribution, as central obesity is associated with increased CVD risk. BMI and BMI z-score can be used in children as an indicator of body fat; however, increasing lean mass due to physical fitness can give a false idea of weight excess, especially in adolescents practicing sports. Total body fat is physiologically more relevant to determine the risk of CVD than BMI, and can be accessed by bio impedance, skin folds and by air displacement plethysmography. Body fat distribution and body composition can be estimated by using skin folds, however, more specific methods are available, such as DEXA, computed tomography and magnetic resonance. Nevertheless, in a pediatric routine appointment, waist circumference (WC) and WC/H ratio are good tools for indirectly evaluate the presence of abdominal obesity, after comparing the values to gender and age adapted charts. Unfortunately, Portugal and WHO do not have population representative charts for WC or WC/H ratio; thus, pediatricians need to use international cut-offs, with the consequent bias in the classification (5). Indeed, pediatric charts for percentage of body fat, abdominal fat and WC would be valuable tools for the diagnosis and management of obesity by pediatricians and other health professionals.

5. White adipose tissue as an endocrine organ

The WAT was considered for some time as a tissue for storage of lipid reserves; nowadays it is known as an active endocrine organ (5). WAT has several secretory functions, namely those associated with the lipid metabolism, such as the release of lipoprotein lipase (LpL), an enzyme that breaks circulating TG to fatty acids. Other lipid particles secreted by the WAT include steroid hormones, retinol, cholesterol and prostanoids (28); however, only some prostanoids are synthesized within the WAT. Retinol and cholesterol are just stored in that tissue and released in the sequence of an adequate stimulus. Some steroid hormones are activated within the adipocytes, e.g. the activation of 11-dehydrocorticosterone (cortisone) into corticosterone (cortisol) (29).

Besides the important role in the lipid metabolism, the WAT is also a source of many other molecules, with several functions.

In the 1980's the adipsin, a complement-related factor, was reported as a protein secreted by adipocytes (30). Following the timeline, the secretion of the pro-inflammatory mediator tumor necrosis factor α (TNF-α) by the WAT was described in the early 90s (7). TNF-α is increased in obesity and has been closely associated with the development of insulin resistance (IR), one of the most common metabolic derangements in obesity (31). TNF-α also influences the lipid metabolism by stimulating lipolysis and apoptosis within the WAT, processes that are intensified in obese individuals (32, 33).

The turning event that definitely changed the way WAT was faced occurred in 1994, with the discovery of leptin. A mutation in the gene responsible for the expression of leptin led to obesity in ob/ob mice (obese / hyperglycemic mice). The homologous human gene was later described as the Obese (Ob) gene (8). Leptin acts in the hypothalamus and helps to control energy intake and expenditure. The absence of leptin increases appetite and decreases energy expenditure, leading to obesity. The discovery of a molecule with such a global influence in the metabolism raised, finally, the WAT to the category of endocrine organ. Later, it was discovered that leptin was secreted by other tissues and cells. Even so, the WAT is the main source of leptin in humans (34). Furthermore, although elevated in obesity, leptin action is deregulated in this disease.

Since the report of adipsin, more than 50 other molecules secreted by the adipose tissue have been described. The secretory function of the WAT is so important that the group of the secreted molecules, the WAT secretome, was named "adipokines" (28, 35).

The adipokines have the most varied functions, and are involved in many biological processes, such as in the lipid metabolism [e.g. adiponectin, apolipoprotein – (apo) – E], angiogenesis [e.g. vascular endothelial growth factor (VEGF)), hepatocyte growth factor (HGF)], energy intake [e.g. leptin, interleukin (IL) -6], hemostasis [e.g. plasminogen activator inhibitor (PAI) -1], blood pressure (e.g. angiotensinogen), and insulin signaling (e.g. adiponectin, leptin, TNF-α) (35).

Several adipokines are also linked to immune and/or inflammatory functions. Indeed, it has been suggested that adipocytes have functions similar to immune cells, and that pre-adipocytes have the capacity of acting, in certain conditions, as macrophages (36).

The secretion of some adipokines can influence the production and action of others in a paracrine, autocrine or endocrine ways (28, 35).

The adipocytes are not the only secreting cell in the WAT; other cells, as the macrophages, vascular endothelial cells and pre-adipocytes, have a relevant role in adipokine production (37).

Considering the large number of substances secreted by the WAT, and their function, it is clear that in the center of obesity related inflammation is the WAT. Table 6 presents some of the most relevant adipokines released by the WAT, the cell(s) type responsible for their synthesis and respective functions.

Table 6. Adipokines produced in the WAT, biological function, association with obesity and with other markers

Adipokines	Plasma levels	WAT locals/cells of synthesis	Biological action	Relation with obesity	Role in obesity related diseases	Relation with other markers or mediators
Adiponectin (24, 38-57)	5-30 µg/ml	Adipocytes VAT > SAT*	- Anti-inflammatory/oxidant - Energy homeostasis ↑ Insulin sensitivity - Improve lipid metabolism ↓ Atherogenesis ↑Adipocyte differentiation	↓ With obesity ↑ After weight loss*	↓ in IR, dyslipidemia, NAFLD, MS, T2DM, atherogenesis, CVD	↑with renal failure, in women. ↓with TNF-α, IL-6, CRP, PAI-1, fibrinogen, UA, age, puberty.
Leptin (4, 24, 55-71)	2-11 ng/ml	Mainly in adipocytes SAT > VAT	-Food intake, energy expenditure and fat storage through central action (Hypothalamus) - Angiogenesis, hematopoiesis	↑ With obesity ↓ After weight loss	↑ in IR, T2DM, dyslipidemia, NAFLD, MS atherosclerosis, CVD	↑with TNF-α, IL-1, CRP, insulin, estrogens women ↓with glucocorticoids and androgens
Resistin (24, 49, 57, 70-84)	5-25 ng/ml	Mainly mononuclear cells. Also adipocytes	-In mice: leads to IR; interferes with glucose metabolism -Humans: not well defined ↑ Adipocyte differentiation	↑ with obesity* ↓ after weight loss*	↑ in IR, NAFLD, T2DM, MS, atherosclerosis, CVD*	↑with TNF-α, IL-1, IL-6,IL-12, CRP, VCAM, ICAM, women ↓with adiponectin*
TNF-α (7, 31, 55, 57, 65, 85-90)	0,5-2,0 pg/ml	Mainly mononuclear cells Also adipocytes SAT > VAT	-Pro-inflammatory cytokine -Studies point to a more local action within the WAT	↑ With obesity ↓ After weight loss	↑ in IR, T2DM, atherogenesis	↑ with resistin, visfatin, IL-1, IL-6, CRP MCP-1, ↓ adiponectin and leptin
IL-1 (55, 91-94)	IL-1α and IL-1β 0,1-0,5 pg/ml	Mainly mononuclear cells. Also by adipocytes VAT > SAT	-Pro-inflammatory cytokine -IL-1α seems to have a more positive effect in glucose´s metabolism, contrarily to IL-1β	↑ With obesity ↓ After weight loss*	↑ in IR, T2DM	↑ with leptin, resistin, TNF-α, CRP ↓ adiponectin
IL-6 (51, 55, 68, 70, 71, 95-105)	0,5-20 pg/ml	Mainly vascular stromal fractions VAT > SAT	-Pro-inflammatory cytokine -Impairs insulin effect -Anti-obesogenic* - ↓ fat mass, ↑ energy expenditure in mice	↑ With obesity ↓ After weight loss*	↑ in IR, dyslipidemia, MS, T2DM, atheriosclerosis	↑ with resistin, TNF-α, IL-1 ↓ adiponectin
IL-18 (57, 106-108)	120-270 pg/ml	Adipocytes and vascular stromal fraction VAT ≈ SAT	-Pro-inflammatory cytokine -Body weight control and glucose metabolism - positive effect in the lean	↑ With obesity	↑ in T2DM	↑ TNF-α, CRP ↓ adiponectin

Adipokines	Plasma levels	WAT locals/cells of synthesis	Biological action	Relation with obesity	Role in obesity related diseases	Relation with other markers or mediators
Chemokines						
MCP-1 (88, 109-117)	70-300 pg/ml	Mainly vascular stromal fraction Also by adipocytes VAT > SAT	-Chemiotatic for monocytes and lymphocytes -Leads to macrophage infiltration in the WAT -Possible role in regulating food intake (centrally)	↑ With obesity ↓ After weight loss	↑ in IR, dyslipidemia, hypertension, NAFLD, MS, atherogenesis	↑ TNF-α, leptin, age ↓adiponectin
Angiogenic factors						
VEGF (57, 118-121)	50-900 pg/ml	Platelets Also by hypoxic pre-adipocytes VAT > SAT	-Angiogenic factor – vascular remodelling -Important in growing WAT vascularization	↑ With obesity	↑ in atherogenesis	↑ insulin, AT II
Acute-Phase Proteins						
CRP (4, 24, 40, 70, 99, 122-129)	0,5-10 μg/ml	Mainly by hepatocytes Also adipocytes VAT IL-6 induces hepatic secretion	-Acute phase protein -Elevated levels are related to bad CVD prognostic	↑ With obesity ↓ After weight loss	↑ in IR, dyslipidemia, hypertension, T2DM, MS, atherosclerosis, CVD	↑ IL-6, man ↓adiponectin, oral anti-diabetics, anti-hypertensive, anti-cholesterolemics
Other Factors						
RBP-4 (57, 130-137)	12-48 μg/ml	Mainly hepatocytes Also adipocytes VAT > SAT	-Linked to retinol metabolism (delivery to tissues, prevent renal depuration, stabilize extra and intra cellular retinol)	↑ With obesity ↓ After weight loss	↑ in IR, NAFLD, MS, T2DM, Atherogenesis	↑ age*, man
PAI-1 (49, 57, 138-145)	0,75-15 ng/ml	Mainly endothelial cells and platelets Also adipocytes VAT > SAT	-Protein of the hemostatic process -Inhibitor of tissue plasminogen activator - Elevated concentrations lead to a pro-coagulation state -Reduces adipocyte differentiation	↑ With obesity ↓ After weight loss	↑ in IR ↑ leads to atherogenesis, CVD	↑ insulin ↓ adiponectin

*, association is not well established. AT-II, angiotensin II; CRP, C-reactive protein; CVD, cardiovascular disease; ICAM, intercellular adhesion molecule; IL, interleukin; IR, insulin resistance; MCP-1, monocyte chemoattractant protein 1; MS, metabolic syndrome; NAFLD, non-alcoholic fat liver disease; PAI-1, plasminogen activator inhibitor 1; RBP-4, retinol binding protein 4; SAT, subcutaneous adipose tissue; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor α; VAT, visceral adipose tissue; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; WAT, white adipose tissue.

6. Adiponectin

Adiponectin is an adipokine with multiple positive effects on metabolism, such as increasing insulin sensitivity and reducing oxidative stress, inflammation, atherogenesis and vascular remodeling (42, 146). Adipocytes are the main source of adiponectin, however its levels are paradoxically reduced in obese individuals (147).

Due to its biological actions, and reduced circulating levels in obesity, adiponectin has been deeply studied by the scientific community, particularly regarding the relation between lower adiponectin levels in obese individuals and obesity-related diseases and comorbidities. Adiponectin levels seems to be already reduced in young obese subjects, nevertheless, the relation between adiponectin and other metabolic markers are not well established, particularly before puberty (42, 148). Moreover, the influence of weight loss therapies, with or without an exercise program, on adiponectin levels is not clear and needs further investigation (42, 51, 148).

Adiponectin is a 244 amino acid protein with 28 kDa, and with 4 domains: a signal peptide in the N terminus, a short variable region, a collagenous domain and, finally, the globular domain in the C-terminal that is homologous to complement C1q (147, 149) (Figure 1).

The adipokine that is now called adiponectin had different names in the past, as it was discovered simultaneously by different groups, working in humans and in mouse: adipose most abundant gene transcript and gelatin-binding protein of 28 kDa (GBP28), in humans; adipoQ and adipocyte complement-related protein of 30 kDa (ACRP30), in mouse (147, 150).

In humans, adiponectin is a protein that is mainly secreted by adipocytes and its gene is mostly expressed in adipocytes (147, 149); its expression increases over 100 times throughout adipocyte differentiation (151). Adiponectin gene is located at the chromosomal band 3q27, a particularly susceptible locus for diabetes and CVD (152).

Adiponectin represents 0.01 % of human plasma proteins and its normal plasma level ranges between 2 to 30 mg/l (51, 151).

6.1. Adiponectin circulating forms

Three different types of adiponectin complexes are formed before secretion from adipocytes: trimers, hexamers (formed by two trimers) and larger structures, resulting from the combination between trimmers and hexamers: 9mers, 12mers and 18mers. The trimers are usually referred as low molecular weight (LMW) adiponectin, the hexamers as medium molecular weight (MMW) adiponectin, and the higher forms as high molecular weight (HMW) adiponectin (153). In humans, the three main forms found in circulation are trimers, hexamers and 18mers. Usually, monomers are not present in circulation (147).

The biosynthesis of adiponectin multimers is a complex process involving extensive post-translational modifications, which are critical for the intracellular assembly and stabilization of its oligomers (147, 154). Close to the N terminus exists, in a conserved region, a cysteine residue, cysteine 36 in humans, that is also crucial for adiponectin multimerization (151). Figure 1 presents a resume of the post-translational multimerization process and the mediators influencing it.

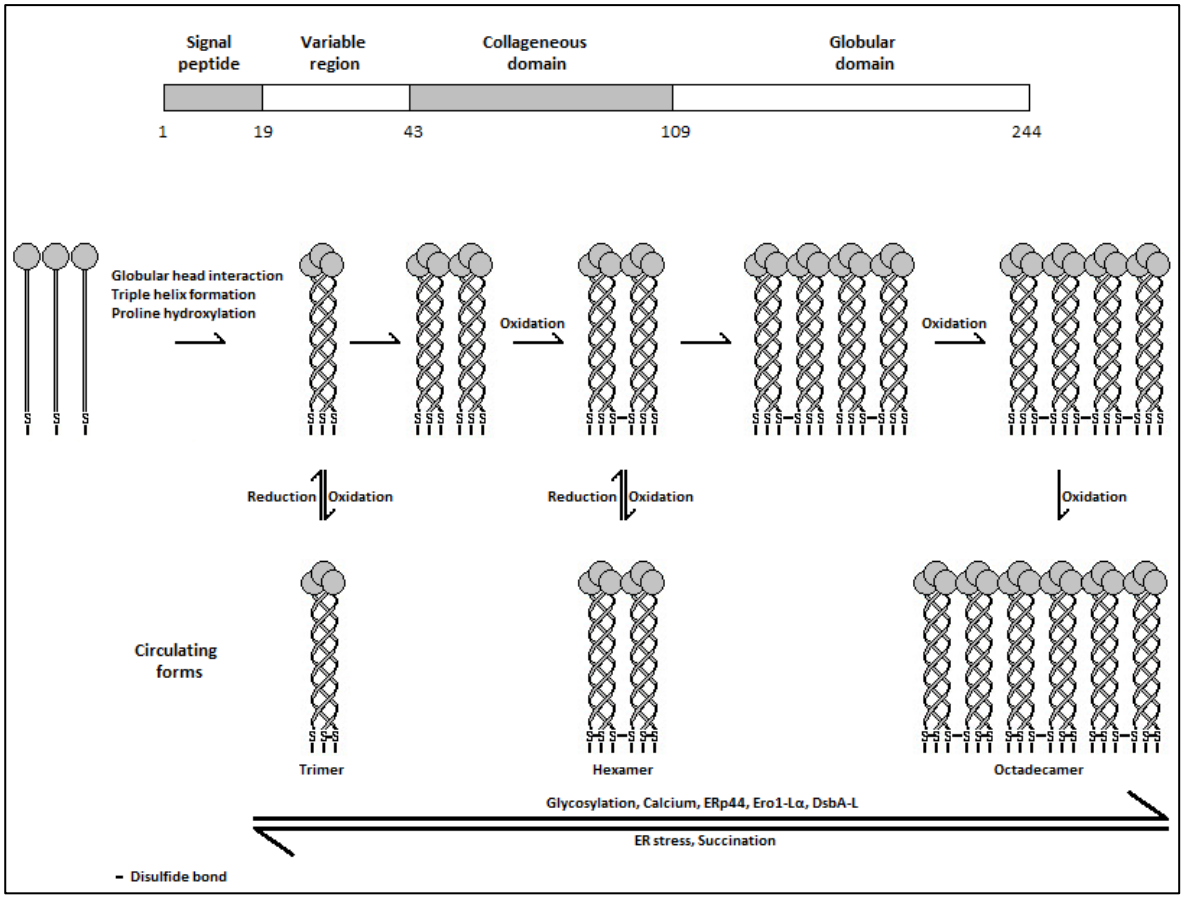


Figure 1. Multimerization process of adiponectin and related mediators
Adapted from (147). DsbA-L, disulfide-bond-A oxidoreductase-like protein; Ero1-Lα, endoplasmic reticulum oxidoreductin-1-Lα; ER, endoplasmic reticulum; ERp44, endoplasmic reticulum resident protein 44.

Trimers are formed by the interaction between the globular domains forming a highly hydrophobic interior in the area where the three monomers interact. Trimers present a ten-strand jelly-roll topology, presenting an interesting homology to TNF-α structure, especially when considering their opposite biological activities (147, 151). Following the interaction of the globular domains, the collagenous domains form triple helix, further stabilizing the trimer. For interaction and stabilization of the triple helix, the hydroxylation of proline residues within the collagenous domain is important (151). The resulting structure resembles a lollypop. A mature trimer, the form found in circulation, presents an intra-trimer disulfide bond in a conserved region close to the N terminus. Theoretically, there is still a free cysteine residue from the third monomer, however, probably due to conformational changes, this cysteine is not available to interact with other adiponectin multimers while still in the endoplasmic reticulum (ER) and, if the dissulfide bond is not reduced, the secretion will happen in the form of a trimer (147).

Hexamers are formed by two trimers in a head-to-head and tail-to-tail configuration and also involve the formation of disulfide bonds between cysteine in the conserved region close to the N-terminus of the molecule. A mature hexamer usually present 3 disulfide bonds, 2 intra-trimer and 1 inter-trimer, presenting no free cysteine for further formation of disulfide bonds (147).

The formation of HMW multimers needs the interaction between trimers and hexamers that are not completely oxidized, still having available cysteine residues to form disulfide bonds. The disulfide bonds are important for stabilizing the intermediate structures. HMW adiponectin has a bouquet-like structure, with all the globular domains in the same side. In fact, the HMW adiponectin form, 18mers, the most common in humans, is in fact a group of three hexamers stabilized by non-covalent bonds. A very important contribution for the non-covalent bonds and formation of 18mers is the glycosylation of amino acid residues. Proline and lysine from the collagenous domain, are extensively hydroxylatated, consisting on the first step to the glucosyl-galactosyl disaccharide glycosylation of the hydroxyl groups of four conserved lysine residues. A cooperative effect is present regarding the relation between lysine glycosylation and the oligomerization of adiponectin, leading to a sigmoidal type curve. In this way, the substitution of one lysine residue has almost no effect, the substitution of 2 residues lead to a medium reduction on HMW adiponectin forming and the substitution of 3 residues drastically reduce HMW levels. The interaction with Ca2+ ions also increase HMW stability (147).

The ER is the place where adiponectin oligomerization takes places. The redox state

within the ER can affect both the non-covalent interactions and the disulfide bond formations. ER stress affects the redox state and can interfere with adiponectin oligomerization. Chaperones, oxidoreductases and molecules with redox potential will influence the interaction between adiponectin multimer and consequently their association. The secretion of adiponectin multimers from adipocytes is tightly controlled by a chaperone in the endoplasmic reticulum, the endoplasmic reticulum resident protein 44 (ERp44). ERp44 has the capacity to bind to cysteine residues in adiponectin, increasing the time of retention in the ER and preventing the formation of disulfide bonds in all cysteine residues and, thus, increasing the formation of HMW forms. In fact, increased ERp44 in the ER is linked with increased HMW form (147, 155). Endoplasmic oxidoreductin-1-Lα (Ero1-Lα), is an oxidoreductase that leads to an oxidizing environment in ER, favoring disulfide bond formation. It catalyzes the transfer of electrons from thiol groups to molecular oxygen during disulfide bond formation, generating hydrogen peroxide. It is a preferred partner for ERp44, as it generates disulfide bonds which it transfers to proteins such as ERp44, releasing adiponectin from ERp44 (151). Ero1-Lα promotes the formation of HMW adiponectin (147, 155). Another interesting molecule is the Disulfide-bond-A oxidoreductase-like protein (DsbA-L), a member of the kappa class of glutathione S-transferase family, presenting a thioredoxin like domain. Increased DsbA-L ER levels are associated with increased HMW adiponectin. The DsbA-L oxidoredutase activity is not well established, however, a putative mechanism of action could include the protection of cysteine residues form excessive oxidation and early intra-trimer disulfide bond formation, by the interaction between DsbA-L thio-redoxin like domain with the thiol groups. Alternatively, the glutathione S-transferase activity associated with DsbA-L might protect cysteines by glutathionylation (147). These ER molecules are known to be regulated by the metabolic state of the cell and peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (e.g. thiazolidinediones), and to display sexual dimorphism, what can help to understand changes in multimer proportions existing in some diseases or between genders (147, 155).

Adiponectin succination is a process by which S-(2-succinyl)Cys is formed, by a non-enzymatic reaction of fumarate with cysteine residues. In the case of human adiponectin, succination occur at cysteine 36 of adiponectin monomers, blocking the oligomerization of adiponectin. As a consequence, a lower amount of adiponectin is secreted. The extent of succination is increased in states as diabetes or high glucose levels, due to accumulation of Krebs cycle intermediates. Increased adiponectin succination might be part of the explanation for the reduction in adiponectin levels in states associated with IR, particularly the levels of HMW adiponectin (151).

The addition of sialic acid is a common post-translational modification to O- and N-linked oligosaccharides present on secreted and cell-surface proteins (147, 151). The addition of di-sialic acid residues to two threonine O-linked oligosaccharides on the variable region of adiponectin have been associated with an increase in the half-life of the molecule in circulation, although no influence on multimerization was detected (147, 151). In fact, desialylation of adiponectin resulted in increased clearance and uptake into primary hepatocytes. The activity of enzymes responsible for adding (sialyltransferases) and removing (sialidases/neuraminidases) sialic acids are upregulated in inflammatory states, including diabetes and CVD. Thus, the hypoadiponectinemia associated with those pathologies might result from a reduced adiponectin synthesis/secretion, and, also, to an increased clearance from the circulation (151).

6.2. Adiponectin receptors

There are two main transmembrane domain receptors for adiponectin: adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). AdipoR1 gene is located in chromosome 1q32 and is expressed predominantly in skeletal muscle, while adipoR2 gene is located in chromosome 12p13 and is expressed mainly in the liver (156, 157).

Yamauchi *et al.* proposed that both receptors mediate anti-diabetic metabolic effects (156). However, an elegant work in mice found that, although these receptors mediate most of the biological functions of adiponectin related to glucose and lipid metabolism, they seem to act in opposite directions. Knock-out mice for each of the receptors were used, and AdipoR1$^{-/-}$ mice showed increased adiposity associated with decreased glucose tolerance, spontaneous locomotor activity and energy expenditure. However, AdipoR2$^{-/-}$ mice were lean and resistant to high-fat diet-induced obesity, presented improved glucose tolerance, higher spontaneous locomotor activity and energy expenditure, and reduced plasma cholesterol levels. Thus, AdipoR1 seems to have more beneficial effects than AdipoR2 (157). T-cadherin has been also considered an adiponectin receptor (158). Besides these receptors, an adiponectin adaptor protein, AAP1, has been described (71, 156).

Adiponectin receptors were also described in other organs and tissues where particular biological actions have been studied, e.g. in the central nervous system, where they may influence eating behavior and body weight regulation (159, 160). Animal studies have

identified adiponectin receptors in the intestine, indicating that adiponectin present in breast milk might influence the energy balance and growth in the first period of life, probably with impact in future metabolism (161).

7. Obesity as an inflammatory disease

As the WAT grows, the adipocytes accumulate lipids, becoming hypertrophic. Hypertrophic adipocytes increase the secretion of mediators that activate resident macrophages. These activated macrophages further produce cytokines and attract other macrophages, helping to sustain a pro-inflammatory cycle. The WAT macrophages are, in fact, the main producers of some adipokines (37).

The growth of WAT leads to the development of areas that become far from vasculature and are under a local hypoxic environment. Thus, it is necessary to create new blood vessels to irrigate the growing areas (angiogenesis). The action of angiogenic factors (e.g. VEGF, HGF) is crucial in this process, but these mediators are, usually, deregulated by the local inflammatory environment and might promote, themselves, inflammatory pathways (118-121).

Adipokines are generally increased in obesity, particularly pro-inflammatory ones, leading to a local increase and, afterwards, to a low-grade inflammatory state (28, 37). Such is the case of IL-6, TNF-α and CRP (28).

This general adipokine increase has an important exception, adiponectin, an anti-inflammatory and anti-oxidant protein. Adiponectin is the most transcript gene in adipocytes and its levels are paradoxically reduced in obese individuals (51).

The rise in the pro-inflammatory mediators and the concomitant decrease of anti-inflammatory defenses, as adiponectin, contributes to the development of the chronic low grade inflammation, common in obese individuals (123, 162). Table 6 summarizes some of the obesity-related changes in adipokines.

The association between obesity and a pro-inflammatory, pro-oxidant and pro-atherogenic profile is evident after weight loss, as the levels of pro-inflammatory markers decrease (125), while circulating anti-inflammatory adiponectin increases (51).

The WAT has an almost limitless growing capacity. The increased mass of WAT in obesity, together with the increase of circulating adipokines suggest that the WAT might be the main source of those molecules. Actually, this is true for some of the circulating adipokines, while for others, there is a more complex mechanism that involves a cross-talk between the WAT and other tissues and organs. For example, PAI-1 is increased in obese individuals and its main source seems to be, in fact, the WAT (139); CRP, for

instance, is mainly produced in the liver. Nevertheless, the production of CRP by the liver is stimulated by the IL-6 produced in the WAT, particularly by the visceral adipose tissue (VAT), that will stimulate the hepatic CRP synthesis through the venous portal system (95, 123).

Hence, the communications between the WAT and other organs have an important role in the metabolism, with considerable relevance in obesity (5). Another example is leptin control of energy intake and expenditure through the hypothalamus. In obesity, a state of leptin resistance, as observed for insulin, may develop and cause the loss of the control mechanism over energy homeostasis. The resistance to leptin action causes a positive feedback mechanism increasing its circulatory levels and contributing to the pro-inflammatory state (162).

In synthesis, the cross-talk between WAT and other organs contribute to the global metabolic changes happening in the obese body, including chronic inflammation (5). The study of each individual product secreted by the WAT is crucial to unveil the global picture and to better understand many of obesity related comorbidities.

7.1. Inflammatory changes in pediatric obesity

The pro-inflammatory changes in childhood obesity are similar to those observed in adults. An increase in WAT, together with increased production of circulating pro-inflammatory mediators, and a decrease in anti-inflammatory molecules, as adiponectin, occurs (44). CRP, a classical marker of inflammation, is 3 to 5 fold increased in obese children, when compared to lean controls (148). Weight loss is generally associated with an improvement of the inflammatory status in children and adolescents (55).

The pro-inflammatory status in obesity increases with the severity of the disease. In fact, an increase in circulating CRP, leptin, resistin and IL-6, and a reduction in anti-inflammatory adiponectin, was observed in severe obese children and adolescents when compared with individuals with moderate obesity. Nevertheless, no difference was found for TNF-α (70). Ogawa *et al.* also found a reduction in adiponectin with increasing severity of obesity in 8 to 13 years old boys (44).

The correlation with adiposity markers is positive for most of the inflammatory mediators. The body fat distribution is particularly important and the correlation with inflammatory

markers is even stronger in case of central obesity (increased VAT), when compared to the correlation with the absolute body mass (4, 24, 56). Indeed, visceral fat accumulation has been linked with a worse metabolic and inflammatory profile characterized by increased leptin, liver transaminases and uric acid, and reduced adiponectin and ghrelin. Leptin is, in fact, a predictor of the visceral fat depot (24).

Besides the association with inflammatory status, VAT is also linked to increased risk of CVD. Leptin, a marker of visceral fat, is also a predictor of MS features in adolescents. Thus, leptin is a potential interesting clinical tool to monitor fat distribution and CVD risk in pediatric populations (4).

Puberty and gender interfere with the inflammatory status in children and adolescents. Before puberty the differences in inflammatory markers according to gender are, usually, moderate or absent. After puberty, besides a less atherogenic lipid profile, females seem to have a different inflammatory profile, with increased leptin and adiponectin, and reduced CRP (4, 24). For the other mediators, as ghrelin, there are no gender related differences, even after puberty (4, 24). Sexual hormones seem to have a significant role of in these post-pubertal changes. Testosterone and derivatives are known to diminish the production and secretion of adiponectin from adipocytes, while leptin increases body fat in post-pubertal females (24).

Several studies reported that pediatric obesity is also associated with an increase in circulating levels of resistin (71, 163), IL-6 (70), IL-8 (55, 57), IL10 (55, 164), IL-18 (57), homocysteine (148), HGF (57), uric acid (6, 24), alanine aminotransferase (ALT) (24, 54), aspartate aminotransferase (AST) (54), complement C3 (163), and with a reduced concentration of ghrelin (4, 24). However, other studies found no differences between overweight/obese individuals and lean controls for circulating levels of ghrelin (148), adiponectin (57, 148, 165), resistin (24, 57), adpsin (57), IL-6 (57), IL-10 (57), PAI-I (57), TNF-α (57), homocysteine (6), retinol binding protein 4 (RBP-4) (57), VEGF(57), soluble vascular cell adhesion molecule (sVCAM) (57), soluble intercellular adhesion molecules (sICAM) (57) and CRP (24, 165).

A moderate increase in bilirubin circulating levels has been reported to be associated with lower inflammation and oxidative stress (166, 167), both in adults and in children (168), and to reduce the incidence of CV events (169, 170). Increased adiposity, in particular central adiposity, has been also associated with lower plasmatic bilirubin (168, 171). However, few reports have addressed bilirubin at pediatric ages.

Increased leukocyte count is positively associated with total and central adiposity in children and adolescents (172, 173) and subcutaneous adipose tissue (SAT) might also have a role in that relation (173). The positive relation between total adiposity and leukocytes appear to be mainly due to an increase in neutrophils. In fact, lymphocyte and basophil counts were reported to be negatively related to BMI and waist circumference (173). Pro-inflammatory adipokines known to be increased in obese individuals, such as leptin, IL-1, IL-6 and TNF-*α*, were related to increased circulating leukocytes (174-177). Nevertheless, it is still somehow uncertain which leukocyte populations are altered in obese children, how early these changes appear, and the relation with other inflammatory and CVD risk factors.

When different inflammatory mediators cluster, a particular inflammatory profile develops which will interfere with other CVD markers, as IR. These profiles are modulated by genetic and/or environmental factors.

The pro-inflammatory changes in pediatric obesity have a broad effect on metabolism and might underlie obese associated comorbidities and diseases. Thus, it should be clear which variables are indeed altered, and which health consequences are associated with such modifications.

8. Obesity and other associated diseases

In adults, obesity has been associated with type 2 diabetes mellitus (T2DM), CVD, metabolic syndrome (MS), infertility, steatohepatitis, gallbladder changes, asthma, dental problems and psychological disorders (5, 19). Consequently, it is not surprising that in Portugal, the patients that use the national health system have an increased prevalence of obesity, when compared to the general population (178). Some of these comorbidities are already present in obese pediatric patients (5, 19, 179, 180). A study in a Dutch pediatric population showed comorbidities in 11% of the overweight and 37% of the obese individuals (19); in another study, in Turkish children, 80% of the obese children presented, at least, one feature of MS (180). Ethnic minorities seem to be particularly at risk for increased prevalence of both obesity and MS (180).

Some of the mechanisms underlying the association of obesity with these pathologies are still unknown and are subject of intense investigations, however, inflammatory and oxidative stress mechanisms are accepted as a common denominator. In fact, inflammation is known to play a vital role in the initiation and progression of many of the referred diseases (181, 182), and the association between increased inflammation and metabolic abnormalities, as increased IR or low density lipoprotein cholesterol (LDLc) concentration, are present since early in life of obese individuals (44). In Table 6 are highlighted associations between changes in some inflammatory mediators, present in obesity, and their influence in diseases as T2DM and CVD.

The link between obesity-related inflammation and comorbidities are already present in early childhood. Children with decreased adiponectin, and thus, with a worse inflammatory profile, seem to present increased prevalence of severe obesity, VAT accumulation, high LDLc, hyperinsulinemia, MS and acanthosis nigricans (44). Moreover, studies in obese children and adolescents showed that the worsening of CV risk markers, such as dyslipidemia, hypertension and IR, is associated to an increase in adiposity (5, 70) and in obese-associated inflammation, presenting the more obese individuals a worse inflammatory and CVD risk profile (70). The worsening of the metabolic profile with increased inflammation highlights that the metabolic derangement in obesity is not only a question between obese/lean individuals, but also that adiposity has a proportional and direct effect (4, 5, 70).

The body fat distribution, besides the influence on inflammatory mediators, has also an impact on metabolic changes. A study in a Mediterranean pediatric cohort reported that a

worse inflammatory profile (e.g. increased leptin and decreased adiponectin levels) was associated with central obesity, high BP, dyslipidemia and IR. Moreover, as the increased inflammatory status correlated with increased adiposity, namely with central adiposity, the relation between the inflammatory mediators (leptin and adiponectin) and the number of MS features presented were associated to an increase in BMI z-score (56).

In addition to the influence of increased adiposity, there is a clustering effect of CVD risk factors in children and adolescents, in a way that the presence of one risk factor facilitates the appearance of another (4, 5). In agreement, similar results were found in a Portuguese pediatric overweight population, presenting increased blood pressure (BP), hyperinsulinemia, IR, dyslipidemia (increased TG, apo B and reduced apo A1), higher levels of leptin, CRP and homocysteine (148).

The importance of the association between obesity, inflammation and IR are even more relevant in children and adolescents, as the presence of obesity in these ages seems to increase the risk of developing T2DM and CVD in adulthood. Actually, overweight is associated with increased CVD mortality (5, 20, 179) and there is an increase in all-cause mortality in adulthood, in case of pediatric obesity (20).

Features of MS are already observed at young ages in obesity (2, 5, 25, 179, 183). The chronic exposition to metabolic abnormalities (e.g. dyslipidemia) might be the link between pediatric obesity and increased risk for CVD and metabolic diseases later in life (5, 179). However, the effect of pediatric adiposity on the onset of the diseases in adults is difficult, as the influence of adult BMI has to be also considered. The tracking of adiposity into adulthood leads to the persistence of risk factors, with a crescendo of metabolic risk (20). Thus, it is complicated to analyze separately those two phases of life.

8.1. Insulin resistance and type 2 diabetes mellitus

Closely linked to the rise in inflammation, IR is also associated with the genesis of many of the obesity-related diseases. Actually, in the development of IR, a critical step towards the appearance of T2DM and dyslipidemia, an important cross-talk exists between the WAT, the skeletal muscle and the liver (5, 33, 179).

In obesity, the increased inflammatory mediators, mainly secreted by adipocytes and macrophages, interfere with insulin signaling and promotes the development of IR. As a consequence of IR, lipolysis within the WAT is augmented (5, 33, 179).

In the skeletal muscle there is also an increase in cytokine secretion and macrophage infiltration, in part due to the increase of inflammatory mediators in the extramyocellular WAT. An increase in free fatty acid uptake is associated with these changes in the skeletal muscle (5, 33, 179).

In the liver occurs an increase in the lipid content, mainly in TG, causing steatosis, an increase in the recruitment and activation of Kupffer cells, and also an amplified cytokine production. In the three referred organs/tissues endoplasmic reticulum stress (ERS) is also present, and has an important part on inflammation perpetuation (5, 33, 179).

Thus, the pro-inflammatory status that appears first at local levels, within WAT, evolves to the whole body, through this metabolic cross-talk. The changes that accompany obesity in these organs, and their relation to IR, are presented in Figure 2.

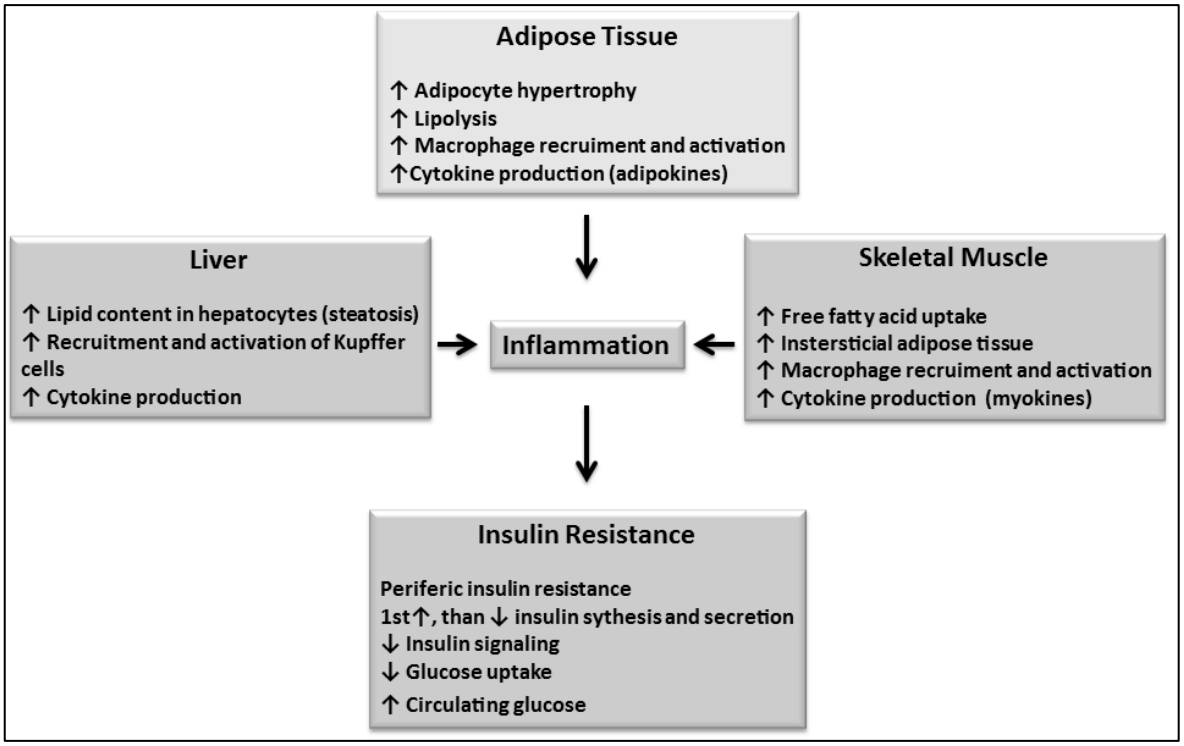


Figure 2. Mechanisms underlying the development of insulin resistance in obesity
Adapter from (33).

A central factor in the relation between obesity related inflammation and IR might be the pro-inflammatory TNF-α. TNF-α is highly increased in obese individuals and is known to interfere and impair insulin signaling (33).

In obese individuals the IR and the consequent hyperinsulinemia, develops earlier than the increase in glucose levels. Solid evidence exists relating childhood obesity with T2DM in adulthood (5). The risk is even stronger when considering obese adolescents, relatively to obese children (20). The risk of evolving from IR to T2DM depends on the duration and magnitude of obesity (5, 20, 25, 179).

T2DM presents nowadays a high prevalence in obese adolescents, particularly when their BMI z-score is above the equivalent to 30 kg/m^2 in adults (179). In the USA, the prevalence of T2DM in children is 0.4%, more than the double of the type 1 diabetes mellitus (T1DM) prevalence. In these ages T2DM is often asymptomatic and it is necessary to pay attention to unspecific symptoms, such as glycosuria or vaginal candidiasis (25). The real predictive power of pediatric onset of T2DM for future CVD in adolescents is not well established (179).

8.2. Dyslipidemia

Risk changes in the lipid profile are a common finding in obese individuals, and are closely connected to IR. As IR develops there is an increase in the hepatic production of very low density lipoprotein (VLDL), contributing to increasing levels of circulating TG and LDLc. Furthermore, peripheral IR reduces the action of local LpL, increasing the plasmatic TG, and reducing HDLc by increasing its degradation and reducing its synthesis (4, 5, 25, 179).

The increase of obesity in children and adolescents is associated with a worse lipid profile (5, 6), with increased TG, decreased HDLc, and normal to moderately elevated total cholesterol (TC) and LDLc (184, 185). These changes in the lipid profile are somehow stable and, without intervention therapy they will persist into the adulthood, together with obesity (25, 179).

Apo E by regulating the hepatic uptake of remnant lipoproteins through the LDL-receptor and LDL-receptor-related protein, and by facilitating cholesterol efflux from foam cells, has an important role in the cholesterol metabolism and in atherosclerosis development (186).

Apo E presents a genetic polymorphism on the gene exon 4, the E2/E3/E4 apo E polymorphism that influence the circulating levels of TC. Individuals carrying the E2 allele present lower values of TC than individuals carrying the E4 allele (187). Thus, besides the increase of TC cholesterol in obesity, the individuals with some specific polymorphisms, such as E4 carriers, might be at increased risk for future CVD and present a worse metabolic profile. The effect of the same polymorphism on TG levels has also been addressed but available results are contradictory; however, it seems that E2 carriers present increased circulating TG in comparison to E4 carriers (188, 189).

Lipoprotein (a) (Lp(a)) is an independent risk factor for CVD (190, 191). It is structurally similar to LDL but presents a different apolipoprotein - apo (a) – that binds to apo B particle in the LDL-like domain of Lp(a), preventing Lp(a) from binding to the LDL receptor and, thus, limiting the catabolism of Lp(a) (191). As a consequence, circulating Lp(a) levels, although widely variable between individuals, are relatively stable in the same subject across time, as its concentration is mainly determined by the synthetic rate, which is mostly genetically determined (191-193). Lp(a) presents a pro-atherogenic role that can be explained by different characteristics of this particle: apo (a) from Lp(a) presents a plasminogen-like domain that prevents fibrinolysis, leading to a pro-coagulant state; Lp(a) has also been reported to function as an oxidant scavenger, preventing the oxidation of LDL and other lipoproteins; nevertheless, the resulting oxidized Lp(a) is an atherogenic factor itself, which is deposited in the arterial walls, contributing to the formation of foam cells. The Lp(a) may circulate in different sizes, being the smaller more atherogenic. The Lp(a) particle size is inversely related the size of the apo (a) (190, 191). The apo (a) particle size is influenced by different genetic polymorphisms that, ultimately, influence the Lp(a) circulating levels. For example, a pentanucleotide repeat (PNR) polymorphism, (TTTTA)n, 1.4 kilobases upstream from the gene reading frame, has been reported to influence Lp(a) levels, presenting individuals with more repeats lower circulating Lp(a) (193). Despite the existing data on lipid profile changes in obesity, little is known about the influence of Lp(a) in the CV risk in pediatric populations, and its relation with other risk markers.

It is important to identify individuals with genetic profiles that associate with increased metabolic risk as these individuals could benefit more from an early intervention. Moreover, a better understanding of the genetic factors might help to identify the individual CVD risk.

8.3. Hypertension

In obese individuals, hypertension is associated with increasing weight and percentage of body fat. Weight loss, usually, induces BP reduction (4, 25). The vascular damages, due to dyslipidemia and the consequent atherosclerosis, have an important part in the increased prevalence of hypertension in obese individuals (194-196). Hyperinsulinemia, a consequence of IR, also has an important and direct role in the development of hypertension. It was proposed that increased circulating insulin causes an increase in sodium retention in the kidneys, and in sympathetic activity and promotes the growth of vascular smooth muscle, thus increasing BP (4, 25).

Increasing BMI is directly linked with increased BP and, consequently, hypertension has accompanied the increase in overweight prevalence in pediatric subjects in the last years (180). Hypertension is one of the most common features in obese children and adolescents, and usually continues until adulthood. In fact, the risk of hypertension in adulthood increases with persistence of pediatric obesity from childhood to adolescence (4, 6, 25, 179).

8.4. Metabolic Syndrome

MS is a cluster of CVD risk factors including obesity, hypertension, dyslipidemia (high TG and low HDLc) and IR (4, 5, 197). These features of MS were, until some years ago, exclusive of adults, but are, nowadays, appearing at younger ages, partially caused by the increased prevalence of obesity in children and adolescents (2, 4, 179, 183).

The prevalence of MS in children and adolescents is high in case of overweight, and even more higher in obese individuals, when compared with lean subjects (179). In fact, MS prevalence seems to be adiposity dependent as by each 0.5 increase in BMI z-score, there is a 50% increase in the probability of developing MS (179); the MS prevalence is increased in severely obese children, when compared with moderately obese individuals (44, 70).

Worsening of the inflammatory profile has been associated with increased MS prevalence in children (44, 70). Actually, in Latin overweight/obese children with a family history of T2DM, lower adiponectin levels were observed and were predictors of MS, independently of gender, age, insulin resistance and visceral obesity; moreover, in overweight children

with MS the adiponectin levels were 25% lower than the values presented by children without MS (53). Plasmatic bilirubin levels, a potential marker of inflammation and oxidative stress, are also decreased in children with MS (168).

The reported MS prevalence in the adolescent general population is 4%, while in overweight adolescents it can rises to values of 30 to 50%. Nevertheless, the prevalence varies widely based on the criterion used to define MS and the studied population (179).

A small study in Portuguese children, 7-9 years old, using the National Cholesterol Education Program (NCEP) criterion, reported a high prevalence of MS in individuals with weight excess (16%), while no cases were found in normal weight individuals (148). In this study MS was predicted by decreased levels of apo A1 (the main protein content of HDL particles), increased circulating insulin and leptin, and by higher Tanner stage (148).

In another study, in a pediatric Mediterranean population (Greek), the prevalence of MS, according to the International Diabetes Federation (IDF) criterion, was 0.7% for the whole population and 7.7% for the obese individuals. Moreover, as observed in the Portuguese study (148), all individuals with MS had increased adiposity. The most common MS features found in this cohort were increased BP (33.3%) and low HDLc (12.3%); surprisingly, no children presented increased TG (56).

A protective effect of normal weight against MS is suggested by the almost absence of normal weight children with MS (56, 148). Actually, in lean individuals there is a sharp drop in the percentage of individuals presenting one MS feature, to individuals presenting two MS features (56). However, the presence of one CVD risk factor (one MS feature) increases the probability of the appearance of a second risk factor. The increased adiposity and consequent hyperinsulinemia and inflammation are basic events that induce the appearance of other MS features (179); abdominal obesity is particularly related to clustering of MS features (4).

Other protective factors, besides low BMI, have been suggested. For example, it was shown that 16 years old obese girls presenting with high adiponectin levels did not develop MS for seven years afterwards. Indeed, the healthy obese phenotype, characterized by healthy values of MS markers in an obese subject, has been associated with the presence of high adiponectin levels (198).

Bilirubin might also work as a protective factor due to its anti-inflammatory and anti-oxidant properties. In fact, a mild hyperbilirubinemia, as occurs in individuals with the

Gilbert Syndrome, has been associated with a reduced incidence of CVD, inflammation, oxidative stress (169, 170, 199) and MS (168, 171). The levels of plasmatic unconjugated bilirubin are controlled by the uridine diphosphate glucuronosyltransferase (UGT) 1A1, a microsomal enzyme responsible for bilirubin glucuronidation, necessary for its excretion (200). A TA duplication polymorphism in the TATA box region of the gene promoter affects the activity of UGT1A1 and, consequently, the bilirubin levels (201). The relation between bilirubin levels and MS features, and the influence of genetic polymorphisms, need further investigations, particularly in individuals at increased risk of developing MS, such as obese children.

8.5. Atherosclerosis

Atherosclerosis is a chronic disease that begins early in life, and obesity is an important risk factor for its development. In fact, atherosclerotic lesions have been described in the vessels of increasingly younger subjects (4, 5, 179). Hyperinsulinemia and dyslipidemia are often present in obese individuals, increasing the formation of atherosclerotic plaques (4, 5, 179, 194, 195).

Circulating TG-rich lipoproteins inflict oxidative damage to vascular endothelium. Furthermore, hypertriglyceridemia, shifts the spectrum of LDL sub-fractions towards smaller, denser species, which are believed to be more atherogenic (185, 196), and the spectrum of HDL to smaller, less stable particles that limits the reverse transport of cholesterol (185).

The deposit of lipids in the vascular walls leads to the formation of foam cells. These cells secret cytokines (e.g. IL-6, TNF-α and CRP) that recruit more macrophages, causing local inflammation, vessel injury and, therefore, the development of atherosclerosis (185, 196, 202).

Atherosclerosis is increased in young obese individuals and has been correlated with the number of MS features (179, 184). Fatty streaks, a pre-clinical manifestation of atherosclerosis, has been identified in children as young as 2 years, presenting a prevalence of around 50% in children until 15 years (184). Obesity itself is particularly relevant, as the increase of the atherosclerotic process with increased BMI is independent from the other risk factors. In fact, coronary artery calcification in young adults was

positively correlated with weight, in childhood, and with BMI, in adolescence. IR was also associated with vessel changes in youth (179).

Leukocytosis, particularly neutrophilic leukocytosis, is also often associated with atherosclerotic disease and an increased risk of CVD (203-205). In fact, activated leukocytes might participate in endothelial injury (203), and monocyte and lymphocyte recruitment to the artery wall is present in the atherosclerotic process (176). The association between obesity related reactive leukocytosis and the development of atherosclerosis needs further clarification.

8.6. Cardiovascular events

Chronic hypertension is associated with left ventricular hypertrophy. In adults, left ventricular hypertrophy is linked to increased risk of coronary heart disease (CHD), sudden death and stroke (179). High BP, increased BMI and fat mass are positively correlated with increased left ventricular hypertrophy in children and adolescents (179). In agreement, a positive association between childhood obesity and increased risk for CHD later in life has been reported. This association is more evident in males than in females, however, it loses some statistical strength when adjusted for adult BMI (20).

The link between the risk of stroke in adulthood and increased adiposity in childhood is not clear. Some studies have reported an increased association, however, other studies did not find any association or have found a reduction in stroke events (20).

8.7. Steatohepatitis and non-alcoholic fatty liver disease

Steatohepatitis and non-alcoholic fatty liver disease (NAFLD) are common in obese individuals. About 30% of obese adults present hepatic fatty changes (5, 25). The increase in obesity, particularly visceral obesity, is associated with changes in liver transaminases and hepatic insulin sensitivity. IR is the main responsible for the development of NAFLD, by causing a rise in lipid (mainly TG) deposits in hepatocytes, and changes in the hepatic lipoprotein production towards TG-rich particles (4, 25, 33). NAFLD is a silent disease and, by affecting liver function, is closely associated with all the

MS classic features. Some authors defend, in fact, that NAFLD should be considered as a part of MS (24, 54).

A study in healthy Japanese adolescents found a high prevalence of fatty liver (23.3% in boys and 30.4% in girls) (24); in another study involving a pediatric population, including only obese and overweight individuals, the prevalence was 35.6% (54). The prevalence of NAFLD is higher in obese children and adolescents (54), and particularly higher in individuals presenting visceral fat accumulation (24). Reduced adiponectin and HDLc levels, characteristic findings in obesity, are further reduced in overweight/obese individual with NAFLD, increasing the overall metabolic risk (54).

8.8. Sexual Hormone changes

Obesity related changes in hormone metabolism are associated with an increase in the bioavailability of sex hormones through increased cortisol production and reduced concentration of the sex-hormone binding globulin, a protein that binds to estrogens and androgens, controlling hormone availability. The increased availability of circulating sex hormones in obesity supports the increased skeletal maturation for chronologic age (although usually adequate for bone age) (5). An increased aromatization of androgens into estrogens is responsible for the increased sexual maturation in girls, while in boys it is responsible for a decreased sexual maturation and gynecomastia. This sexual hormone excess in obese girls favors the development of polycystic ovary syndrome, characterized by acne, hirsutism, and menstrual irregularity (5).

8.9. Asthma and allergic diseases

Many studies have addressed the relation between obesity and the development of asthma in children. Actually, the concomitant increase in asthma and obesity in the last years suggest a linkage between them, which has been considered by many authors (20, 180). The pro-inflammatory state and leukocyte activation present in obesity might cause the increase in allergic manifestations. In agreement, besides asthma, allergies and eczemas are also reported in association with obesity. Nevertheless, conclusive results have not been reached, especially in some specific populations. Differences in eating habits might underlie the differences in asthma prevalence found in different cohorts. The

causual relation of asthma and obesity is not clear. Indeed, asthmatic children may reduce physical activity to avoid asthma symptoms and the reduced activity leads to weight gain; or obese children develop more asthmatic symptoms, due to the inflammatory process associated with obesity (180). The relation between increased adiposity in pediatric ages and an increase in the incidence of asthma later in adulthood is also not clear, with studies reporting opposite results, especially after adjusting for adult BMI (20).

8.10. Orthopedic comorbidities

Orthopedic comorbidities are, in general, a direct result of weight excess, especially in morbidly obese children. The knees are a particularly susceptible joint (25). The weight excess on the locomotor apparatus, especially during growth phases, is associated with orthopedic pathologies that will persist into adulthood. Orthopedic compensatory changes, to balance adiposity excess and distribution, are frequently present and might include curving of the femur (*genu valgo*), changes in the normal column curvature (with associated back pains and disc degeneration), slipped capital femoral epiphysis, pain in the lower members and foot problems (5, 180). Furthermore, the increased mass makes, *per se*, harder to execute physical exercise, and even daily activities, and may cause pain with certain movements. As a consequence, obese individuals, usually, respond to the referred limitations with lower activity levels, creating a vicious cycle towards sedentarism (25).

8.11. Obstructive sleep apnea

Increased BMI has been associated to an increased risk of obstructive sleep apnea in both adults and children (179). The excess weight in the thoracic wall and the increased size of the tongue in obese individuals make more difficult to breathe normally, particularly when lying down. During sleep, the reduction of the muscular tonus, together with the mentioned changes, increases the risk of obstructive sleep apnea (25, 179). The sleep duration and its quality were reported to be decreased in obese children and adolescents, with lower number of sleeping hours and of sleep spent in rapid eye movement phase, a sleep phase important for energy recovery and cognitive functions (180). Nevertheless, this same report found no relation between increased prevalence of sleeping apnea with the quality of sleep and obesity (180).

8.12. Psychological comorbidities

It is not clear the relation between obesity and psychological comorbidities. A direct association between increased BMI and depression or lower self-esteem in obese individuals has been reported, however, it is not clear which is the cause and which is the consequence (25).

Obese children have lower self-esteem, lower number of friends and higher incidence of depression, when compared to their lean counterparts (25, 179); moreover, adults with increased BMI are more likely to have been diagnosed with depression in youth. In agreement, adolescents with depressions tend to increase BMI through time (179). Some studies, however, found no difference between lean and overweight children regarding depression (180). In addition, a decrease in anxiety and depression in extremely obese children was reported (180).

Bullying might be an important part of the problem. Overweight children and adolescents victims of bullying are more prone to have suicidal idealization and attempts (179). The lower satisfaction with their body makes difficult for obese children to engage in physical exercise and group activities, fearing rejection or bulling from pairs and, therefore, persisting with inactivity and obesity (25, 179).

Regarding other psychological disorders, the prevalence of attention deficit hyperactivity disorder (ADHD) was reported to be higher in obese and overweight children and adolescents. The increase in deregulation of the behavior of those patients would extend to their eating habits and contribute to obesity. However, some studies found no association or even a decreased prevalence of this disease in overweight youth (180).

The cause-consequence order between behavior problems and obesity is also not clear. Obese children presented increased incidence of behavior problems in school, and behavior problems were associated with the development of overweight in lean children (180).

In synthesis, there is controversy regarding the linkage of obesity and psychological disturbances. Nevertheless, an interesting work in Chinese youth, studying the interactions between psychological factors and obesity, reported that it is not the BMI but the individual perception of weight excess that should be associated with psychological disorders (206).

8.13. Other associated diseases and comorbidities

Childhood and adolescent obesity have also been associated with different types of cancer. Increased pediatric overweight and obesity have been linked to increased risk for colorectal, kidney, cervical and ovarian cancers (20, 207-209). On the other hand, breast cancer presents opposite trends, namely, a reduced risk in case of adolescent obesity. However, other studies found no association between pediatric obesity and cancer development in adulthood (20). Other factors influencing the development of cancer, such as genetics, lifestyle and environment, can modulate and mitigate the effect of obesity, particularly at young ages.

Biliary lithiasis is also common, particularly in obese women following diet-induced weight loss, due to an increase in biliary saturation of cholesterol, dehydration, vesicular hypomotility (due to low fat diets) and estrogenic action (25).

Obesity and overweight have been associated with worsening of dental health, with an increase in the development of caries. Nevertheless, results are not conclusive. The usual sugar rich diet followed by obese children, associated with poor hygiene, could be key factors linking weight excess and caries. Other factors, such as age, race, poverty and income ratio might also influence that association (180).

Other comorbidities reported in obese and overweight pediatric patients include increased gastro-esophageal reflux, *pseudotumor cerebri*, glomerulopathy and respiratory infections, among others (5, 180).

Figure 3 resumes some of the most relevant obese associated comorbidities.

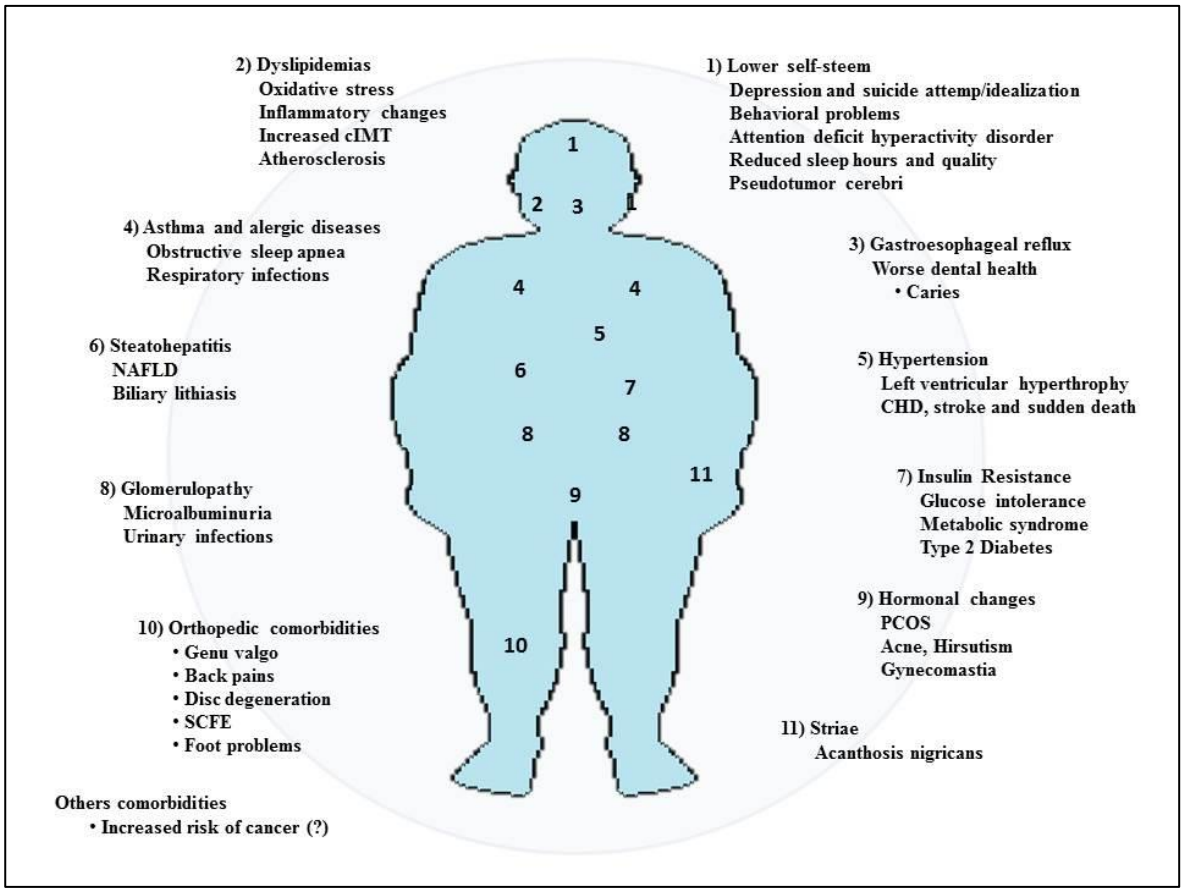


Figure 3. Obesity associated comorbidities.

CHD, coronary heart disease; cIMT, carotid internal media thickness; NAFLD, Non-alcoholic fatty liver disease; PCOS, polycystic ovarian syndrome; SCFE, slipped capital femoral epiphysis.

The association of obesity with many diseases is a very relevant issue nowadays, as the prevalence of overweight and obesity is growing markedly. The majority of the comorbidities present in pediatric obese patients are the same observed in obese adults (179). The severity of the comorbidities is positively linked with the excess of adiposity. The chronic exposure of the body to metabolic derangements might have a cumulative effect and represent a fundamental problem, probably more important than obesity itself, particularly in pediatric patients. Thus, investing in health programs nowadays, is most likely to invest in the future, both at human and financial levels, by reducing the prevalence of obesity and its comorbidities, as well as by reducing the associated costs. Intervention of decision makers is crucial to change this paradigm.

9. Diagnosis of Metabolic Syndrome in children

The diagnosis of MS in children is not consensual, even less consensual than in adults. Particularly difficult is to find cut-off values, as those usually used in adults are not adapted for children and adolescents. In fact, features of MS, as lipid profile, fat distribution, blood pressure, insulin sensitivity, vary through pediatric ages, with a particularly strong fluctuation during puberty (5, 210, 211).

There are several MS criteria, varying on how the variables are used (age and sex adjusted percentiles or their absolute values), on the use of specific established cut-offs and on ethnicity of the studied population. These variations in MS definition make difficult to compare the results from different studies, particularly the prevalence of MS in pediatric population (5, 56, 210, 211).

A study involving an obese pediatric population, that was classified as presenting or not MS, according to three of the existing criteria, showed the variability of the obtained results. The prevalence of MS, by any of the criteria, was 3 fold higher in the severely obese individuals than in the moderately obese. Nevertheless, the prevalence of MS ranged from 8% in moderately obese to 25% in the severely obese using IDF criterion, to 21% and 57%, respectively, using Ferranti's criterion (70).

Some attempts to find a more consensual characterization of pediatric MS have been made. The IDF criterion divides the pediatric population in 4 groups: bellow 6 years, from 6 to 10 years, from 10 to 16 years and above 16 years. It uses the WC percentile as an indicator of visceral obesity, and establishes that central obesity is a necessary condition for the diagnosis of MS. The 90^{th} percentile of WC, adjusted for age and sex, was chosen as the cut-off value. Regarding the remaining MS components, the IDF recommended cut-offs are close to those used for adults. The IDF does not recommend the diagnosis of MS in children under 10 years of age; however, it is recommended lifestyle intervention and further examination for those children, when they present a high WC, for age and gender. For children under 6 years, cut-off values are not available due to scanty data. Adult cut-off values are used in adolescents over 16 years old (210). The fact that for the diagnosis of MS using the IDF criterion is mandatory the presence of central obesity, accompanied by other two MS features, is the main reason why MS prevalence using this criteria is lower (56). Indeed, other criteria accept the presence of 3 MS features as equally relevant for the MS diagnosis. In Table 7 are resumed the IDF criterion for the diagnosis of MS.

Table 7. **International Diabetes Federation criterion for diagnosis of Metabolic Syndrome**

Age (years)	≥6 and <10	≥10 and <16	≥16
Obesity (WC)	• ≥90th percentile	• ≥90th percentile (or adult cut-off if lower)	• Male: ≥94cm • Female: ≥80cm (ethnicity specific values should be used for other groups if available)
Triglycerides (mM)	MS should not be diagnosed but child follow up should be done, especially if other risk factors are present.	• ≥1.7 (≥150mg/dl)	• ≥1.7 (≥150mg/dl)
HDLc (mM)		• <1.03 (<40mg/dl)	• Males: <1.03 (<40mg/dL) • Females: <1.29 (<50 mg/dL) • Treatment for lipid abnormalities
Blood pressure (mmHg)		• Systolic ≥ 130 or diastolic ≥ 85	• Systolic ≥130 or diastolic ≥85 • Treatment of previously diagnosed hypertension
Glucose (mmol/l)		• ≥ 5.6 (≥100 mg/dl) • Previously diagnosed T2DM (If ≥ 5.6 an OGTT is recommend)	• ≥5.6 (≥100 mg/dl) • Previously diagnosed T2DM (If ≥ 5.6 an OGTT is recommend)

Adapted from (165, 167). HDLc, high-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; T2DM, type 2 *diabetes mellitus*; WC, waist circumference.

Brambilla *et al.*, in a study involving the European Childhood Obesity Group (ECOG), suggested a totally different set of factors regarding the identification of children with metabolic risk. This approach was called by the authors “MIRACLE” (metabolic individual risk factor and clustering estimation) and involves the study of the individual and family history (past), the clinical data (present) and the potential future outcomes (future). The MIRACLE approach includes the selection of 10 variables that are easy to determine in routine medical appointments, involving family and individual history, clinical features and the presence of metabolic abnormalities of the glucose metabolism. Some variables, usually used in other studies, such as WC, TG, HDLc and insulin, were not used, as the authors argued that neither the scientific evidence supporting their association with metabolic risk is clear, nor the reference values for children and adolescents are available (211). Table 8 lists the relevant data used in the MIRACLE (ECOG) approach.

Table 8. Factors used in the MIRACLE approach to define Metabolic Syndrome

Factors	Criteria
Family history	o Early cardiovascular diseases – one relative with cardiovascular disease before 55 years (man) or 65 years (woman); o T2DM - one first-degree relative affected; o Hypertension - one first-degree relative affected.
Individual history	o Small for gestational age - birth weight for length <10th percentile for gender and gestational age; o Ethnic origin - Indo-Asians, Hispanics, African-Americans.
Clinical features	o BMI - corresponding to adult value of ≥30 kg/m^2; o Waist circumference - ≥90th percentile for age, gender, ethnic-specific; o Hypertension - systolic or diastolic BP ≥ 95th percentile for age, gender and height-specific; o Acanthosis nigricans - one lesion.
Metabolic abnormalities	o IGT - fasting glucose 100–126 mg/dl or glucose at 120 min. 140–200 mg/dl (OGTT); o T2DM - fasting glucose ≥126 mg/dl or glucose levels at 120 min ≥ 200 mg/dl (OGTT).

Adapted from (211). BMI, body mass index; BP, blood pressure; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

Correct and accurate anthropometric measurements and specific data from blood analysis are mandatory for the correct diagnosis of MS. The lack of guidelines for pediatricians might influence and under-estimate the diagnosis of MS in children and adolescents. A study in the Netherlands verified that although all of the pediatricians measured height and weight, and almost all measured BP, only 42% of them measured WC, blood sampling was only taken in 2/3 of the cases, and different analysis were requested; about 70% of the children were tested for lipid profile and fasting glucose, while less than 50% had fasting insulin measured. Insulin is particularly important, as abnormalities in fasting insulinemia appear before changes in glucose levels in the pathway of IR (19).

The traditional definition of MS has some gaps, both in adults and children: the lack of NAFLD in the criteria, a factor involved with all the classical MS features; the lack of consideration for the family and individual history; the lack of use of new markers of adipose tissue and chronic low grade inflammation (e.g. CRP); and the way the factors have all the same weight in the classification, although it is unlikely that they all influence the development of MS in the same way, among other flaws (56, 211).

Thus, further studies are needed to clarify which factors should be used and their relative weight when analyzing metabolic risk in pediatric patients. It is also necessary to clarify the associations between metabolic changes in childhood and comorbidities in adulthood and to construct age and gender adjusted databases of risk factors, in order to provide evidence-based guidelines for pediatricians, to perform a correct risk assessment.

9.1. Portuguese recommended values for Metabolic Syndrome risk factors

The international guidelines for the classification of MS in pediatric patients are standardized and facilitate comparisons between different studies and countries. Nevertheless, each country may choose to use specific pediatric cut-offs for MS features, even when there is not an official criterion to define MS.

In Portugal, the clinical entity of pediatric MS has not an official definition. Nevertheless, the NCAHP highlights the importance of screening dyslipidemia and hypertension in youth, starting at young ages. The values that establish an increased risk differ from those stipulated for MS diagnosis by the IDF.

9.1.1. Lipid profile assessment in Portuguese children and adolescents

The National Heart, Lung and Blood Institute (NHLBI; USA) guidelines recommend universal screening of dyslipidemia in children between the ages of 9 and 11 years. Children 2 to 8 years old with increased risk for dyslipidemia (with diabetes, hypertension, BMI > 95th percentile, another relevant medical condition or family history associated with CVD risk) are also target for screening of dyslipidemia (184).

The recommendations in Portugal are not much different from that of NHLBI. According to the NCAHP, a screening of dyslipidemia should be performed in the following situations (18):

- In children between 2 and 4 years old, with family history (first and second degree relatives) of:
    - Early CVD (before 65 years in women and 55 in men)
        - Stroke or CHD
        - Sudden death
        - Peripheral vascular disease
        - Cerebral vascular accident
    - Dyslipidemia: TC >240 mg/dl and/or LDLc >130 mg/dl and/or TG >170 mg/dl and/or HDLc < 35 mg/dl
- Any child or adolescents with personal history of:
    - Weight excess or obesity
    - Diabetes
    - Hypertension
    - Renal or cardiac disease
    - Hormonal or metabolic disease
    - Taking medicines that interfere with the lipid profile (corticosteroids, anticonvulsants, antidepressives, …)

Even in children and adolescents without risk factors is desirable to perform a screening above 2 years of age. At least one analysis of the lipid profile in each decade of life should be performed in all children (18).

A good clinical practice is to perform “opportunist” analysis and to introduce the study of lipid profile when other analysis are made with a different purpose (e.g. pre-surgical analysis) (18).

A list of the lipid variables that should be screened and the reference values are presented in Table 9. Due to the absence of reference values for the Portuguese pediatric population the recommended cut-offs at the NCAHP are based on values of the NCEP (TC and LDLc) and the American Heart Association (AHA) (TG and HDLc), both entities from the USA (18).

Table 9. Reference values for dyslipidemia screening in Portuguese children and adolescents

	TC*	LDLc*	HDLc*	TG*
Normal (<P75)	**< 170**	**< 110**	**> 35**	**< 150**
Borderline (P75-95)	**170-199**	**110-129**		
High (>P95)	**< 200**	**< 130**		

*, values in mg/dl. HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; P, percentile; TC, total cholesterol; TG, triglycerides.

The pediatrician or family doctor should recommend the children and adolescents with risk changes in the lipid profile, as follows (18):

- **Children with dyslipidemia**: advised to follow a healthier diet, to increase physical exercise levels and to be referred to a specialist;
- **Children with borderline risk changes**: advised to follow a healthier diet and physical exercise. After four to six months, if the values do not normalize, the child should be referred to a specialist.

The risk values for TG and HDLc in Table 9 are very similar to those used by IDF to classify MS in children, with a slight difference in HDLc (IDF presents a higher cut-off) (18), 165, 167).

Although the used values are in agreement with the values from other countries, the use of cut-offs built specifically for the Portuguese population could increase the accuracy of the identification of individuals at increased risk of MS.

9.1.2. Glycemia assessment in Portuguese children and adolescents

There is no specific screening schedule for the determination of glycemia and insulinemia in children and adolescents in Portugal (18). However, fasting glycemia is suggested to be analyzed together with the lipid profile screening recommended in the NCAHP, due to the high prevalence of diabetes in our country and the confluence of dyslipidemia and diabetes risk factors. (18).

Although the changes in insulin levels are the first to appear in childhood obesity, before abnormal levels of glucose can be detected, insulinemia is not usually used as a routine

determination for tracking changes in glucose metabolism. Only the evaluation of glucose levels are, usually, used in routine clinical practice (19).

Portugal uses the cut-off levels of glycemia recommended by the American Diabetes Association (ADA). These reference values, the same used for adults, are presented in Table 10.

Table 10. Reference levels for plasma glucose

Criteria	Normal	Pre-diabetes	Diabetes[1]
Fasting plasma glucose (mg/dl)	**< 100**	**≥100;<126[2]**	**≥126**
Casual plasma (mg/dl)			**≥200[3]**
OGTT 2 hour plasma (mg/dl)	**<140**	**≥140;<200[4]**	**≥200**

Adapted from (212). 1, In the absence of unequivocal hyperglycemia with acute metabolic decompensation the diagnosis should be confirmed, on a subsequent day, by any one of the criteria. 2, classified as impaired fasting glucose. 3, with classic diabetes symptoms: polyuria, polydipsia and unexplained weight loss. 4, classified as impaired glucose tolerance.

In the absence of unequivocal hyperglycemia with acute metabolic decompensation, the diagnosis of diabetes should be confirmed, on a subsequent day, by any of the three criteria presented on the third column of Table 10. Nevertheless, fasting glucose is the preferred test, due to its simplicity and applicability (212).

The ADA recommends the screening of glucose levels in children above 10 years, or at onset of puberty, if puberty took place in an earlier age, in children with increased metabolic risk. Increased risk is defined as an overweight child (BMI ≥85th percentile for age and sex, weight for height ≥85th percentile, or weight ≥120% of ideal weight for height) together with any of the following risk factors:

- Family history of T2DM in first-or second-degree relatives;
- Race/ethnicity: Native American, African American, Latino, Asian American, Pacific Islander;
- Signs of IR or conditions associated with IR (acanthosis nigricans, hypertension, dyslipidemia, or PCOS).

The screening should be repeated every 2 years (212).

The use of the same cut-off values for glycemia in children and adults is widely accepted. Nevertheless, more studies are necessary to analyze the sensibility of these values to identify IR onset before T2DM is clinically diagnosed. A good option would be to use insulin plasmatic values, as insulinemia is more sensible for detecting early IR changes in pediatric patients.

9.1.3. Blood pressure assessment in Portuguese children and adolescents

The guidelines of NCAHP for hypertension screening in children and adolescents follow the international recommendations and should be performed in routine appointments in all children, starting at 3 years old.

BP measurements should be compared against tables presenting systolic blood pressure (SBP) and diastolic blood pressure (DBP) values adapted for gender, age and height percentile. These tables present cut-off values for specific BP percentiles that are used in the classification of BP; the limits defining BP are presented in Table 11.

Table 11. Blood pressure evaluation in children and adolescents – cut-off values.

	DBP[1]	SBP[1]
Normal (P)	**< 90th**	**< 90th**
Pre-hypertension (P) [3]	**≥ 90th; < 95th or ≥ 80mmHg**[2]	**≥ 90th; < 95th or ≥ 120mmHg**[2]
Hypertension grade I (P)[3,4]	**≥95th; <99th+5mmHg**	**≥95th; <99th+5mmHg**
Hypertension grade II (P) [3,4]	**≥99th+5mmHg**	**≥99th+5mmHg**

P, percentile. 1, blood pressure values should be adjusted to gender, age and height. 2, even if the values fall under the adjusted 90th percentile. 3, for the diagnosis of pre-hypertension or hypertension is sufficient that SBP or DBP fall above the maximum limit of the previous category. 4, hypertension should be confirmed in three separate occasions.

The diagnosis of hypertension should be confirmed in 3 separate occasions. The standard technique to measure the BP is the auscultatory. Digital measurement can be used, but must be confirmed by the auscultatory method if BP values are high. The measurement in the right arm, contrary to the general habit, is recommended (18).

Similarly to the lipid profile, Portugal does not have reference BP tables for children and adolescents. The recommended tables are those from the National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Children and Adolescents, USA. The use of reference values based on populations from different countries might misdiagnose or underdiagnose this problem in Portuguese youth (18).

The cut-offs IDF recommends for BP, to use in the classification of MS, are higher than those used in Portugal, what could lead to underdiagnosis of MS, when following IDF guidelines (18), 165, 167).

10. Therapeutic strategies for pediatric obesity

The use of therapeutic strategies in obese children aims weight normalization and reduction of the future risk of CVD. A behavioral approach, towards lifestyle modification, is the main pillar of obesity treatment, both in children and adults. The main objectives are to increase physical activity and improve diet in order to obtain an enhanced energy balance. Two strategies are usually used: non-interventional programs, involving nutritional counseling and motivation to exercise; and interventional programs, involving the use of diets and/or physical exercise programs. Pharmacological drugs have also been tested as adjuvants in the treatment, with limited but consistent results (e.g. orlistat and metformin) (210, 213-215).

To improve weight, three points are common to adults and children's treatment: motivation, diet and physical exercise. Usually, childhood obesity is treated by a regular pediatrician. As obesity increases, and comorbidities or psychological consequences appear, a multidisciplinary approach becomes necessary, including a nutritionist, behavior modification specialist, psychologist and an obesity specialist pediatrician (210, 213-215).

The treatment of obesity is a long process presenting different phases that should not be hurried. In the beginning of the treatment, several visits might be necessary, to build the trust between the doctor/team and the child. Also, it is beneficial for the children to be followed by the same doctor throughout the treatment (5).

The IDF 2007 recommendations for the prevention and treatment of MS in children and adolescents focus on weight improvement and management, tackling obesity as a MS underlying cause. IDF recommendations are (210):

- Moderate caloric restriction;
- Moderate increase in physical activity;
- Change dietary composition.

According to IDF, the use of adjuvant pharmacologic therapy should also be considered, if safety is proved (210). IDF recommendations are similar to other international guidelines, such as those recommended by the WHO, the American Association of Pediatricians (AAP), and the Associacíon Española de Pediatría (AEP) (25, 214, 216).

Obesity reduction programs in children should be adapted to age, degree of obesity and presence (or not) of comorbidities. The treatment should start immediately and do not wait

for older ages (5, 179, 210, 214). Children and young adolescents have a linear growth potential that can help on BMI normalization and should be considered when defining a treatment strategy (5, 210, 215).

Depending on the age and the levels of adiposity, children with a BMI higher than the 85th percentile and lower than the 95th percentile (overweight children), or above 95th percentile but without comorbidities (obese children without complications), could follow 3 basic options (179, 214, 215):

- Slow the rate of weight gain – used in very young children (2-4 years old), as they present a very rapid growth rate that might lead to BMI normalization;
- Weight maintenance – used for children over 4 years;
- Gradual weight loss (1 to 2 kg/month) to improve BMI.

For children with BMI above the 95th percentile with comorbidities, an approach based on the severity of the situation should be used. Weight loss is usually necessary but, even in severe obesity, the weight loss program should be gradual for several reasons (5, 179, 215):

- It should be used a goal that the children can achieve to avoid demotivation;
- Even slow weight gain involves a great reduction on habitual caloric intake, especially if the children is still growing;
- Treatment of weight excess is a long term process and gradual weight loss is easier to be sustained for longer periods.

Adolescents who have reached growth limit and have a BMI higher than 30 Kg/m^2 should be submitted to a program similar to that used for obese adults, to minimize CVD risk (179, 213).

It is necessary to approach the child/adolescent in his culture, social and family environment, as it would be harder to tackle childhood obesity without the help of parents and caretakers (165, 179, 213-215).

The NCAHP provide guidelines and check points to follow the pediatric development that, if correctly followed, might help to identify obesity and associated comorbidities while still in early stages, when the chances for a successful treatment are higher (18). It also focuses on the promotion of healthy lifestyle habits, improving diet and increasing physical

activity. Moreover, anticipatory care instructions are provided to parents and caretakers to guaranty that they are aware of the most common health issues in each particular age, or of any particular health problem of the child, and have the skills and knowledge necessary to support the child along the treatment. A gradual responsabilization, first of the parents and, afterwards, of the child or of the adolescent, by their health maintenance and success of therapy should be done (18).

In Portugal, there are no clear guidelines on how the pediatric obesity should be approached, only general recommendations are given for a healthier lifestyle. Nevertheless, many hospitals in Portugal have specialized pediatricians and provide obesity appointments to which children can be referred. The obese appointments, usually, involve besides the doctor, a visit to a nutritionist.

Clearer guidelines regarding obesity and overweight treatment and prevention should be provided worldwide, and particularly in Portugal. It seems unreasonable that the NCAHP focus on dyslipidemia, hypertension and oral health programs, known comorbidities of obesity, while obesity is only referred twice along the 121 pages of the document.

10.1. Prevention and interventional programs

The prevention of overweight and obesity can be considered as population based programs, involving national or global initiatives, regional or community interventions, or individualized programs (179, 215). According to the WHO, the use of population based programs to prevent obesity in combination with local-based community, school or individual programs are successful in fighting obesity (151, 170).

The population based programs intend to promote and support healthy habits, by reducing unhealthy habits and by interfering with the social determinants of health. The use of local-based actions is helpful, but needs the support of national government policies to ensure their sustainability and efficacy. Thus, population based prevention of obesity transfers part of the responsibility on reduction of health risks, from the individual to the government (151, 170). The main stakeholders in this kind of approach are the governments, who are responsible for creating policies, implementing interventions, and monitoring their results (179, 215). The WHO itself is responsible to provide technical and scientific basis, the necessary tools and resources for the government's actions (216).

Other key stakeholders include regional and local level administration, non-governmental organizations, academic and civil society (216).

The government, usually through the Ministry of Health, is responsible for the coordination of the various stakeholders and for the development of different approaches that must globally influence the society, allowing the achievement of optimal results: the upstream approach refers to the improvement of the economic physical and social environments; the midstream approach is to increase the efficacy in influencing individual behavior and lifestyle; and the downstream approach, is related to the availability of health services (216). By coordinating the population based programs through these different approaches, the government objective is to make a change in the social paradigm and make the healthier choices the easier ones (179, 216). The WHO Technical Meeting for Population Based Prevention of Childhood Obesity in 2009 highlighted some points, key issues, which should be addressed by governments while tackling obesity:

- **Globalization** – driving force responsible for much of the changes in diet:
    - To redefine policies with food supply, focusing on quality and quantity. To give particular attention to food marketing directed to child and adolescents;
- **Urbanization** – urban environments are, in general, associated with unhealthy lifestyle. However, increasing obesity occurs both in rural and urban areas:
    - Create friendly environments that facilitate healthy options. Political options include building and maintaining in good conditions bicycle and pedestrian pathways; offering a good network of public transportation; providing the schools with adequate sports equipment, among others;
- **Socioeconomics and demographics** – many issues affecting obesity can be included in this group. Children of ethnic minorities and low-income families are in increased risk of obesity. Furthermore, children from low-income families appear to achieve worse responses in interventional programs. Also, the risk of obesity can vary, depending on the gender in some societies.

  Early personal history can influence body weight. Children of obese mothers, of mothers who had gestational diabetes, or children who were exclusively breastfeed for a period shorter than 6 months present a higher prevalence of weight excess:
    - Identify and direct interventions to children in higher risk (risk factors can be specific of a certain society). A healthy beginning of life should never be denied to a children, due to their social status;

    - The lifecycle approach to obesity consider that to guaranty health in the future life the prevention of disease should start early, still during pregnancy and continue afterwards;
- **Children with disability** – these children are in greater risk of obesity due to physical, environmental and psychological limitations, and to the lack of knowledge about real capacities of health professionals, parents and the children themselves:
    - Build directed programs and encourage disabled children to do physical activity, if possible integrated with non-disabled children;
- **Cost-effectiveness** – the cost-effectiveness of programs in inducing changes in lifestyle and lowering obesity prevalence is not well established, limiting evidence-based policies changes:
    - Most of the programs are cost effective. Intervention success is increased when large populations are approached and the target is not set in small groups or individuals. Considering this, the national health system, due to its broad reach, can achieve maximum results.

The other stakeholders, besides the government, also have an important function, to transform and apply the guidelines in the field. While national governments are responsible for the design of the programs, regional and local governments should adapt the general guidelines to their particular context, in order to increase success rates (216).

The private sector should help to create and support healthy environments. A very specific social role is that of the food industry, by enabling healthy food options and supply. Partnerships with commercial entities, regarding the programs funding, can be done and might increase the sustainability and quality of those programs. Nevertheless, they have to be carried carefully as conflicting interests might be present. Thus, the WHO advises that the commercial partners should be more involved in the delivery of the objectives than in their lining (216).

Non-governmental associations should advocacy towards policies tackling obesity, good practices, transparency and resourcing of programs. Universities and researchers, on their turn, should provide scientific evidence for the development of more effective programs (216).

Successful implementation also depends on other players, as parents, health professionals, and teachers. These players are in the field and interact directly with the

obese children and adolescents, being the real face of the struggle against obesity (151, 170).

To face this public health problem, the best approach is to act early and prevent the development of weight excess. Besides general prevention, directed programs can still act in other levels of the population, including the prevention of overweight children to become obese - primary prevention; and in the treatment of obese children, to prevent and reduce obesity-related comorbidities – secondary prevention (179). Community based prevention programs can be more or less geographically embracing, presenting maximum effect with minimum cost. On the other hand, individualized prevention of overweight and obesity has much higher costs and is less likely to have a great impact in public health (179, 215).

When developing a program is essential to do the "tailoring" of the strategies, adapting them to the socio-cultural reality of the target group. Approaches for preventing obesity in school age should not be only focused on the obese youth and weight loss, but also to encompass cultural aspects, community, schools, family and peers. Afterwards, and as the program is applied in the ground, it is necessary to pay attention to individual differences that were not considered in the first approach, but might compromise the program success (179, 215).

Interventional strategies to treat obesity are using nowadays a behavioral approach, particularly in children. The behavioral modification is aimed to create, or increase, healthy habits, by changing diet and physical activity routines and increasing motivational levels (5).

One of the first steps in the behavioral treatment of obesity is to detect unhealthy habits and identify the favoring situation, the driving force, and clarify the conditional link between them (for example, nutritional excesses and television watching). Afterwards, it is necessary to break the perverse association and build new ones with healthy habits (5, 216). Through a process of conditioning, it is important to associate the new habits with enjoyable experiences and good outcomes, proportioning a positive reinforcement, as gratifying experiences are more likely to be repeated. Stress, on the other hand, is a potential source of unhealthy behaviors. The identification and prevention of stress sources in obese individuals under treatment is crucial. An important point to avoid unnecessary stress is to target realistic objectives regarding weight improvements (5).

Several interventional programs to reduce overweight and obesity have been conducted with various results. Different study protocols, designs and studied population are on the

basis of this variation. An interesting study in Australia found that multi-angled school-based programs involving a physical active component were (together with reduction in TV advertising of high fat and/or high sugar foods and drinks and laparoscopic adjustable gastric banding) the interventional measures with the greatest health benefit in the prevention of childhood obesity (216).

There are international and national guidelines towards fighting obesity and a variety of approaches are used, even within the same country. In a small country as Netherlands 30 different interventional programs were identified, 28 of them were used exclusively in one pediatric department. The strategies in those interventional programs varied broadly, though the majority lack established scientific basis, or methods to evaluate the efficacy (19).

Regarding the effectiveness of interventional programs there are still doubts in many areas, to define which is the best strategy for weight reduction. Pedrosa *et al.*, studying overweight Portuguese children (7-9 years) response to weight losing therapy, based in nutritional counseling and motivation to exercise, found a greater impact of group (family) therapy, when compared to the individual approach. Although both treatments lead to the improvement in body weight and lipid profile, the effects of the group based treatment in the reduction of adiposity, IR, leptin and in the improvement of lipid profile were more marked (148). Children, especially in young ages, are included in family and school environment having access almost only to food in the extent that is allowed by parents or caretakers, and apply just to the physical activities that the parents are willing to promote (5, 148). In this way, a group or family intervention, in which is transmitted information about healthier lifestyles, are more likely to promote lasting results, even after the end of the intervention (165). This interconnection between the individual and the group/family is more complicated in adolescents, as they are in a phase of personality forming and have more freedom of choice, regarding diet and physical activities (165).

10.2. Diet

Unbalanced diet and increased energy intake is a common problem nowadays and a major contributor to the epidemic obesity. Lower income families have particularly increased difficulty on having a healthy diet, as it is more expensive than other unhealthy options (179, 213, 216).

Obtaining data about a patient's diet can involve different strategies. The use of questionnaires is common, but lacks objectivity; moreover, obese children and their parents, usually, underreport diet excesses, introducing analytical bias. Weekly tables to discriminate the number of servings and respective amounts can also be used, as can the 24 hour food recall. However, both share the lack of objectivity of questionnaires (25).

Some interventional programs, in order to better control energy intake, choose to provide at least one balanced meal per day. Although this is a good strategy, it has the predictable logistic, children compliance and financial problems, particularly in longer studies (25).

To design a healthy diet the children's nutritional habits and their socio-cultural reality should be considered. The basic idea of the diet in the obese pediatric patient is to adjust the energy intake to their necessities, in order to allow the correct development, as children and adolescents are in a period of constant changes, both physical and psychological. Thus, providing an adequate nutrition, to allow the child to reach his full development potential is essential (25, 179, 215). The traditional recommendations are, usually, overestimated in obese, due to the decreased physical activity, and should be adjusted (25).

The first approach in obese children and adolescents is to reduce the traditional nutritional mistakes. Over the last decades it has been observed a trend to increase the caloric density of foods, the percentage of fat and carbon hydrates (CH), and to reduce the content of micronutrients and fibers. Thus, there is an absolute and relative increase in fat intake. Many children, for example, do not have breakfast. It is known that skipping breakfast increases the incidence of obesity and decreases school performance (24). Other common mistakes include an excessive amount of candies and pastries, particularly as the mid-morning and afternoon snack, and an excessive intake of soft drinks with a high concentration of CH (25, 213, 215). The substitution of this type of snacks by other healthier options, e.g. a fruit, is beneficial (5).

Not only the type of food is inadequate, but also the number of meals, the daily energy distribution, the serving sizes and their weekly basis (5, 25). In western cultures there is an excess of meat intake, especially red meat, with a reduction of fish based meals. The intake of meat should be reduced to 3 times a week, preferably lean meat (e.g. turkey, chicken), and the intake of fish should be of at least of 2-3 meals per week (25). Fruit and vegetables intake usually do not reach the recommended 4-5 portions per day (25, 214) and the consumption of whole grains is also reduced (25). Due to the reduced intake of

fruits, vegetables and cereals there is a lower intake of fibers, a macronutrient that is associated with the reduction of obesity and with other beneficial effects, as the regulation of intestinal function, reducing the risk of colon-rectal cancer (25). In adolescents, the importance of alcohol ingestion in the energy balance should be considered (25).

Other healthy changes in food habits include the reduction of serving sizes and the increase in the time reserved for meals as, by prolonging the length of the meal, there is an increase in satiation through the release of anorexogenic and satiation mediators, as some gastro-intestinal peptides (e.g. Y peptide) (5).

The way food is prepared is important to stimulate children to eat healthy. Cooking with less fat and the substitution of frying by grilling and baking help to reduce fat intake (25, 215). It is also important to reduce the use of oil and industrial sauces (e.g. mayonnaise, ketchup). The use of salt should be limited, as many of the obese children have increased BP; aromatic herbs are a good substitute. Food presentation should not be forgotten, as it motivates children to eat better and forget that they are in a diet, if that is the case (25).

The majority of the diets target changes in the amount and percentage of macronutrients. When the approach to reduce nutritional mistakes in overweight and moderated obesity is not successful, a nutritionist can prescribe a hypocaloric diet, with the reduction of daily energy intake of about 30%. A balanced hypocaloric diet might also involve the increase in fiber intake, the reduction of CH with high glycemic levels and the substitution of saturated by mono and poly-unsaturated fats (25, 215). Hypocaloric diets and low CH diets have shown similar results in short term studies (5).

In the last years a great number of fast diets and supplements have become available to help weight loss. The scientific data supporting these treatments are scanty and they should not be used by pediatric patients. Adolescents are a particular vulnerable group due to their autonomy to make some decisions (5).

The nutrition committee of the AEP recommends a mixed, balanced and quantitatively limited diet to treat obese individuals. In Spain, to help children to learn the best nutritional options, different strategies have been used, as the traffic light diet. In this diet the foods are classified according to their content in fat. The foods labeled green are low in fat and could be consumed freely, while the consumption of food of the red group, high in fat, should be limited. Similar campaigns have been used in Portugal with interesting results (5).

When a weight-losing diet is followed, besides the decrease in fat mass, a decrease in lean mass also occurs and, consequently, a reduction of the energy expenditure. Thus, it is important to add protein sources of high biological value to diet and to increase physical exercise levels, in order to maintain and improve the lean mass throughout the diet (25).

In overweight and moderately obese younger children the main objective is to maintain the body weight without compromising the normal body development, as the BMI, usually normalizes with height increase (25, 215). Also, the energy restriction should be moderate, in a way that it can be sustained in time (5). Very low caloric diets (500-600 kcal/day) and the use of liquid formulas to substitute entire meals should not be used in children and adolescents, and are only recommended in cases of increased health risk and morbid obesity (25). In fact, some centers specialized in the treatment of morbidly obese or obese with comorbidities have successfully used that approach (5). These diets need special attention to avoid the development of acidosis, through an adequate CH intake, and to prevent the extensive loss of lean mass, by controlling the protein ingestion (5, 25).

10.2.1. Biochemical outputs of diet

Diet restriction is associated with improvements in adiposity and in lipid profile, namely a reduction in TC and LDLc. The influence of diet in HDLc appears to be limited, presenting physical exercise more pronounced effects (217). This fact might be explained by a small impact of diet on IR (217).

An improvement in inflammation, following diet-induced weight loss has also been reported. In fact, following a diet restriction program, total adiponectin levels were increased, while inflammatory mediators such as leptin, MCP-1, IL-15, and IL-18, were reduced (218, 219). A cumulative effect of diet and physical exercise on the improvement of the inflammatory status was reported (218), however, there are conflicting results (219).

The use of specific components in the diet, besides the energy restriction, might lead to different outcomes regarding metabolic changes. The different types of fats, for instance, are associated with different effects. Increased fat intake is associated with a reduction of adiponectin (220), while children receiving n3 fatty acids supplementation presented an improvement of the inflammatory status (increased adiponectin and reduced TNF-α and

leptin). The improvement in inflammation is probably on the basis of the reduction in IR (221).

The type of diet recommended its caloric content and individual components should be defined according to the individual degree of obesity, presence or not of comorbidities and specific metabolic risk profile, in order to obtain the expected benefit from it.

10.3. Physical activity

Southern European countries, including Portugal, present lower physical activity levels, among children and adolescents, than countries from Centre and Northern Europe. Part of the increased prevalence of obesity in the Southern countries might be related to this fact (222).

An inadequate energy intake accompanied by reduced physical activity is very likely to result in weight excess. The increase of the physical activity levels is mandatory in obesity treatment and has multiple benefits. To achieve an improvement, two points should be considered: to reduce the time spent on sedentary behaviors and to increase physical activity levels.

The daily energy expenditure is calculated by the sum of the energy expended in rest, the diet induced thermogenesis and the energy expended on physical activities (25). Physical activity, besides the energy used during the activity itself, increases lean mass and, consequently, increases the energy expend during rest (25).

The strategies to access information about individual physical activity levels are similar to those used for diet. Questionnaires, filled by children or parents, do not have enough accuracy, as there is a trend to underestimate sedentary behaviors. Also, questionnaires do not discriminate different levels of energy expenditure that can occur in the same type of activity, depending on the engagement of each individual (25). The use of accelerometers is a good option for objectively control the level of physical activity, even during entire days. Its use has, however, considerable financial costs (25).

Strategies and interventional programs to increase the physical activity among pediatric populations have flourished in the last years. School based programs might be particularly interesting as they take advantage of children availability and school sports equipment

(25, 213). However, programs involving physical exercise should choose stimulating and new activities, especially for the younger children, as their interest is harder to maintain (179, 215). The simple increase of the hours spent on curricular physical exercise do not present significant improvements (179).

The guidelines for physical activities in children and adolescents state that 30 to 60 min a day should be dedicated to moderate or intense physical activity (25, 179, 213, 214). Physical activities should be adequate for age and gender, and should also be stimulating, in order to prevent the loss of interest. The general recommendations have to be adjusted for each children and the exercise plan adequate to their socio-cultural-economic background (25, 179, 213-215).

Increasing activity levels with everyday activities, as gardening, walking to school, riding a bicycle and traditional children games, as hide and seek, might be effective in increasing the metabolic level and should be encouraged (5, 179, 215). On the other hand, sports groups, although helpful, may not provide enough activity, due to the limited training period and weekly schedule. Moreover, the ingress in a sport group of an unfit obese child might have harmful results caused by the initial incapacity to perform requested exercises and by eventual bulling of their peers, what could, somehow, traumatize the child and lead to withdraw. Thus, group sports should be considered carefully, or postponed until the patient has reached minimum skills (179, 215).

Nevertheless, there are conflicting results regarding the success of interventional programs involving exercise alone or combined with diet. Doubts exist, particularly regarding the long term effectiveness on weight maintenance (25, 179). Further studies on the influence of less “aggressive” programs following (e.g. a motivational approach towards the improvement of lifestyle habits) would be important.

Regarding sedentary behaviors, such as screen time, parents should define the period of duration and its content, while outdoor playing should be encouraged, if safeness is granted (5).

Once again, each obese individual is a particular case and should be addressed in that way. The child should be challenged and motivated to increase physical activity gradually and to achieve realistic goals. Unpleasant situations should be avoided.

10.3.1. Biochemical outputs of physical exercise

An adequate performance of physical exercise presents several beneficial effects. For instance, there is an improvement of the lipid profile, with the reduction of TG and the increase of HDLc. A key factor underlying this improvement seems to be the increase in insulin sensitivity that follows the increased physical fitness. In fact, the improvement of the hepatic insulin sensitivity is associated with changes in the lipoproteins produced by this organ, towards the production of less atherogenic lipoproteins (181, 182, 223, 224). Nevertheless, there are still some contradictory results (55). Diet, on the other hand, although presenting a significant role in the reduction of TC and LDLc, seems to have limited impact on HDLc and insulin sensitivity, being the increase in physical exercise the main pathway to achieve those changes (217).

The increase in regular physical activity is also associated with an improvement of the inflammatory status, with the reduction of inflammation markers, such as IL-6, CRP, leptin and TNF-α (55, 225-227) and the increase in anti-inflammatory adiponectin (228, 229). Moreover, the exercise-related improvement in the IR is likely to be associated with the reduction in pro-inflammatory mediators and the increase in anti-inflammatory mediators, such as adiponectin, namely the HMW adiponectin (230, 231). However, there are still some controversy regarding changes in inflammatory mediator levels with physical exercise and weight loss, particularly on which markers vary and in the extent of the modification. The results obtained with adiponectin multimers are particularly confusing (42, 232, 233)

The variation in the training protocol used in an intervention program can lead to different results. Although both aerobic training (AT) alone, and aerobic combined with resistance training (ART) improved adiposity measures, as BMI, BMI z-score, visceral fat and subcutaneous fat, and were effective in reverting MS in obese individuals, the improvement achieved with ART, namely in adiposity markers (with exception of subcutaneous adipose tissue (SAT)), TC, glucose and adiponectin levels was higher than with AT (234).

Cardiorespiratory fitness (CRF) is inversely associated with CVD risk markers and total mortality (235) and one of the main determinants of the physical activity levels in children and adolescents (222). In fact, a lower CRF is related to increased sedentary behaviors and weight gain (236). Although CRF has a considerable genetic background, it increases with exercise (222). The use of training protocols aiming to increase CRF, as well as the

use of CRF as an outcome of exercise programs, might be a good strategy to control the effectiveness of interventional programs.

Some studies suggest that improvements in IR, lipid profile (e.g. HDLc) and inflammatory status (e.g. adiponectin) are only present in the case of a substantial improvement in BMI (e.g. a reduction of BMI z-score > 0.5) (226, 227, 237). Besides the weight reduction that might accompany the increase in physical exercise, a key factor to explain the metabolic changes that occur, are the changes in the body composition, particularly the reduction in central adiposity, known to be associated with worsening of the risks factors for CVD (4, 39, 44). Thus, some of the conflicting results regarding the effects of physical exercise and diet on metabolic parameters might be related to the effective changes in body composition. More than considering only changes on weight or BMI to evaluate the success of an interventional program, the analysis of markers of adiposity distribution, such as WC, WC/H, skin folds and DEXA, would, probably, be a better option

Study the impact of small reductions of adiposity on CVD risk markers would be important. Smaller improvements might be a more realistic target, which could be more easily achieved and sustained, avoiding the loss of motivation in longer, stricter programs.

10.4. Pharmacological strategies

Pharmacological strategies to improve weight loss in pediatric obese patients have been proposed. Nevertheless, these strategies must be always an adjuvant to lifestyle changes, including diet and increased physical exercise. The weight loss obtained by the use of drugs are, usually, small but significant (25, 213, 238).

The use of an adjuvant pharmacologic therapy should be considered carefully and in specific cases, when the relation risk/benefit is favorable. Patients that could be considered for pharmacological therapy include obese individuals that do not respond to behavioral treatments or present comorbidities, and overweight children presenting comorbidities. Overweight or obese children with a strong family history of T2DM or CVD should also be considered, even when comorbidities are still not present (25, 213, 238).

In Portugal the only drug approved for the treatment of obesity is Orlistat, however it is not recommended for children and adolescents (239-241).

The drugs used have specific actions and can be included in the following groups: drugs reducing energy intake (anorexigenics), drugs that interfere with dietary nutrients and drugs interfering with the metabolism (25, 238). Table 12 resumes some of the drugs that have been studied and/or used in pediatric obese patients.

Other drugs have been used in adults with interesting results regarding weight reduction, however the information about their applicability in pediatrics is limited or absent. This list of drugs enclose bupropion (anti-depressive), lorcaserin (selective 5-HT2C receptor agonist), tesofensine (monoamine re-uptake inhibitor), pramlintide (amylin analogue), exenatide and liraglutide (GLP-1 analogs); other drugs, as acarbose (pseudotetrasaccharide), have still limited support, even in adults (238). Rimonabant (CB1 cannabinoid receptor inhibitor), an apparently promising drug, was withdraw due to the association to the increased risk of suicide idealization and attempt (5).

Another therapeutic option for the treatment of obesity, available in Portugal, is a medical device called XL-S Medical®. The functional component of this device is Litramine, a complex of soluble organic and vegetal fibers that jellify in the stomach, capturing the fats and lipids present in the meal, diminishing their absorption, with effects similar to orlistat (although the mechanism of action is different). It is not recommended for individuals under the age of 18 years or with a BMI lower than 18.5 kg/m^2 (241, 242).

Obesity is a multiorgan pathology, and weight control is mediated by a large number of mechanisms in the organism. In this way, the use of therapies combining different drugs, acting by different mechanism, to treat or prevent weight gain, such as the combination of peripheral and central acting drugs, can enhance the success of the pharmacologic therapy (213, 238). Some of the combinations used in adults are phentermine and topiramate (approved by FDA for the treatment of obesity in adults, in 2012), bupropion and naltrexone (recommended for approval by FDA Metabolic Drugs Committee), amylin and leptin analogues, and pramlintide and phentermine or sibutramine (238). None of these combinations are available in Portugal (239-241).

Table 12. Pharmacological options for obesity treatment

Drug	Mechanism of action	Side effects
	Centrally acting anorexigenic	
Phentermine[1] and diethylpropion[1] (238-241)	Anorexogenic effects by increasing adrenergic tone, decreasing energy intake and increasing resting energy expenditure	Euphoria, palpitations, hypertension, cardiac arrhythmias, dizziness, blurred vision and abuse potential.
Fluoxetine, chlorphentermine[1], fenfluramine[2] and dexfenfluramine[2] (238-241)	Reduce appetite by increasing serotonergic release or inhibiting re-uptake	Cardiac valvulopathies, increased risk of primary pulmonary hypertension, headache, abdominal pain, drowsiness, insomnia, dry mouth, increased activity and irritability
Methylphenidate and dextroamphetamine[1] (238-241)	Anorexogenic effect by inhibiting dopamine reuptake	Agitation, insomnia, tachycardia, hypertension, hyperhidrosis and abuse potential
Sibutramine[2] (25, 179, 213, 214, 238-241)	Inhibits serotonin, norepinephrine and dopamine reuptake, presenting an anorexogenic effect and increasing energy expenditure	Cardiovascular events (myocardial infarction and stroke), hypertension, increased heart rate, headache, dry mouth, anxiousness, insomnia, depression, constipation and cholelithiasis
Leptin[1,3] (25, 179, 238-241)	Transmit information to hypothalamic centers of the adipocyte lipid reserves and of nutrients and energy of meals – satiation effect	Increased inflammation
Topiramate (25, 213, 238-241)	Anorexogenic effects by GABA-ergic stimulation	Psychomotor disturbances, difficulties with concentration, sedation, reduction of learning skills, paresthesias and taste impairment.
	Drugs affecting dietary nutrients	
Orlistat[4] (25, 179, 213, 214, 238-241)	Inhibit gastrointestinal and pancreatic lipases, reducing the absorption of ingested dietary fats	Intestinal gases, oily stools, oily spotting, oily evacuation, abdominal pain, fecal urgency, diarrhea and gallstones. Lipid soluble vitamins deficiency.
	Drugs affecting the metabolism	
Metformin (25, 179, 213, 214, 238-241)	Modulation of insulin action. Inhibit intestinal glucose absorption, reduces hepatic glucose production and increases peripheral insulin sensitivity by improving glucose uptake and utilization	Diarrhea, nausea, vomiting and flatulence. Vitamin B12 and folic acid deficiency
Octreotide[5] (213, 238-241)	Modulation of insulin action. Octreotide inhibits the release of the growth hormone, thyrotropin and corticotropin from the hypophysis; and of insulin and glucagon from the pancreas and ghrelin from the stomach.	Transient elevation of blood glucose, diarrhea, abdominal pain, discomfort, flatulence influenza-like symptoms, constipation, headache, fatigue, dizziness, nausea, anemia, hypertension, and gallstones
Growth hormone[6] (213, 238-241)	Modulation of lipolysis. Inhibits LpL, increases hormone-sensitive lipase levels and stimulates lipolysis in adipocyte, stimulates protein synthesis and increased fat-free mass	Increased risk of tumor, potential adrenal insufficiency, insulin resistance, increased risk of sleep apnea and increased cardiac diameter
Ephedrine combined with caffeine[1] (238-241)	Modulation of energy expenditure. Ephedrine increases catecholaminergic tone while caffeine acts by inhibiting phosphodiesterases. Ephedrine presents a thermogenic effect that is enhanced with the administration of caffeine	Cardiac effects, nausea, insomnia, tremor, dizziness and palpitations

1, not commercially available in Portugal. 2, withdraw from Portuguese market. 3, Used only to treat syndromic obesity related to lack of leptin production. 4, only pharmacological option approved in Portugal. 5, Used only for the treatment of patients with hypothalamic obesity. 6, used only to treat syndromic obesity characterized by reduced synthesis of growth hormone – e.g. Prader Willi Syndrome.

The use of pharmacologic treatment should always be considered with caution, particularly in pediatric patients, and faced as adjuvant of lifestyle modification strategies. Nevertheless, it should not be a taboo and, when a positive risk benefit balance is considered, the use of drugs can be a helpful tool. Portuguese and European regulatory agencies have adopted a particularly restrictive position towards possible pharmacological options, particularly when compared to the USA's FDA. As a direct consequence, off-label use is a growing reality in obese treatment. More flexible legislation and clearer guidelines would probably guarantee the safety and efficacy of obesity treatments. When considering the pros and cons of pharmacological options it is important to consider not only the comorbidities presented by the patient, but also the risk for future obese-related complications. Thus, the balance between the benefits and risks of controlling adiposity, especially in young ages, is very important, as obesity is a chronic condition that tracks into adulthood. Moreover, the drugs to treat obesity, both in adults and in children, have to demonstrate long-term safety and efficacy, particularly when treatment is started in pediatric ages (213, 238).

10.5. Surgical treatment and other options

Surgical treatment in obese children and adolescents is controversial and the last therapeutic option, used only when the traditional approaches failed. It might be considered in case of extreme obesity (BMI > 40kg/m^2) with associated comorbidities (hypertension, dyslipidemia, IR, NAFLD…), and in case of BMI > 35 kg/m^2 with associated serious comorbidities (T2DM, sleep apnea, endocraneal hypertension or serious NAFLD) (5, 25, 179). Moreover, as adolescents are still in development, bariatric surgery should be carefully considered. The recommendations for bariatric surgery include:

- A minimum Tanner stage of 4 or 5. Less mature individuals could be considered if severe comorbidities are present;
- Have reached at least 95 % of growth potential, especially if malabsorptive surgery is considered;
- Psychological maturity and capacity to understand the limitations that will follow the procedure:
    - Dietary and activity changes;
- Family and social support;

- Exclusion of genetic or syndromic obesity (5, 214, 215);
- Failure of lifestyle intervention, with a duration of at least 6 months (5).

Bariatric surgery is also contraindicated in adolescents with history of alcohol or drug abuse in the last year, pregnancy or planning to get pregnant in the next 2 years, and in patients who did not correctly followed previous lifestyle treatment (215).

The most relevant bariatric techniques in pediatric obesity are gastric bypass, gastric banding and gastric balloon.

The gastric bypass in Y of Roux, due to its malabsorptive and restrictive nature, is one of the most used surgical options in obesity. In fact, it is the most used technique in the USA. This surgery consists of a reduction of stomach size and a reduction of intestinal absorptive capacity via the creation of a gastrojejunal anastomosis (5, 214). The gastric bypass allows the loss of 50-60% of the body weight, as well as improvements in several metabolic parameters, such as TG, TC and IR (5, 25, 179, 214). Side effects involve nutritional deficiencies, due to malabsorption. Patients submitted to this surgery need to be followed by a multidisciplinary team, including doctors, psychologists and nutritionists before and after surgery (25, 179, 214).

The gastric banding is a less invasive surgical technique, when compared to gastric bypass. This option has increasing acceptance nowadays, due to its reversibility and lower number of side effects. The gastric banding is, in fact, the most used bariatric surgery in Europe for the treatment of obese adolescents, presenting a considerable success. It consists in the laparoscopic placing of a silicon ring around the proximal part of the stomach that will limit food ingestion. This ring can be regulated by introducing saline in a subcutaneous reservatory. However, the weight reductions are more limited than with gastric bypass, reaching around 20-30%. The side effects, as malabsorption, are less frequent and have less impact in the development of the adolescents than gastric bypass (25, 214, 215).

The endoscopic implantation of a gastric balloon is the lowest invasive option, when compared to the other two. Similarly to the gastric banding this is a reversible technique with lower number of side effects. Achieved weight reductions are comparable to those obtained with the gastric banding (20-30% of weight reduction) (5, 25, 214, 215).

The temporary nature of the gastric balloon, and the reversibility of gastric banding, come with the common problem of weight rebound, once the balloon or the banding are

removed. A multidisciplinary team with psychologists and dietitians, beside pediatricians, need to follow the patient, before and during the whole process, and particular attention after the removal of the restrictive device is needed (215).

II. Aims

Obesity is a disease characterized by a state of low grade inflammation, and increased visceral adiposity. The WAT, an active endocrine organ, secrets several molecules collectively known as adipokines. These adipokines are mainly involved in primary inflammatory processes and, therefore, in inflammatory diseases. While most of the inflammatory markers are increased in obese individuals, adiponectin, an important anti-atherogenic, anti-diabetic and anti-inflammatory molecule, is reduced.

Inflammatory mediators have been studied as potential markers of CVD. In fact, increased inflammation has been linked to an increase in other CVD risk markers, such as IR, dyslipidemia, obesity and hypertension. These known markers for CVD risk are also often present in obesity.

In pediatric obesity an increased inflammatory process with systemic changes are already present. This is particularly important in Portugal, as our country presents a high prevalence of obesity and overweight in young ages. Considering the high prevalence of CV events in Portugal, we found as important to better understand the biology of children and adolescent's obesity, the relation between obesity and the classical and new CV risk markers, as well as the clinical value of lifestyle intervention in improving the risk profile.

To achieve these aims, we designed a work involving a Portuguese obese pediatric population that consisted of three parts:

- A transversal analysis: with the objective of improving the knowledge about the risk profile and the values of classical and new CV risk factors, as well as their inter-relation, in young obese patients (Papers I to V);
- A longitudinal analysis: intending to evaluate the influence of an improvement in the BMI on CV risk markers. Two approaches to achieve weight loss were:
    - Lifestyle motivational program (Paper VI)
    - Interventional program (Paper VII)
- Analysis of the criteria used to classify obesity in Portugal (Paper VIII)

12. Methods

12.1.Subjects

Children and adolescents participated in the cohort study, after informed and written consent of their parents. Two recruitment strategies were used. Part of the population was identified from medical records, at two outpatient clinics of pediatric obesity in Porto, and invited to participate. A second group of children was involved in a school-based physical exercise (PE) promotion program, carried out in 5 primary and 2 middle and high public schools from Porto suburban setting.

The longitudinal study consisted of either a lifestyle modification motivational program in the hospitals, taking advantage of routine obesity appointments with the paediatricians; or a physical exercise program of 8 months that took place in the aforementioned schools.

Only children and adolescents who completed the evaluation protocols, pre and post-program participation, and who were not participating in any other formal sports or physical exercise program were included in the longitudinal approaches. Individuals who did not complete the entire programs, were included only in the cohort study.

12.2. Exclusion criteria and ethical approval

Smokers, subjects under regular medication or with diabetes mellitus, endocrinologic disorders, hereditary, inflammatory or infectious diseases were excluded from the study.

The protocol used for all participants was approved by the Committees on Ethics of the University Hospital S. João and of the Children's Hospital Maria Pia, Central Hospital of Porto. The Regional Education Board approved the study protocol, and students, parents and school responsibles agreed to participate. The project, its benefits and risks were explained to the volunteers.

12.3. Procedures and assays

12.3.1. Anthropometric, nutritional and clinical evaluation

Height, weight and waist circumference were measured. Obesity was defined by a BMI z-score higher than +1.65 for age and gender, according to 2000 Centre for Disease Control and Prevention (CDC) growth charts (27). Children's BMI z-score was also classified according to the WHO criterion (6). Body composition was evaluated by dual-energy X-ray absorptiometry (DEXA).

The development of puberty was clinically assessed on the basis of Tanner stages.

12.3.2. Lifestyle modification motivational program

All obese individuals involved in the lifestyle modification program were motivated to change their lifestyle habits (conventional weight loss programs based on dietary counseling and encouragement to exercise). Clinical and biochemical data were obtained at the beginning, as well as at the conclusion of the one year follow-up.

12.3.3. Physical activity promotion program

The participants were involved in a physical exercise program over a period of 8 months, from October to May. Besides regular classes of PE at school 3 times a week, participants were enrolled in an extra-activity PE program twice a week, resulting in a total of 5 hours per week of moderate to vigorous PE, matching the international recommendations. The PE proposed by the program aimed to increase moderate-vigorous PE intensities. All the activities were performed in indoor schools´ sports facilities, under the supervision of Physical Education teachers, after school time. The evaluation methods and procedures were approved by the Scientific Board of the Faculty of Sports of the University of Oporto.

Clinical and biochemical data were obtained at the beginning and at the end of the exercise program.

12.4. Laboratory analysis

12.4.1. Blood sampling

Blood samples were obtained on a fasting basis and processed within 2h of collection. Blood was obtained by venipuncture in ethylenediaminetetraacetic acid (EDTA) containing tubes. Plasma and buffy coat aliquots were made and immediately stored at – 80ºC until assayed.

12.4.2. Biochemical determinations

The determination of circulating levels of glucose, insulin, TG, TC, HDLc, LDLc, apo A1, apo B, apo E, Lp(a), bilirubin and CRP were performed by using routine automated technology.

LDLc and VLDLc concentrations were calculated using Friedwald formulas (243): LDLc = TC – HDLc – (TG/5); VLDLc = TG/5. Homeostasis model assessment – insulin resistance ($HOMA_{IR}$) was used to detect the degree of insulin resistance, by using the following formula (244): insulin resistance ($HOMA_{IR}$) = (fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5).

Plasma concentration of adiponectin and adiponectin multimers were evaluated by using standard commercial enzyme-linked immunoassay (ELISA).

12.4.3. Hematological data

Total leukocyte count, red blood cell count, hematocrit, hemoglobin concentration and hematimetric indices (mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration) were measured by using an automatic blood cell counter.

Blood cell morphology and leukocyte differential count were evaluated in Wright stained blood films.

12.4.4. DNA analysis

Genomic DNA was extracted from white blood cells (buffy-coat) by proteinase K/salt precipitation method (25, 26). Genotyping was performed by polymerase chain reaction (PCR) followed by electrophoresis in polyacrylamide gel in a Tris/Borate/EDTA buffer.

In the specific case of Apo E E2/E3/E4 polymorphism we used a PCR - Restriction Fragment Length Polymorphism (PCR-RFLP) analysis, as described by Hixson and Vernier (245). The restriction enzyme used, before electrophoretic analysis, was *Hha*I.

12.5. Statistical analysis

The distributions of continuous variables were analysed using Kolmogorov-Smirnov tests to assess significant departures from normality. Normally distributed variables are presented as mean±SD. Variables non-normally distributed are presented as median (interquartile range) and were log transformed, when necessary for further analyses. Comparisons between two groups were performed using Student's unpaired t-test or Mann Whitney U-test. Multiple comparisons between groups were performed by one-way ANOVA supplemented with Tukey's HSD post hoc test. Adjustment of statistical differences for confounding factors was performed using ANCOVA. The association between categorical variables was analyzed using chi-squared test and Fisher exact test.

The strength of the association between the variables was estimated by Pearson or Spearman correlation coefficient. Multiple regression analysis was performed to evaluate the contribution of different variables to a specific output using stepwise selection, with an entry criterion of $p < 0.05$.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) (IBM, Armonk, NY, USA). Statistical significance was accepted at p less than 0.05.

13. Specific objectives

13.1. Paper I

Hypoadiponectinemia and obesity are risk factors for the development of CVD (84, 246). Low levels of adiponectin were already observed in obese children and have been associated with increased CVD risk later in life, but some results are still controversial (84, 246).

To clarify this association, suggesting a central role of adiponectin, we reviewed in pediatric patients:

- How adiponectin is affected by gender and physiological changes occurring at young ages;
- The association between circulating adiponectin levels and MS features in obese children and adolescents;
- How adiponectin levels vary following different types of interventional programs in obese pediatric patients.

The results of the review are presented in Paper I.

13.2. Paper II

Atherosclerosis is a chronic disease that begins early in life. Apolipoprotein E has an important role in the atherosclerotic process, by facilitating cholesterol efflux from foam cells and increasing the hepatic uptake of remnant lipoproteins, through the LDL receptor and the LDL-receptor related protein (186, 187). Apo E gene polymorphism originates three possible isoforms (E2, E3 and E4), which have been related to changes in lipid profile. The individuals with the E4 allele present higher TC levels than the E3 and E2 allele carriers (187).

Adiponectin, by improving hepatic insulin sensitivity, is associated with a better lipid profile (increased HDL and reduced TG) and presents positive effects on the vasculature, by preventing foam cell formation and smooth cell proliferation.

We evaluated the interaction between obesity, apo E polymorphism and adiponectin levels, as well as the impact of apo E polymorphism and adiponectin levels on the lipid profile of the obese children and adolescents.

The results of this study are presented in Paper II.

13.3. Paper III

Lp(a) results from the combination of LDL with a plasminogen like protein, (apo (a)). Increased levels of circulating Lp(a) are associated with CV risk (191). The plasmatic levels of Lp(a) are, mainly, genetically determined. A apo (a) PNR polymorphism (TTTTA)n has been reported to be associated with Lp(a) levels (193). The number of repeats vary from 5 to 12, and the smaller number of repeats are associated with increased Lp(a) circulating levels.

The aim of this study was to access the levels of Lp(a) in obese pediatric patients, and to evaluate the influence of the PNR polymorphism on Lp(a) circulating levels.

Results are presented in Paper III.

13.4. Paper IV

Bilirubin, the final product of haem catabolism, is a water insoluble molecule that circulates in plasma bound to albumin. To be excreted, bilirubin needs to be glucuronised by a microsomal enzyme, the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). A common polymorphism in the *UGT1A1* gene (*UGT1A1*28*), known to affect bilirubin levels, is a TA duplication polymorphism in the TATA box region of the gene promoter. The 6 and 7 repeats genotypes are the most relevant. The homozygous individuals carrying the A(TA)7TAA allele present higher levels of unconjugated bilirubin (170, 201). Although the toxicity of high levels of unconjugated bilirubin has been reported in some conditions, namely in newborns, a moderate bilirubinemia has been associated with beneficial effects, due to its anti-oxidant and anti-inflammatory capacities (171, 199). In fact, serum bilirubin levels have been inversely associated with MS and inflammation,

both in adults and in children. Obesity, particularly abdominal obesity, has been related to a decrease in bilirubin circulating levels (168, 171).

The objectives of this study were to evaluate the influence of *UGT1A1*28* polymorphism, hematological, biochemical and anthropometric variables on total bilirubin plasmatic levels, in Portuguese obese children and adolescents.

Data is presented in Paper IV.

13.5. Paper V

Obesity is associated with increased inflammation and reactive leukocytosis (172, 173), however, little is known about the contribution of the different circulating leukocytes to the inflammatory process in obese individuals. It has been reported that increases in BMI and WC are accompanied by an increase in neutrophils and a decrease in lymphocytes (173).

We evaluated circulating leukocytes (total and differential leukocyte count) in young obese patients, and studied the relation between the changes in the leukogram and those in anthropometric variables and inflammatory status, to clarify the role of leukocytes.

Results are presented in Paper V.

13.6. Paper VI

Obesity, including pediatric obesity, is associated with metabolic risk changes, such as dyslipidemia, IR and a pro-inflammatory state (5, 28, 37). Weight loss, conversely, lead to an improvement on those markers (181, 182, 223, 224), however different weight reduction interventional programs reported different outcomes, probably due to different design protocols, population studied and goals (218, 219).

Participants in this study were only asked and recommended to improve lifestyle habits. We evaluated the impact of a non-interventional approach in children and adolescents, on weight loss, lipid profile, glucose metabolism and inflammatory markers (CRP and adiponectin).

Data is presented in paper VI.

13.7. Paper VII

As already referred, adiponectin is an insulin-sensitizer, anti-inflammatory, anti-oxidant and anti-atherogenic adipokine that is reduced in obesity. Adiponectin circulates in three forms, HMW, MMW and LMW adiponectin (153). The HMW form seems to be the most protective, with positive effects on IR and lipid profile, while little is known about the biological functions of the other multimers. The reduction of total adiponectin in obese individuals has been associated, mainly, with the reduction of the HMW oligomer, what might contribute to the risk changes present in obesity (84, 246).

Weight loss, through diet and exercise, is said to improve total and HMW adiponectin, in children and adolescents (84, 246). Nevertheless, it seems that it is necessary a significant reduction in adiposity, with a change in body composition, towards a reduction of central adiposity, to achieve those results (42, 51, 148).

We studied the circulating levels of total adiponectin and multimers, as well as their association with CV risk markers (lipid profile, IR, inflammation) in an obese pediatric cohort. We also analyzed how a regular physical exercise program influences those variables.

Results are presented in Paper VII.

13.8. Paper VIII

As referred in the Introduction section, the most used criteria for the determination of the BMI z-score in pediatric patients are those from CDC and WHO. We used the CDC criterion as it was the recommended criterion when this study was started. Currently, the recommended criterion is the WHO's criterion.

Obesity is part of the cluster of risk factors for CVD, known as the MS. The classification of children according to their nutritional status is important. Therefore, we studied the impact of this change in the identification of children at risk for CVD and MS.

In this way, we evaluated and compared, in a Portuguese obese pediatric population, the relation between the BMI z-score, calculated by both criteria, with MS ad with its features.

Data is presented in Paper VIII.

14. Results

The results of this work are presented in the form of articles/papers, published or under consideration for publication.

14.1. Paper I

Nascimento H, Quintanilha A, Santos-Silva A, Belo L. “Adiponectin relation with inflammation and metabolic syndrome features in pediatric obese patients - impact of interventional studies”. Submitted.

Adiponectin relation with inflammation and metabolic syndrome features in pediatric obese patients - impact of interventional studies

Henrique Nascimento[1,2*], Alexandre Quintanilha[2,3], Alice Santos-Silva[1,2], Luís Belo[1,2]

[1]Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal; [2]Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, 4150-180 Porto, Portugal; [3]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4099-003 Porto, Portugal.

Abstract

Pediatric obesity is a major health problem nowadays and has grown worldwide, accompanying the trend in general obesity. Obesity in childhood is usually associated with increased risk of type 2 diabetes, metabolic syndrome (MS) and cardiovascular diseases in adulthood. Nevertheless, changes in features of metabolic syndrome are already present in young ages and tend to increase with augmenting adiposity.

Children increased body weight is associated with reduced adiponectin, an adipokine associated with beneficial effects, particularly regarding MS components (dyslipidemia, high blood pressure and insulin resistance), inflammation and oxidative stress, all common findings in obese children.

With the objective to tackle obesity many interventional studies have been done and different results were obtained. This variability probably come from the different studies designs and strategies used, including age and pubertal stage of the studied subjects, exercise practice, diet counseling, group or individual therapy…

Although the general positive effect on weight loss, inflammatory markers and MS of the different interventional studies, changes in adiponectin levels are more inconstant. In fact, improvements in adiponectin might only occur with great weight loss or intensive physical exercise, particularly in pediatric individuals experiencing puberty.

Keywords: Pediatric Obesity, Adiponectin, Metabolic Syndrome, Inflammation, Interventional Studies

1. Introduction

The increase in obesity worldwide at pediatric ages has been accompanied by the appearance of diseases that were considered exclusive of adults, namely type 2 diabetes mellitus (T2DM), dyslipidemia and hypertension. These pathologies are commonly accompanied by central obesity, representing a metabolic derangement usually referred as the metabolic syndrome (MS), a cluster of factors that are associated with increased risk of cardiovascular disease (CVD) (15, 19).

Obesity is closely associated with both hypoadiponectinemia and MS. Also, low levels of circulating adiponectin are a potential predictor of MS development and, therefore, adiponectin has been studied as a possible link between obesity and MS. Furthermore, growing evidence support a relation between obesity in childhood and low levels of adiponectin and increased CVD risk factors in adulthood (20, 21).

The objectives of this review are:

1. To describe physiological and external factors affecting adiponectin;
2. To present up-to-date information on the relation between adiponectin and features of MS in childhood and adolescence;
3. To analyze the impact of interventional studies in adiponectin levels.

2. Influence of age and gender on adiponectin levels

Gender, age and puberty play a role on the regulation of adiponectin levels.

The results of different studies on the concentration of adiponectin in pre-pubertal (PP) children vary widely (Table 1). Nevertheless, some studies unveil alterations, even in such young ages.

Table 1. Reported studies assessing the relation between total adiponectin levels and different features associated with metabolic syndrome in pre-pubertal children

Population	MS	Adiposity	IR	Dislip	BP
215 British-born of European (138; 3y; 49f) and South Asian (77; 3y; 27f) origins. UK. (24)		(-)		(-)#	
34 ob (9.4±0.4y; 11f). 20 lean cts. Spain. (18)		(-)*	(-)	(-)	
56 ob (6-18y; 29f). 29 lean cts. Mexico. (17)		(-)*			
159 boys (9y). China. (11)		(-)*	NS	NS	(-)
70 ob (8.92±1.80y; 22f). 61 lean cts. Spain. (25)		NS	(-)		
305 (5-13 y; 161f). Italy. (2)		NS			
30 ob (7.8±1.3y). 35 lean cts. Poland. (26)		NS			
61 ow and ob (7–9y 34f) 22 lean ct. Portugal. (5)	NS	NS		NS	NS

*, correlation with total and central adiposity; #, positive correlation with HDL. BP, Blood Pressure; Dislip, Dislipidemia; IR, Insulin Resistance; MS, metabolic syndrome; (-), negative association; NS, not significant.

A study in Polish children found that T, HMW and MMW adiponectin were decreased in PP OB children, when compared to control (CT), suggesting that adiponectin multimers distribution might already be altered at young ages (26). Another study in PP children showed that HMW adiponectin was lower in OB girls when compared to their lean counterparts, while no difference was observed for boys (2).

The difference in adiponectin levels according to gender is well established in adults. Women have higher adiponectin concentrations than men (29, 30), showing that sexual hormones might play an important role. Before puberty no differences were found between genders (31, 32), strengthening the hormonal influence in adiponectin levels (Figure 1). In accordance, Martos-Moreno found no difference in total and HMW adiponectin levels between PP boys and girls (25). Interestingly, in this study, PP OB children presented with increased total adiponectin levels, in spite of the decreased level of HMW isoform, when compared to CT. This finding might be related to weight gain at this age, accompanied by adipocyte hyperplasia, rather than hypertrophy, leading to an increase in adiponectin synthesis (25). In a 4 years longitudinal study in PP children (4 to 8 years) no difference in adiponectin levels was also observed between girls and boys, although it was already visible a trend toward a reduction in adiponectin with age, despite the increasing metabolic control during the studied period, as shown by gradually improving insulin sensitivity and the lipid profile (33).

Several studies have shown an increase in adiponectin levels with age in adults (43); however adiponectin levels are usually presented as negatively correlated with age at young ages (6, 29). Longitudinal studies showed that adiponectin was reduced thought-out the study period (20, 33). In agreement, adiponectin decreases with puberty for both genders and stabilizes afterwards; these changes through puberty have been associated to a rise in IR (32, 35). This effect is more marked in boys (35), presenting the post-pubertal girls higher adiponectin plasmatic concentrations than post-pubertal boys (32). In contrast, a study in Chilean children found no difference between genders, before and after puberty (6), in contrast with others reports (7, 17).

Sexual hormones are the most probable cause of adiponectin changes during puberty. The influence of sexual hormones involves peroxisome proliferator activator receptor γ (PPAR γ). PPAR γ regulates adiponectin expression *in vitro* (44), and drugs, such as thiazolidinediones, that activate PPAR γ, are known to increase adiponectin concentrations (45). Animal studies showed that estrogens activate more efficiently PPAR γ than androgens, what could explain part of the sex related differences in adiponectin values (30).

In a study involving obese adolescents, total adiponectin concentration and adiponectin-to-leptin ratio increased following a 6 months lifestyle intervention program. This increase was higher in the individuals taking a daily dose of metformin (1500 mg), a known PPAR γ stimulator (46). This result is in agreement with another study in obese and insulin resistant children and adolescents (47).

On the other hand, children with Kawasaki disease have lower values of total and HMW adiponectin, despite the increased PPAR γ gene expression in peripheral leucocytes. This fact can be due to an unbalance in the inflammatory pathways in the chronic inflammatory state present in these patients. Similar mechanisms can be present in OB individuals (48). It is important to emphasize that different pathways can influence adiponectin levels.

Figure 1 presents the reports concerning the differences in total adiponectin according to gender, separated by the pubertal status of the studied population. The lack of differences between boys and girls before puberty is almost consensual, while it is ambiguous in more mature subjects. The different findings in mixed populations, including PP and pubertal individuals, are probably due to the influence of the pubertal status in the final result, diluting putative differences.

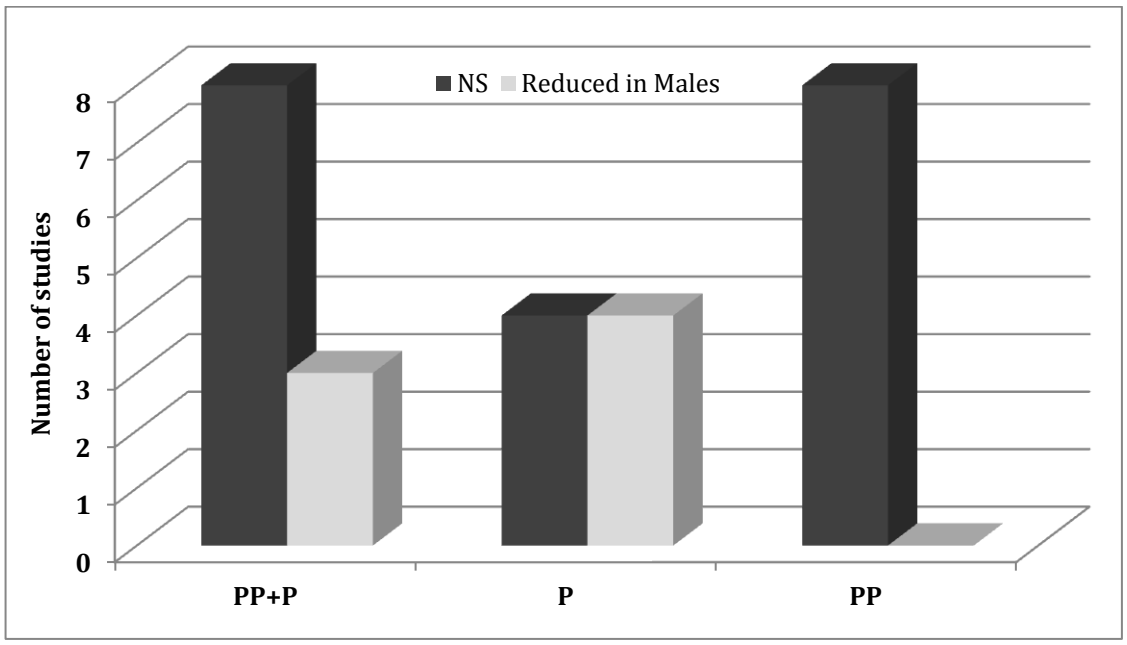


Figure 1. Number of reported studies comparing adiponectin levels between genders separated by the pubertal status of the population

Included articles: (2, 5-7, 10, 12-18, 20, 23, 24, 28, 33, 35-42, 49) . NS, no significant difference between male and females in adiponectin concentration; P, post-pubertal inidviduals; PP, pre-pubertal individuals.

The impact of puberty itself on adiponectin levels is also not very clear, with almost half of the reviewed studies finding no difference between pre and post-pubertal individuals, while the other half found a decrease in adiponectin with puberty (5-7, 13, 23, 35, 38, 49, 50).

Another aspect that should be considered, particularly in PP children, is that IR is not the only factor related to adiponectin. Murphy and colleagues, in a 3 years longitudinal study, found that adiponectin decreased with age and adiposity in PP children, despite the better metabolic control, as suggested by the increased insulin sensitivity and HDLc throughout the study (33). Metcalf also described that, despite the decrease in adiponectin in the group with increased physical activity (PA), IR did not differ in PP lean children with different levels of PA (51). Thus, in PP individuals adiponectin relation with IR and adiposity is not as clear as in older individuals.

The controversial results are probably influenced by the different study designs, populations, the use of statistical adjustment or even to other factors that influence adiponectin, e.g. adiposity and IR.

3. Adiposity and Metabolic Syndrome in obese children and adolescents

Animal and human studies showed that adiponectin levels decrease as the body weight rises. This is, somehow, a reversible mechanism, as levels of adiponectin increase after weight loss (52).

Some contradiction exists in literature regarding adiponectin levels in young obese patients. Total adiponectin levels are generally accepted to be lower in OB chidren and adolescents when compared with control (CT) and overweight (OW) subjects (22, 35). However, Medina-Bravo found no differences in adiponectin concentration between OB and lean pubertal children, while pre-pubertal (PP) OB children presented decreased levels, when compared to their lean counterparts (17) (Table 1). Roth, on the other hand, found that adiponectin was increased in OB children, although HMW adiponectin was decreased (3) (Table 2).

The MS is a cluster of factors that are associated with increased risk of CVD, such as dyslipidemia, hypertension, central obesity and IR (15, 19). These risk factors are already altered in early ages in OB individuals, and changes in adiponectin levels may underlie such risk. Adiponectin appears to be associated with several features of MS. Shaibi found adiponectin to be negatively related to waist-circumference (WC), triglycerides concentration (TG), 2h-glucose tolerance and to systolic blood pressure (SBP), and positively related to high density lipoprotein cholesterol (HDLc) concentration in OW Latin children (53). Similarly, a studies in Japanese adolescents showed that adiponectin levels decreased with increasing number of CV risk factors (15).

Adiponectin has been proposed as a marker of MS. Calcaterra found adiponectin to be lower in OB, when compared to CT, and even lower in OB adolescents presenting MS (13). In fact, in a pediatric study comparing OB children and adolescents with and without MS, only adiponectin differed between those groups, while leptin and TNF-α presented no difference (54).

Mexican children in the lower tertiles of adiponectin levels presented an increased prevalence of MS and worsening of MS markers. This association was not exclusive of obese individuals, and was present both in OB and eutrophic individuals (36). Adiponectin was also found as an independent predictor of MS in an Italian pediatric cohort (49).

An association between adiponectin levels and the probability of developing MS in the future has been raised. Lower levels of adiponectin, together with increased levels of adipocyte fatty acid binding protein (A-FABP), predicted the development of MS in Korean boys, in a 3 year prospective study (11). Similarly, lower levels of adiponectin in 16 years old females were related with the development of MS at the age of 23 years (55). Another study also showed adiponectin as a predictor of MS, in OW children with family history of type 2 diabetes (T2DM), even when adjusted for age, gender, Tanner stage, body mass index (BMI), visceral fat and insulin resistance (IR) (53). Nevertheless, a meta-analysis found no association between total adiponectin and coronary heart disease and stroke in adult individuals without clinically manifest vascular disease (56).

T2DM is commonly present in individuals with MS. In T2DM OB adolescents, adiponectin levels were found to be lower than in normal individuals, and even lower than in OB individuals without T2DM. In patients with T2DM, IR, a central feature of MS, appeared to be a main determinant of adiponectin concentrations, more than BMI itself (19).

Regarding adiponectin multimers, Araki stated that HMW adiponectin is a better metabolic marker than total adiponectin, and that HMW is negatively associated with MS (37).

Besides the amount of weight excess, in obesity it is important to consider also body fat distribution. Increased abdominal obesity was associated with lower levels of adiponectin (15, 22, 57). A study in healthy adult women showed that abdominal visceral adipose tissue (VAT), rather than abdominal subcutaneous adipose tissue (SAT), is inversely correlated with adiponectin levels (58). Also, a relation between high adiponectin and low waist-to-hip ratio, an indirect measure of central fat distribution, was described (59). However, adiponectin messenger ribonucleic acid (mRNA) was found to be more expressed in SAT than in VAT in adult humans (60).

Table 2. Reported studies assessing high (HMW), medium (MMW) and low (LMW) molecular weight adiponectin levels in obese children and adolescents. Association with some features of the metabolic syndrome

Population	Obese (vs. control)	MS	Adiposity	IR	Dyslip	BP
38 f (mean 16y) with AN. 13 lean cts. Israel. (8)	+ (HMW%)					
44 ob (9.97y (5.2–13.9), 16f). Ct: 28 (10.4y; 15.9–14.9; 13f). Japan. (61)			(-) (HMW)			
14 ob with PWS (11.35y (7.13–14.61); 9f). 14 cts. USA. (62)			(-) (HMW)	(-) (HMW)		
59 ob (10.3±0.3y; 21f). 28 lean ct. (37)	(-) (HMW, HMW%)	(-) (HMW, HMW%)	(-)* (HMW,HMW%)	(-) (HMW, HMW%)	(-) (HMW vs TG) NS (HMW vs HDLc) NS (HMW%)	
	(-) (MMW) (+) (MMW%)	NS (MMW,)				
	(+) (LMW, LMW%)	NS (LMW)				
70 ob (12.9±3.3y; 40f). 55 lean cts. Austria. (1)	(-) (HMW, HMW %)		(-) (HMW)	(-) (HMW)	(-) (HMW)	
	(+) (LMW%)					(+) (LMW vs DBP)
71 ob (12.9±3.3y; 44f). 75 lean cts. Austria. (4)	(-) (HMW, HMW%)					
	(+) (LMW%)		(+)* (LMW%)		(+) (LMW% vs HDLtg)	(+) (LMW% vs SBP)
70 pp ob (8.92±1.80y; 22f). 61 lean cts. 18 months follow up. Spain. (25)	(-)º (HMW)		(-)º (HMW)	(-)º (HMW)		
30 pp ob (7.8±1.3y). 35 lean cts. Lifestyle intervention program (3-months). Poland. (26)	(-)º (HMW, HMW%)		NSº (HMW)			
	NSº (MMW)		NSº (MMW)			
	(+)º (LMW%) NSº (LMW)		NSº (LMW)			
305 pp subjects (5-13 y; 161f; Tanner stage 1). 105 lean (46f); 60 ow (28f) and 140 ob (70f). Italy. (2)	NSº (HMW boys) (-)º (HMW girls)		(-)º* (HMW)	(-)º (HMW)	(-)º (HMW vs TG)	

(-), negative association; (+), positive association; *, correlation with total and central adiposity; º, pre-pubertal individuals. AN, nervous anorexia; ct, controls; f, female; BP, Blood Pressure; Dyslip, Dyslipidemia; IR, Insulin Resistance; MS, metabolic syndrome; NS, not significant; ob, obese; ow, overweight; y, year

The association between VAT accumulation and lower adiponectin levels was also found in children (63). Actually, adiponectin was negatively associated to visceral-to-subcutaneous fat ratio (while TNFα was positively associated), and significantly predicted that ratio (42). The association between hypoadiponectinemia and increased VAT seems to appear early in life, as Medina-Bravo found it to be particularly strong in PP children (17). Central fat distribution is also associated with a worse metabolic control. In fact, accompanying the increased abdominal obesity and reduced adiponectin, adolescents also presented increased TG levels and decreased levels of HDLc (15).

Inappropriate/pathological eating habits do not seem to interfere in the relation between adiponectin and adiposity. In a group of Ballet dancers presenting decreased fat mass, due to an energy intake inappropriate to the exercise intensity, adiponectin was found to be increased (50). Furthermore, in extremely malnourished anorexic female adolescents total adiponectin and HMW adiponectin were increased (8). Likewise, adiponectin did not differ between OB adolescents who had binge eating or nervous bulimia, and those who had not such disturbances (64).

The importance of ethnicity should also be considered, as some studies suggest that adiponectin levels might differ between ethnicities. Actually, in a study involving Indian teenagers, adiponectin did not correlate with any marker of MS (14). Furthermore, adiponectin levels were not related to family history of diabetes or levels of physical activity. These findings are somehow in agreement with results in Indian adult populations (14), but greatly different from the results found in Hispanic children, to whom adiponectin was a predictor of MS (53).

The negative relation of adiponectin with MS appears to be accepted, especially in pubertal subjects. In figure 2 is shown that the proportion of reports corroborating these associations is higher than those who do not. However, these relations are not so clear in PP individual (table1). Nevertheless, it is important to highlight that the diagnosis of MS in young children (under 10 years) is not recommended by the International Diabetes Federation. Reasons include the lack of age and gender adjusted cut-offs for MS components and the ambiguous causality evidence in such young ages relating MS and increased risk of CVD later in life (65).

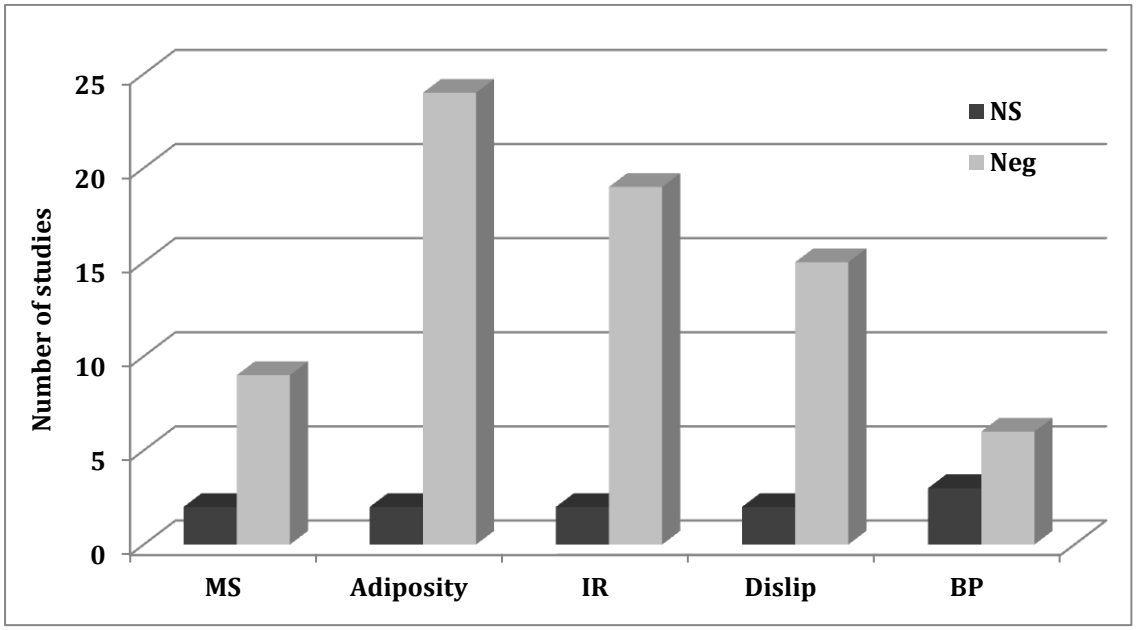


Figure 2. Number of reported studies in mixed (pre and post-pubertal) pediatric populations assessing the association between total adiponectin levels with metabolic syndrome and with its common features

Included articles: (1-3, 5-7, 10-18, 22-27, 29, 35-37, 39, 42, 49, 53-55, 61, 63, 64, 66-70).BP, Blood Pressure; Dislip, Dilipidemia; IR, Insulin Resistance; MS, metabolic syndrome; Neg, negative association; NS, not significant.

4. Adipokines and other inflammatory mediators

Different mediators are known to influence adiponectin levels such as other adipokines, hormones (e.g. insulin, insulin-like growth factor 1 (IGF-1), growth hormone (GH)), or other type of molecules, produced within the adipose tissue or in other different organs and tissues.

Obesity in pediatric ages, as in adults, has been considered a low grade inflammation state, which is usually accompanied by a rise in pro-inflammatory mediators, and a decrease in anti-inflammatory molecules, especially adiponectin. In accordance, many studies have shown that adiponectin is, in pediatrics, negatively associated with pro-inflammatory mediators, such as interleukin (IL) IL-1β, IL-6, and IL-8 and TNF–α (3). In a large cross-sectional study, Zhang found that adiponectin was reduced in OB children and adolescents, together with increased levels of pro-inflammatory leptin, resistin and complement C3; a particular worse risk profile for the “mixed” type of obesity was observed, when compared to obesity with central and peripheral adipose tissue distribution types (23).

Inflammatory status appear to vary within obesity grades, as severely OB children and adolescents, when compared with individuals presenting a moderate type of obesity, have increased MS features and inflammatory markers, including reduced adiponectin and

increased C-reactive protein (CRP), leptin, IL-6 and resistin (68). However, other studies did not find differences in adiponectin levels when comparing different degrees of obesity, although a positive correlation between BMI and CRP was present (29).

TNF-α is a particularly important mediator, especially regarding IR. Adiponectin reduces TNF-α secretion by macrophages, marking its anti-inflammatory action (52). On the other hand, TNF-α, and well as IL-6 and CRP, reduce adiponectin mRNA expression, which may explain, at least in part, the relations of these cytokines to IR (52, 71). Actually, TNF-α is known to inhibit insulin signaling (72), while adiponectin is an insulin sensitivity agent.

Roth found that TNF-α was negatively correlated to adiponectin levels in children (3). In agreement, in an OB and OW adolescent population with IR, one-month supplementation with n3 fatty acids led to the increase in adiponectin, accompanied by a reduction of TNF-α and leptin, and an improvement in IR (73).

The relation between adiponectin and TNF-α in PP children are less clear. A study in PP children did not find any correlation between adiponectin and TNF-α, in both OB and CT (18). Also, in a Romanian OB population, adiponectin did not correlate with TNF-α (7). Moreover, in OB and OW adolescents, presenting a decrease in TNF-α, IL-10 and IL-8 after weight and fat loss, following a physical exercise program, no changes in adiponectin were present (74).

Leptin, a product of the Ob gene, is one of the adipokines more closely associated to adiposity. Leptin is known to correlate inversely with adiponectin and positively with BMI. Actually, children whose BMI increased the most in a 3 years longitudinal study, also presented lower values of adiponectin and increased levels of leptin (20). Likewise, adiponectin levels were negatively associated with leptin in female adolescents (8). In OB Romanian children a worse adipokine profile was observed, when compared to lean CT, with increased IL-6, leptin and resistin and decreased adiponectin concentration. In this study, adiponectin was only correlated, negatively, with leptin. Nevertheless, this relation with leptin disappeared when corrected for WC and BMI z-score. Actually, WC has been proposed as the good predictor of adiponectin levels, highlighting the influence of adiposity distribution in adipokines (7).

In contrast, Papoutsakis did not find any correlation between adiponectin and leptin, despite the association of both with IR and adiposity (leptin with an increase, and adiponectin with a decrease, of those variables) (27). Similar results were found in either post-puberty (69) and PP (5) OW and OB children. Other studies also did not find any association between total adiponectin and leptin. Nonetheless, HMW adiponectin correlated negatively with leptin, while LMW and LMW % adiponectin correlated positively with leptin (1, 4).

CRP is a classic marker of inflammation and a risk marker for CVD in adults. It is usually increased and associated to the low grade inflammatory state present in childhood obesity (66). The correlation between adiponectin and CRP is not clear in pediatric OB individuals, although there are strong evidence that both are related to obesity and IR (29, 66).

A study in OW and OB PP Portuguese children found a negative correlation between adiponectin and CRP (5). In Chilean children, however, adiponectin was only negatively correlated to CRP in post-pubertal children, with no association in PP individuals (6). Moreover, in another Portuguese study, involving PP and pubertal individuals, no correlation was observed (29, 66). In agreement, a study in obese children and adolescents showed that, lower adiponectin levels was correlated with increased IR, dyslipidemia, adiposity, PAI-I, fibrinogen and uric acid, however, no correlation was found between adiponectin and CRP or IL-18 (49). Mangge also did not find any association between either CRP and total adiponectin, or between CRP and HMW and LMW adiponectin (1, 4).

IL-6, a pro-inflammatory mediator, is usually increased in obesity. In lean individuals IL-6 controls energy intake but, in obese individuals, a state of resistance to IL-6 actions appears to develop. IL-6 stimulates CRP secretion in the liver. The IL-6 synthetized by the abdominal VAT has a particular importance in this mechanism, working through the portal system (3). In a similar way to what is described for adults, adiponectin was found negatively associated with IL-6 in children (3). However, there are still contradictory results (7).

Resistin influence in IR has been studied in animal models, showing a positive association between them. In human studies, especially in children, the results are not conclusive. No association between adiponectin and resistin in children (1, 7), or in PP obese patients, were found (2). Also, no link between resistin and HMW and LMW adiponectin was reported (1).

Part of the anti-inflammatory effect of adiponectin is linked to IL-10, an anti-inflammatory and anti-atherogenic interleukin, as adiponectin induces the synthesis of IL-10 by macrophages. Considering that in obesity there is a reduction in adiponectin and an increase in the infiltration of macrophages in the adipose tissue, it would be expected that the hypoadiponectinemia would diminish circulating IL-10. However, it has been reported an increase in serum IL-10 in obese adults, probably as a mechanism to reduce the increased inflammation in those individuals. Another explanation is that a state of resistance to IL-10 actions may develop in OB individuals, as occurs for resistance to IL-6, leptin and insulin) (13, 75). Contrarily to adults (75), no correlation was observed between adiponectin and IL-10, in OB children with or without MS (13).

5. Other factors affecting adiponectin

Exogenous factors, as diet and drugs, can also interfere with adiponectin concentrations. It has been negatively correlated with dietary fat intake in OB adolescent; an increase in adiponectin has been found following an intervention program to reduce that macronutrient intake (12). An increased fat intake seems to deregulate the microbiological intestinal system, increasing the passage of endotoxin from intestinal lumen into the blood stream. This increase in circulating endotoxin would lead to activation of the inflammatory mechanisms. In agreement with this, adiponectin levels are inversely related with circulating endotoxin levels in OB adolescents. (12).

Growth hormone (GH), is relevant due to its global influence on metabolism, particularly during childhood and adolescence. Growth hormone diminishes adiponectin secretion by adipocytes and increases visceral fat deposition, a fat depot associated to hypoadiponectinemia. In agreement, in diseases presenting lower values, or a reduced activity, of GH, like Prader Willi (PWS) and Laron syndromes, respectively, adiponectin is, in fact, increased. Besides, the increased adiponectin, and probably because of it (at least in part), these patients also present a reduction in IR, conferring these changes a "protection" for CVD complications, when compared to BMI matched individuals (62, 76).

Insulin-like Growth Factor 1 (IGF-1) has been reported to decrease adiponectin expression in the adipose tissue (77). As adiponectin presents an insulin sensitizer action, this effect of IGF-1 could represent a negative feedback inhibition. However, there are still conflicting data in children (76).

6. Adiponectin isoforms in pediatric obese patients

Adiponectin is important as a mediator of other inflammatory factors and as an anti-atherogenic, anti-dyslipidemic and insulin sensitizer factor. Adiponectin has a particular difference to most of the other mediators: each one of its 3 circulating isoforms appears to be linked with different, sometimes opposite, actions in the organism (1, 4). Thus, studies should focus also on its multimers, instead of looking only to total adiponectin concentration.

Total and HMW adiponectin concentration were reported to be lower in OB children and adolescents, when compared to lean CT (1, 26). Besides, the reduced multimer absolute concentration, the relative percentages of the isoforms were also altered: HMW% adiponectin was decreased, while LMW% adiponectin was increased. Furthermore LMW% adiponectin, a multimer that has not been much studied, was positively correlated with adiposity, particularly with SAT. Moreover, LMW % adiponectin was also positively correlated with HOMA, leptin, oxLDL, and liver transaminases. Nevertheless, no correlation was found with CRP (4). These deleterious associations of LMW adiponectin

are opposite to those reported for HMW adiponectin. Actually, HMW adiponectin multimer is usually linked to an improvement in IR and lipid profile in children (1), and it has been negatively associated with MS (37). The negative association of HMW adiponectin with IR and adiposity appear to be present even in PP individuals (25). In another study, in PP children, HMW adiponectin was negatively associated with BMI z-score, IR, TG, leptin, and sICAM, however, after adjustment for age and sex, only BMI z-score and TG kept their association with HMW adiponectin (2). In this same study, there was no correlation between HMW adiponectin and pro-inflammatory mediators, such as resistin, IL-8 and IL-18 (2). This lack of correlation with inflammatory mediators suggests that the influence of adiponectin multimers on metabolism are more linked with IR and with lipid profile.

As already referred, in children with PWS HMW adiponectin present a higher concentration, as compared with age and BMI matched controls, and present a negative association with IR. In fact, the high circulating HMW adiponectin in PWS probably underlies the protection against dyslipidemia and IR, despite the severe obesity observed in those patients (62).

The figure 3 resumes the reviewed association between total, HMW and LMW adiponectin with inflammatory mediators, hormones, and other factors.

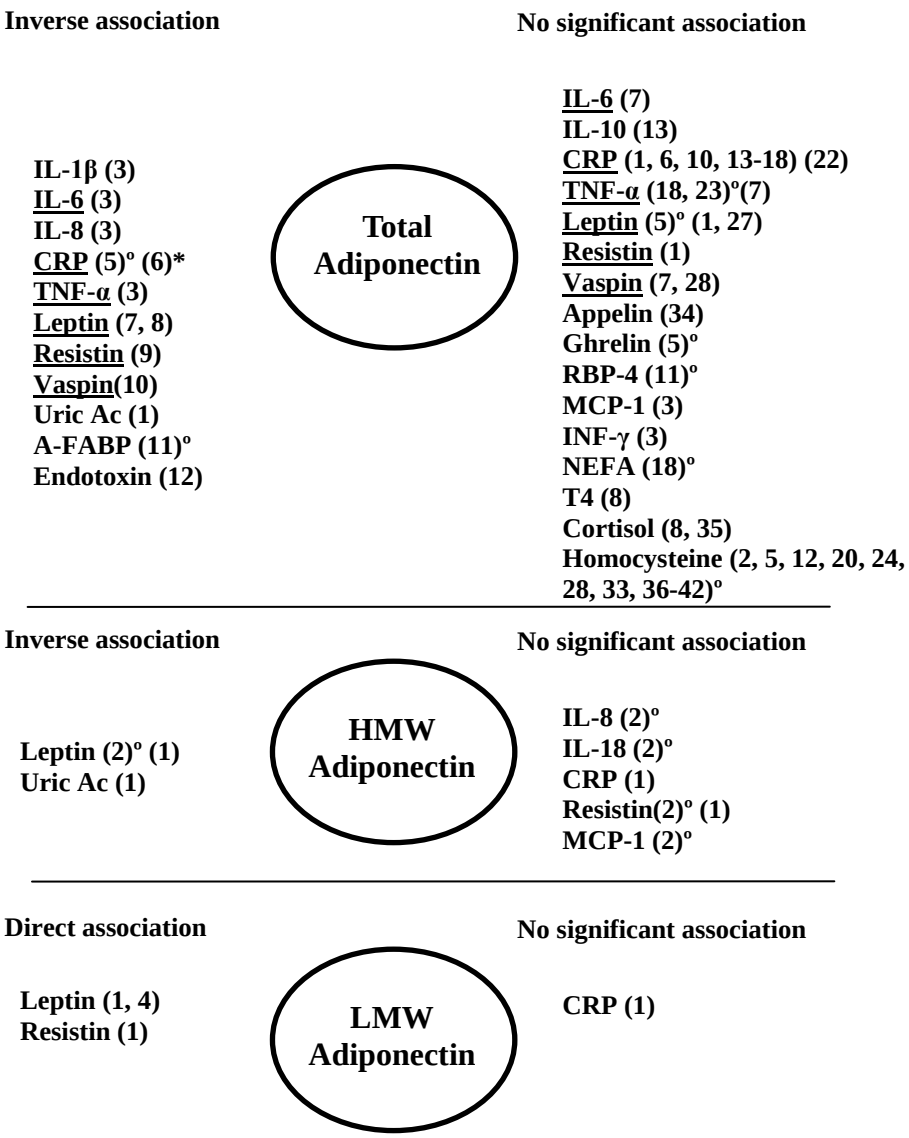


Figure 3. Studies reporting associations between inflammatory markers and total, high molecular weight (HMW), and low molecular weight (LMW) adiponectin

Underlined are the inflammatory factors that appear in both columns (ambiguous results). º, pre-pubertal; *, post-pubertal; Ac, acid; A-FABP, adipocyte fat acid binding protein; CRP, C-reactive protein; IL, interleukin; INF, interferon; MCP, monocyte chimeo attractant protein; NEFA, non-esterified fatty acid.

7. Lipid profile

The effect of adiponectin on lipid metabolism has been studied by many research groups. Adiponectin is known to lower the synthesis of free fatty acids and to stimulate β-oxidation (78). Furthermore, HMW adiponectin seems to lower apo B and apo E release from the liver, decreasing, in this way, the release of TG rich lipoproteins [(e.g. very low density lipoprotein (VLDL)] and increasing high density lipoprotein cholesterol (HDLc) levels (79).

The positive link between adiponectin and an improved lipid profile has been confirmed by many studies (Figure 2). The most consensual effects for adiponectin are a positive association with HDLc and a negative correlation with TG (6, 15, 27, 66). These associations are present even in PP ages (18, 24). A recent study from our group, involving obese children and adolescents, also showed that adiponectin may modulate the effect of less favourable apo E genotype on blood lipids, namely on total cholesterol/HDLc and apo B/apo A1 ratios. Higher levels of adiponectin were associated with an improvement in those atherogenic ratios (29). Furthermore, adiponectin in obese 16 years old girls were positively correlated with HDLc both at baseline and after 7 years of a follow-up study, raising the possibility of a long term modulation of the metabolism. On the other hand, in the same work, no correlation was found between adiponectin and TG or LDL (55). Snehalatha also found a positive association between adiponectin and HDLc, in boys (14). Conversely, other studies did not found any association between adiponectin and the lipid profile, neither in OW and OB Portuguese children (5), nor in PP Chinese children (11).

Regarding adiponectin multimers, HMW has been linked with improvements in the lipid profile (1), even in PP individuals (2). LMW, oppositely, seems to have a deleterious impact, presenting a positive association with TG (4).

In the longitudinal studies reviewed, no association between changes in lipid profile and variations in adiponectin levels were reported.

8. Blood Vessels and Blood Pressure

The positive effects of adiponectin in the vasculature appear to be independent from its relation to IR (30). Adiponectin improves vessels status and reduces atherogenesis by inducing nitrous oxide production by endothelial cell, which induces vasodilatation and reduces platelet aggregation. It also reduces vascular smooth muscle cells proliferation (80, 81) and prevents macrophage activation and, therefore, the development of foam cells (82). Through these effects it prevents the development of atherosclerosis while still in early stages. An inverse correlation between the levels of adiponectin and the internal media thickness (IMT) of carotid arteries (cIMT) (83, 84), and a positive association between adiponectin and brachial artery distensibility (30), has been observed in pediatric

cohorts. On the other hand, Arnaiz did not find any correlation between adiponectin with cIMT or flux mediated vessel distensibility in Chilean children (including OB, OW and CT) (6).

The development of oxidative stress is a predictive risk factor for CVD events (e.g. coronary artery disease, myocardial infarction) (85). Adiponectin presents anti-oxidant activity reducing the production of reactive oxygen species (ROS) that are deleterious to endothelial cells (86). Increased markers of oxidative stress in OB children have been described. These markers are usually increased in the presence of features of MS and lower values of adiponectin (87). OB children also present lower anti-oxidant defenses(88), leading to increasing HDL and LDL oxidation (89). Increased oxLDL is linked to the formation of foam cells, a key step in atherosclerosis initiation. The PON1, an anti-oxidant enzyme, usually presents a reduced enzymatic activity alongside with lower adiponectin values in OB. In fact, adiponectin concentration directly and positively correlates with PON1 arylesterase activity in OB children, and was found to be its main determinant (89).

Decreased levels of adiponectin are also related to other oxidative stress markers as adipocyte fatty acid binding protein (A-FABP) and lipocalin-2 (90). A negative association between adiponectin and A-FABP was also found in children (11). Moreover, higher concentrations of A-FABP and lower levels of adiponectin were described as predictors of MS development in a 3 year longitudinal study (11).

Opposite effects of HMW and LMW adiponectin multimers in oxidative stress have been reported. In Japanese OB children, HMW adiponectin was the only adiponectin multimer that was negatively associated with isoprostane, an oxidative stress marker, which is increased in OB individuals (61). LMW% adiponectin, on contrary, was positively associated with increased oxidative stress (as measured by oxLDL), cIMT, SBP and oxLDL (1).

Conflicting results exist in literature regarding the association of adiponectin levels with blood pressure (BP) (Figure 2). Some studies point to a negative association between adiponectin and both SBP and diastolic blood pressure (DBP) (6, 7, 36), or with SBP alone (22, 49, 53), having these relations been observed even in PP children (11). Conversely, other works describe no associations between adiponectin and BP (5, 14, 27).

Regarding adiponectin multimers, LMW was associated with increased SBP and DBP in Austrian children (1, 4) (Table 1).

On the subject of longitudinal studies, no associations were found between fluctuations of adiponectin and changes in BP. Although Choi found in PP children, a positive relation between baseline adiponectin and BP changes during a three years follow-up, the

differences were lost when adjusted to Tanner stage (11) (Table 1). Morrison also did not find any association between adiponectin concentration at 16 years and the values of BP 7 years later (55).

9. Insulin Resistance

IR is known as the unifying factor of MS, underlying and linking all the other features (dyslipidemia, obesity and hypertension). IR is also closely associated with inflammation.

Apart from PP individuals (Table 1), the relation between adiponectin, obesity and IR appears to be clear (Figure 2). Total adiponectin is negatively correlated with IR, and increases following a lifestyle intervention, leading to a decrease in IR (70).

Some studied showed that total adiponectin, and HMW in particular, have a closer relation with IR markers (as HOMA) than with BMI or body fat, which was particularly noticeable in OB children (1), especially in PP subjects (3). On contrary, a positive association between LMW % adiponectin and HOMA was reported (1) (Table 2). In fact, a study by Martos-Moreno did not find any correlation between total and HMW adiponectin with adiposity markers in OB PP children, although a negative association was already present between total adiponectin and HOMA (25).

Nevertheless, the association of adiponectin with IR might not be present from birth. Indeed insulin levels increase until one year of age, while a reduction in adiponectin occurs being present no association with HOMA (91). Actually, the inverse association between total and HMW adiponectin with IR seems to appear only after 2 to 6 years of age (18, 92). In accordance, a study in Portuguese children showed that adiponectin levels did not differ between PP OW and CT, despite the increased dyslipidemia and IR in OW subjects (5). Another study from our group found similar results in OB and CT PP individuals (H Nascimento *et* al - unpublished data). An Italian PP cohort study, also did not find any association between HMW adiponectin and IR, after adjustment for age and BMI, despite the increased IR present in the OB individuals, and that lean girls presented increased levels of HMW adiponectin when compared to OW and OB girls (2).

HMW adiponectin has been proposed to be more closely related to IR than total adiponectin. Children and adults with PWS (genetic syndrome characterized by severe obesity and cognitive deficiency) are known to present decreased insulin and HOMA, when compared to age and BMI matched OB CT. These findings might be due to the increase in total and HMW % adiponectin observed in these individuals (62). A study of girls with Laron syndrome (a syndrome characterized by dwarfisms and severe obesity) showed similar observations. A common feature to both of these syndromes is the lower concentration of GH (in PDW), or its absence, or loss of action (in Laron syndrome). The diminished level or activity of GH in these two syndromes might explain their paradoxical

hyperadiponectinemia, increased insulin sensitivity and lower percentage of visceral fat, when compared to OB matched CT (76).

Adiponectin and leptin are closely related, and oppositely associated to adiposity and IR. In obese individuals an increased inflammatory cluster profile was present associating increased leptin and leukocyte activation with IR, on the other hand, lean individuals presented a profile characterized by increased adiponectin, lower leukocyte activation and increased insulin sensitivity (93). Adiponectin-to-leptin ratio has appeared as a relevant marker of IR presenting a strong negative association with HOMA and insulin. In fact, it might be a better marker than HOMA to predict IR, as was shown in OB adolescent cohorts (9, 94). In opposition, a cross-sectional study in Chinese children found that leptin was the best predictor of IR (grouped both by Tanner stage and weight), while adiponectin only predicted IR in OB and OW boys and OW girls, and had no influence when regarding lean individuals (35).

Adiponectin levels in childhood and adolescence can also be used as a marker of risk for developing MS or CVD later in life. High adiponectin levels in girls at the age of 16 were a negative predictor of HOMA and insulin levels at 23 years. This relation was particularly notable regarding OB girls that had paradoxically high adiponectin (higher than the median for lean CT of the same age) (55).

There is some debate regarding if decreased adiponectin is a cause or a consequence of IR. Indeed, lower values of adiponectin might be the cause, not the consequence, of IR, at least in some ethnic groups, as in South Asians (sub continental India), who showed reduced adiponectin levels in infants, despite the normal levels of insulin or lipid profile (24). Lower adiponectin values are also present in both adult (52) and adolescent black individuals (55), despite the better lipid profile. Associated with the lower adiponectin levels, black adolescents also presented an increased IR (55). It must be highlighted, once again, that the link between adiponectin and other metabolic parameters should always be considered regarding the population background.

10. Interventional studies and adiponectin levels

Longitudinal studies are crucial as they allow us to obtain causal associations, in this case between adiponectin and other factors. Table 3 presents a revision of interventional studies involving physical exercise and/or nutritional counseling programs.

Improvements in adiponectin levels may be obtained by energy restriction and exercise, and, the combination of both approaches appears to have a greater impact, as was observed in OB adolescent boys (9). The magnitude of the weight loss does not seems to be a confounding factor as, in this study, the group practicing only exercise had no significant weight loss, but the adiponectin levels increased even so. Thus, changes in

body composition might be the real factor behind adiponectin improvement, being weight loss and exercise two complementary ways to achieve these changes (9). This is in agreement with a lifestyle interventional study in children, in which adiponectin variation was negatively associated with changes in total percentage of fat and this change was present even in the absence of an effective weight loss (70). Lazzer and colleagues, likewise, found that a weight reduction program for OB adolescents, involving physical exercise and diet restriction, lead to an increase in adiponectin together with an increase in lipid oxidation rates. The adiponectin levels were kept in the individuals that maintained weight after the program, and reduced in the others (41).

Interestingly, in a study involving OB females, with and without eating disorders (binge eating and nervous bulimia), adiponectin did not differ between those two groups, before and after the exercise and diet intervention program. The presence of eating disorders does not seem to be a bias regarding the response of metabolism to interventional programs, as both groups presented an increase in adiponectin following the program but, in spite of that, only the group without eating disorders presented a negative correlation between adiponectin variation and the changes in total percentage of fat and HOMA (regardless of no correlation between adiponectin and HOMA, at baseline) (64). This increase in adiponectin, regardless of changes in body fat, highlights that other factors, as inflammatory mediators and exercise *per se*, must influence adiponectin changes.

Some studies in pediatric ages support the fact that a considerable amount of adiposity must be lost before any improvement in adiponectin concentrations occurs (22). In fact, several studies showed that just a reduction of BMI z-score higher than 0.5 lead to changes in IR and adiponectin in children (3, 95, 96). In an interventional study, involving increased exercise and nutritional counseling (Obeldicks program), only children who reduced their BMI z-score in more than 0.5 presented increased total adiponectin and reduced HOMA (3). Strengthening the importance of weight loss magnitude, Lira found that OB adolescents following exercise and dietary intervention, who reduced more than 5 % of their fat mass increased adiponectin. Besides the fat mass reduction, the increase in adiponectin might be influenced by the fact that all subjects in Lira's study were already in the final stage of pubertal development and, therefore, no longer under the age and puberty associated reduction in adiponectin (12).

A study by our group reported that reductions in BMI z-score, as small as 0.3, even without an interventional program, are already associated with an improvement in HOMA and with the maintenance of adiponectin levels, while a reduction of adiponectin was found in individuals who did not achieve that reduction value (66).

Table 3. Interventional studies evaluating the effect on adiponectin levels, and the relation between changes in adiponectin levels and the variation of adiposity and IR

Population	Adiposity	IR	Effect
ENCP. 26 severely ob (12–16y; 14f). 9m program. France. (41)	(-)		(+) Program
ENCP. 37 ob (10y (8-12); 24f). 16 lean ct. 1y program. Germany. (97)			(+) Program (D-BMIzsc>0.5)
ENCP. 21 ob boys (13.1±0.7y). 2m program (exercise and energy restriction, separately or combined). Tunisia. (9)	(-)	(-)	(+) Program (more in energy restriction and exercise combined)
ENCP. 50 ow and ob (12.0±0.9 y; 25f). 7d program. Korea. (28)			(+) Program
ENCP. 115 ob (10.7±0.29; 57f). 30 lean cts. 62 ob (11±0.5y; 34f) participated in 1y program. Germany. (3)	(-)	(-)	(+) Program (D-BMIzsc>0.5)
ENCP. 30 ob (16.71±1.47y; 10f). 1y program of aerobic only (AT) or aerobic and endurance training combined (AET). Brazil. (98)			(+) Program (AET) NS (AT)
ENCP. 25 ow and ob (13–16 y; 12f; 18 ob). 13m program. Spain. (74)	NS		NS Program
ENCP. 80 ob (10.9±0.3; 42f). 40 lean ct. 1y program. Germany. (34)	(-)		(+) Program
ENCP. 18 ob (16.6±1.67y; 11f). 1y program. Brazil. (12, 42)	(-)		(+) Program
ENCP. 28 ob (15-19y, 21f) with (binge eating or bulimia nervous). 55 ob ct. 1y intervention. Brazil. (64)	(-)	(-)	(+) Program
ENCP. 402 ow and ob (13.9±2.3; 238f). 4-6w program. Germany. (69)	(+) (BMIbs)		(+) Program
MENCP. 104 ow or ob (10.7±3.2y; 53f). 55 lean ct. 1y lifestyle intervention (48 participated). (70)	(-) (D-%fat mass) NS (D-weight)		(+) Program
MENCP. 25 ob (10–16y; HOMA >3.0). 6m lifestyle intervention. Two groups (receiving, or not, a daily dose of 1500mg of metformin). Canada. (46)	(-)		(+) Program
MENCP. 60 ob (11.0±2.4, 29f). 1y lifestyle intervention. Portugal. (66)	(-)		(+) Program
MENCP. 70 pp ob (8.92±1.80y; 22f). 61 lean cts. 18 ms follow up. Spain. (25)	(-) (T, HMW)		(+) Program
MENCP. 30 pp ob (7.8±1.3y). 35 lean ct. 3m lifestyle intervention. Poland. (26)			(+) Program (T, HMW, HMW% and MMW) (-) Program (LMW%)
NT. 38f (mean 16y) with AN. 13 lean cts. Israel. (8)			(-) Program (T, HMW%)
MENCP. 40 obese individuals (13.3±2.0y; 22f), 39 OB CT and 34 lean CT without lifestyle intervention. 3 m, 1 and 2 y follow-up. Netherlands. (22)			NS Program
NCP. 61 pp ow and ob (7–9y 34f). 22 lean ct. 1y individual or group-based treatment. Portugal. (5)		NSº	NS Program
PI. 151 insulin resistant ob (13.±2.3y; 102f). Double blind placebo controlled study (metformin). No lifestyle intervention. UK. (47)			(+) Program

ENCP, Exercise and Nutritional Counseling Program; MENCP, Motivation to Exercise and Nutritional Counseling Program; NCP, Nutritional Counseling Program; NT, Nutritional therapy; PI, Pharmacological Intervention. HMW, High Molecular Weight adiponectin; LMW, Low Molecular Weight adiponectin. +, positive increase with intervention program; º; pre-pubertal individuals; AN, nervous anorexia; bs, baseline; ct, control; BMI, body mass index; f, female; HOMA, Homeostasis Model Assesment insulin resistance; IR, insulin resistance; ob, obese; overweight, ow; PP, pre-puberty; T, total adiponectin; y, year.

Regarding the influence of lifestyle interventions on multimeric distribution of adiponectin, a study in PP children found that total and HMW adiponectin were lower in OB children when compared to lean CT, with no differences for MMW and LMW adiponectin. The HMW % adiponectin was lower and LMW % adiponectin was higher in OB children, suggesting that adiponectin multimerization, that take place within the adipocyte, is probably turned towards the LMW form, with a decrease in the formation of MMW and HMW adiponectin (26). Although no correlation was found between adiponectin, or its multimeric forms, with BMI for the OB patients, the lifestyle intervention, induced an average reduction of 10 % in the BMI, increased total, HMW, MMW adiponectin, as well as HMW % adiponectin, while a reduction in LMW % adiponectin was observed (26).

Controversial results are found not only in adolescents but also (and particularly) in children (Table 3). Adiponectin did not improve after a lifestyle intervention with nutritional counseling, but no exercise program, despite BMI z-score reduction and lipid profile improvement being achieved (however HOMA also did not improve) (5). As well, Romeo and colleagues did not found adiponectin increase, even after BMI improvement and fat reduction, following a diet and exercise program (EVASYON) (74). It is possible that the improvement in total adiponectin, especially in children, requires a greater reduction of adiposity than in adults, as there is an age related trend towards adiponectin reduction (mainly in peri-pubertal period), or a shorter duration intervention with high intensity, leading do increase weight loss over a short period (66). Indeed, in an intensive intervention involving 7-day exercise and nutrition program, Lee and colleagues managed to significantly increase adiponectin, and decrease BMI, in a cohort of OB children (28). In another short term intervention (4-6weeks) adiponectin increased following BMI weight loss (69).

Different types of training may have different impact in adiponectin (74, 98). A study by Mello on OB adolescents suggested that aerobic combined with endurance training (AET) increases adiponectin and improves IR, both in short (6 months) and long term (1 year), while aerobic training (AT) alone does not. Both AET and AT were accompanied by an improvement in some of the MS features (BMI, visceral fat, dyslipidemia) however, the global improvement was higher with AET. It was hypothesized that the greater improvement in dyslipidemia observed in the AET group was caused by the increased adiponectin found in these individuals, but absent in the AT group (98).

Ballet, for instance, is characterized by intense physical activity and increased lean mass. Adolescent female ballet dancers present increased adiponectin levels when compared to non-dancers lean CT, and an increase in adiponectin through puberty, which was not observed in the CT group. This increase in adiponectin was present despite an increase in BMI z-score and a trend towards a central distribution of fat mass (50).

In a non-intervention 3 years longitudinal study in PP lean children (5-8 years old) it was found that increased PA was linked to a decrease in adiponectin, despite no changes in IR, leptin and CRP were observed. The relation between adiponectin and IR varied among children with different PA patterns and a stronger negative association was found in the less active individuals. The authors postulated that in response to increased PA the adiponectin, an insulin sensitizer agent, might decrease due to the decrease in IR induced by exercise, by a kind of negative feedback inhibition (51).

These changes in lean PP children might be also triggered in response to interventional programs in OB children, particularly in those programs involving exercise. As the OB children engage in a more regular PA, after an initial increase in adiponectin, as reported by Lee (28), the IR reduction could "signal" to reduced adiponectin synthesis. This increase of adiponectin in response to IR can be seen in T1DM children (38, 99).

Regarding pharmacological intervention, metformin appear to increase adiponectin, particularly adiponectin-to-leptin ratio, in obese pediatric patients both after 3 and 6 months of treatment, accompanying reduction in BMI z-score, without noticeable side effects. Still, long-term maintenance of metformin effect on adiponectin levels in children and adolescents is still unknown, particularly in children, an so its use should be considered carefully (46, 47).

In synthesis, the negative association between adiponectin, adiposity and IR appears to be well established. Furthermore, the impact of the majority of the interventional studies on adiponectin levels is positive and pharmacologic strategies could be good adjuvants of the treatment. Nevertheless, there are factors that might influence that impact, such as the study design, population studied and the type and intensity of exercise practiced. The amount of lost adiposity, and the magnitude of the changes in body composition, appears to be a particular importance.

11. Conclusions and Final Remarks

The relation between adiponectin and MS in children and adolescents has been studied for some time.

Obesity, and particularly, abdominal obesity, a component of MS, is closely associated with lower adiponectin values in pediatric ages. Adiponectin acts as an insulin sensitizer, decreasing as the IR rises. Regarding the lipid profile, an increase in circulating adiponectin is particularly associated with increased HDLc and lower levels of TG, with limited effect on LDLc. Adiponectin also acts in the vasculature and prevents atherosclerosis through multiple mechanisms, as reduced foam cell formation, inhibition of the synthesis of inflammatory mediators by macrophages,... Adiponectin is also associated with smaller IMT of the arteries and increased vessel elasticity, in children. Likewise, increased adiponectin is linked to reduced, healthier, BP.

Considering the different adiponectin multimers, HMW adiponectin appears to be, associated with a better metabolic control, mainly by improving IR, while the LMW multimer presents opposite effects. It is important to consider not only the absolute concentrations of these multimers, but also their relative percentage, as potential markers of CVD risk.

Combining the specific effects of adiponectin and its association with the lipid profile, BP, IR and obesity, adiponectin was shown to predict the future development and help diagnosis MS in pediatric individuals.

HMW adiponectin might be a better predictor of MS than total adiponectin. An increase of total and HMW adiponectin seems to occur following interventional programs, particularly those programs involving exercise and diet interventions. Changes in body composition, with reduction of total and central body fat, appear to be the key to the therapeutic success, more than absolute changes in body weight.

The positive effects of adiponectin on general metabolism are not so clear in PP individuals, particularly its relation with IR. No significant differences are usually observed between genders in PP individuals. The OB PP children present a trend towards lower adiponectin concentration than pubertal individuals. After puberty, and possibly through a mechanism involving sexual hormones, adiponectin lowers with age in both genders but more markedly in boys.

The controversial of results obtained by different groups are, probably, related to the study design, subjects studied and population background. Different outcomes between different populations and ethnic groups might be, in fact, caused by genetic differences. Diet, as well, can be particularly specific from a certain population, influencing adiponectin levels.

The study design of interventional studies is especially important, as many factors can

vary: the presence and type of the exercise (eg. aerobic, endurance, strength), the duration, the age of participants and pubertal status, diet control or counseling,…

The complex cross-talk between adiponectin, obesity and IR makes hard to unveil which factors are causes, and which are consequences. The use of statistical strategies that allow adjustment for confounding factors is important; however longitudinal studies are the best option to find casual relations.

Future studies should focus on longer longitudinal studies to clarify controversies regarding physiological changes and the role of adiponectin, as well as the stability of the obtained results. Particularly important is the inclusion in those studies of low age participants (controls and obese patients), as well as the measurement of adiponectin isoforms, considering that such information is scanty.

Acknowledgements: This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-028613 (PTDC/DTP-DES/0393/2012). A PhD grant was attributed to H. Nascimento by FCT (SFRH/BD/48060/2008).

References

1. Mangge H, Almer G, Haj-Yahya S, Pilz S, Gasser R, Moller R, et al. Preatherosclerosis and adiponectin subfractions in obese adolescents. Obesity (Silver Spring), Md. 2008;16(12):2578-84..
2. Murdolo G, Nowotny B, Celi F, Donati M, Bini V, Papi F, et al. Inflammatory adipokines, high molecular weight adiponectin, and insulin resistance: a population-based survey in prepubertal schoolchildren. PLoS One. 2011;6(2):e17264.
3. Roth CL, Kratz M, Ralston MM, Reinehr T. Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. Metabolism: Clinical and Experimental. 2011;60(4):445-52.
4. Mangge H, Almer G, Haj-Yahya S, Grandits N, Gasser R, Pilz S, et al. Nuchal thickness of subcutaneous adipose tissue is tightly associated with an increased LMW/total adiponectin ratio in obese juveniles. Atherosclerosis. 2009;203(1):277-83.
5. Pedrosa C, Oliveira BM, Albuquerque I, Simoes-Pereira C, Vaz-de-Almeida MD, Correia F. Metabolic syndrome, adipokines and ghrelin in overweight and obese schoolchildren: results of a 1-year lifestyle intervention programme. European Journal of Pediatrics. 2011;170(4):483-92.
6. Arnaiz P, Acevedo M, Barja S, Aglony M, Guzman B, Cassis B, et al. Adiponectin levels, cardiometabolic risk factors and markers of subclinical atherosclerosis in children. International Journal of Cardiology. 2010;138(2):138-44.
7. Gherlan I, Vladoiu S, Alexiu F, Giurcaneanu M, Oros S, Brehar A, et al. Adipocytokine profile and insulin resistance in childhood obesity. Maedica. 2012;7(3):205-13.
8. Modan-Moses D, Stein D, Pariente C, Yaroslavsky A, Ram A, Faigin M, et al. Modulation of adiponectin and leptin during refeeding of female anorexia nervosa patients. The Journal of Clinical Endocrinology and Metabolism. 2007;92(5):1843-7.
9. Elloumi M, Ben Ounis O, Makni E, Van Praagh E, Tabka Z, Lac G. Effect of individualized weight-loss programmes on adiponectin, leptin and resistin levels in obese adolescent boys. Acta Paediatrica. 2009;98(9):1487-93.
10. Suleymanoglu S, Tascilar E, Pirgon O, Tapan S, Meral C, Abaci A. Vaspin and its correlation with insulin sensitivity indices in obese children. Diabetes research and clinical practice. 2009;84(3):325-8.
11. Choi KM, Yannakoulia M, Park MS, Cho GJ, Kim JH, Lee SH, et al. Serum adipocyte fatty acid-binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: a 3-y prospective cohort study. The American journal of clinical nutrition. 2011;93(1):19-26.
12. Lira FS, Rosa JC, Pimentel GD, Santos RV, Carnier J, Sanches PL, et al. Long-term interdisciplinary therapy reduces endotoxin level and insulin resistance in obese adolescents. Nutrition journal. 2012;11:74.
13. Calcaterra V, De Amici M, Klersy C, Torre C, Brizzi V, Scaglia F, et al. Adiponectin, IL-10 and metabolic syndrome in obese children and adolescents. Acta bio-medica : Atenei Parmensis. 2009;80(2):117-23.
14. Snehalatha C, Yamuna A, Ramachandran A. Plasma adiponectin does not correlate with insulin resistance and cardiometabolic variables in nondiabetic Asian Indian teenagers. Diabetes care. 2008;31(12):2374-9.
15. Yoshinaga M, Takahashi H, Shinomiya M, Miyazaki A, Kuribayashi N, Ichida F. Impact of having one cardiovascular risk factor on other cardiovascular risk factor levels in adolescents. Journal of atherosclerosis and thrombosis. 2010;17(11):1167-75.
16. Tadokoro N, Shinomiya M, Yoshinaga M, Takahashi H, Matsuoka K, Miyashita Y, et al. Visceral fat accumulation in Japanese high school students and related atherosclerotic risk factors. Journal of atherosclerosis and thrombosis. 2010;17(6):546-57.
17. Medina-Bravo P, Meza-Santibanez R, Rosas-Fernandez P, Galvan-Duarte R, Saucedo-Garcia R, Velazquez-Lopez L, et al. Decrease in serum adiponectin levels associated with visceral fat accumulation independent of pubertal stage in children and adolescents. Archives of medical research. 2011;42(2):115-21.
18. Gil-Campos M, Ramirez Tortosa MC, Aguilera CM, Canete R, Gil A. Fasting and postprandial adiponectin alterations anticipate NEFA and TNF-alpha changes in prepubertal obese children. Nutr Metab Cardiovasc Dis. 2011;21(1):62-8.
19. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. The Journal of Clinical Endocrinology and Metabolism. 2009;94(10):3687-95.
20. Nishimura R, Sano H, Matsudaira T, Morimoto A, Miyashita Y, Shirasawa T, et al. Changes in body mass index, leptin and adiponectin in Japanese children during a three-year follow-up period: a population-based cohort study. Cardiovascular Diabetology. 2009;8:30.
21. Toprak D, Toprak A, Chen W, Xu JH, Srinivasan S, Berenson GS. Adiposity in childhood is related to C-reactive protein and adiponectin in young adulthood: from the Bogalusa Heart Study. Obesity (Silver Spring), Md. 2011;19(1):185-90.
22. Vos RC, Wit JM, Pijl H, Houdijk EC. Long-term effect of lifestyle intervention on adiposity, metabolic parameters, inflammation and physical fitness in obese children: a randomized controlled trial. Nutrition & Diabetes. 2011;1:e9.
23. Zhang M, Zhao X, Li M, Cheng H, Hou D, Wen Y, et al. Abnormal adipokines associated with various types of obesity in Chinese children and adolescents. Biomedical and Environmental Sciences. 2011;24(1):12-21.
24. Bansal N, Anderson SG, Vyas A, Gemmell I, Charlton-Menys V, Oldroyd J, et al. Adiponectin and lipid profiles compared with insulins in relation to early growth of British South Asian and European children: the Manchester children's growth and vascular health study. The Journal of Clinical Endocrinology and Metabolism. 2011;96(8):2567-74.
25. Martos-Moreno GA, Barrios V, Martinez G, Hawkins F, Argente J. Effect of weight loss on high-molecular weight adiponectin in obese children. Obesity (Silver Spring), Md. 2010;18(12):2288-94.
26. Gajewska J, Weker H, Ambroszkiewicz J, Chelchowska M, Wiech M, Laskowska-Klita T. Changes in concentration of serum adiponectin multimeric forms following weight reduction programme in prepubertal obese children. Medycyna Wieku Rozwojowego. 2011;15(3):298-305.
27. Papoutsakis C, Yannakoulia M, Ntalla I, Dedoussis GV. Metabolic syndrome in a Mediterranean pediatric cohort: prevalence using International Diabetes Federation-derived criteria and associations with adiponectin and leptin. Metabolism: Clinical and Experimental. 2012;61(2):140-5.
28. Lee MK, Jekal Y, Im JA, Kim E, Lee SH, Park JH, et al. Reduced serum vaspin concentrations in obese children following short-term intensive lifestyle modification. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2010;411(5-6):381-5.
29. Nascimento H, Silva L, Lourenco P, Weinfurterova R, Castro E, Rego C, et al. Lipid profile in Portuguese obese children and adolescents: interaction of apolipoprotein E polymorphism with adiponectin levels.

Archives of Pediatric & Adolescent Medicine. 2009;163(11):1030-6.
30. Urbina EM, Khoury P, Martin LJ, D'Alessio D, Dolan LM. Gender differences in the relationships among obesity, adiponectin and brachial artery distensibility in adolescents and young adults. International Journal of Obesity. 2009;33(10):1118-25.
31. Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, et al. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes. 2006;55(7):1954-60.
32. Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. The Journal of Clinical Endocrinology and Metabolism. 2004;89(8):3790-4.
33. Murphy MJ, Hosking J, Metcalf BS, Voss LD, Jeffery AN, Sattar N, et al. Distribution of adiponectin, leptin, and metabolic correlates of insulin resistance: a longitudinal study in British children; 1: Prepuberty (EarlyBird 15). Clinical Chemistry. 2008;54(8):1298-306.
34. Reinehr T, Woelfle J, Roth CL. Lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children: a longitudinal analysis. Metabolism: Clinical and Experimental. 2011;60(9):1349-54.
35. Xu L, Li M, Yin J, Cheng H, Yu M, Zhao X, et al. Change of Body Composition and Adipokines and Their Relationship with Insulin Resistance across Pubertal Development in Obese and Nonobese Chinese Children: The BCAMS Study. International Journal of Endocrinology. 2012;2012:389108.
36. Klunder-Klunder M, Flores-Huerta S, Garcia-Macedo R, Peralta-Romero J, Cruz M. Adiponectin in eutrophic and obese children as a biomarker to predict metabolic syndrome and each of its components. BMC Public Health. 2013;13:88.
37. Araki S, Dobashi K, Kubo K, Asayama K, Shirahata A. High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. The Journal of Clinical Endocrinology and Metabolism. 2006;91(12):5113-6.
38. Galler A, Gelbrich G, Kratzsch J, Noack N, Kapellen T, Kiess W. Elevated serum levels of adiponectin in children, adolescents and young adults with type 1 diabetes and the impact of age, gender, body mass index and metabolic control: a longitudinal study. European Journal of Endocrinology / European Federation of Endocrine Societies. 2007;157(4):481-9.
39. Larnkjaer A, Schack-Nielsen L, Molgaard C, Ingstrup HK, Holst JJ, Michaelsen KF. Effect of growth in infancy on body composition, insulin resistance, and concentration of appetite hormones in adolescence. The American Journal of Clinical Nutrition. 2010;91(6):1675-83.
40. Hitze B, Bosy-Westphal A, Plachta-Danielzik S, Bielfeldt F, Hermanussen M, Muller MJ. Long-term effects of rapid weight gain in children, adolescents and young adults with appropriate birth weight for gestational age: the Kiel Obesity Prevention Study. Acta Paediatrica. 2010;99(2):256-62.
41. Lazzer S, Vermorel M, Montaurier C, Meyer M, Boirie Y. Changes in adipocyte hormones and lipid oxidation associated with weight loss and regain in severely obese adolescents. International Journal of Obesity. 2005;29(10):1184-91.
42. Lira FS, Rosa JC, Dos Santos RV, Venancio DP, Carnier J, Sanches Pde L, et al. Visceral fat decreased by long-term interdisciplinary lifestyle therapy correlated positively with interleukin-6 and tumor necrosis factor-alpha and negatively with adiponectin levels in obese adolescents. Metabolism: Clinical and Experimental. 2011;60(3):359-65.
43. Dekker JM, Funahashi T, Nijpels G, Pilz S, Stehouwer CD, Snijder MB, et al. Prognostic value of adiponectin for cardiovascular disease and mortality. The Journal of Clinical Endocrinology and Metabolism. 2008;93(4):1489-96.
44. Verma S, Szmitko PE. The vascular biology of peroxisome proliferator-activated receptors: modulation of atherosclerosis. The Canadian Journal of Cardiology. 2006;22 Suppl B:12B-7B.
45. Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes. 2002;51(10):2968-74.
46. Clarson CL, Mahmud FH, Baker JE, Clark HE, McKay WM, Schauteet VD, et al. Metformin in combination with structured lifestyle intervention improved body mass index in obese adolescents, but did not improve insulin resistance. Endocrine. 2009;36(1):141-6.
47. Kendall D, Vail A, Amin R, Barrett T, Dimitri P, Ivison F, et al. Metformin in obese children and adolescents: the MOCA trial. The Journal of Clinical Endocrinology and Metabolism. 2013;98(1):322-9.
48. Fukunaga H, Kishiro M, Akimoto K, Ohtsuka Y, Nagata S, Shimizu T. Imbalance of peroxisome proliferator-activated receptor gamma and adiponectin predisposes Kawasaki disease patients to developing atherosclerosis. Pediatrics International : Official Journal of the Japan Pediatric Society. 2010;52(5):795-800.
49. Gilardini L, McTernan PG, Girola A, da Silva NF, Alberti L, Kumar S, et al. Adiponectin is a candidate marker of metabolic syndrome in obese children and adolescents. Atherosclerosis. 2006;189(2):401-7.
50. Donoso MA, Munoz-Calvo MT, Barrios V, Garrido G, Hawkins F, Argente J. Increased circulating adiponectin levels and decreased leptin/soluble leptin receptor ratio throughout puberty in female ballet dancers: association with body composition and the delay in puberty. European Journal of Endocrinology / European Federation of Endocrine Societies. 2010;162(5):905-11.
51. Metcalf BS, Jeffery AN, Hosking J, Voss LD, Sattar N, Wilkin TJ. Objectively measured physical activity and its association with adiponectin and other novel metabolic markers: a longitudinal study in children (EarlyBird 38). Diabetes Care. 2009;32(3):468-73.
52. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, et al. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. American Journal of Physiology. 2003;285(3):E527-33.
53. Shaibi GQ, Cruz ML, Weigensberg MJ, Toledo-Corral CM, Lane CJ, Kelly LA, et al. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. The Journal of Clinical Endocrinology and Metabolism. 2007;92(5):1809-13.
54. Alikasifoglu A, Gonc N, Ozon ZA, Sen Y, Kandemir N. The relationship between serum adiponectin, tumor necrosis factor-alpha, leptin levels and insulin sensitivity in childhood and adolescent obesity: adiponectin is a marker of metabolic syndrome. Journal of Clinical Research in Pediatric Endocrinology. 2009;1(5):233-9.
55. Morrison JA, Glueck CJ, Daniels S, Wang P, Stroop D. Paradoxically high adiponectin in obese 16-year-old girls protects against appearance of the metabolic syndrome and its components seven years later. The Journal of Pediatrics. 2011;158(2):208-14 e1.
56. Kanhai DA, Kranendonk ME, Uiterwaal CS, van der Graaf Y, Kappelle LJ, Visseren FL. Adiponectin and incident coronary heart disease and stroke. A systematic review and meta-analysis of prospective studies. Obesity Reviews. 2013;14(7):555-67.
57. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma

lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(4):459-69.
58. Kwon K, Jung SH, Choi C, Park SH. Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. International Journal of Cardiology. 2005;101(3):385-90.
59. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obesity Research. 2003;11(3):368-72.
60. Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. Circulation. 2004;109(18):2181-5.
61. Araki S, Dobashi K, Yamamoto Y, Asayama K, Kusuhara K. Increased plasma isoprostane is associated with visceral fat, high molecular weight adiponectin, and metabolic complications in obese children. European Journal of Pediatrics. 2010;169(8):965-70.
62. Haqq AM, Muehlbauer M, Svetkey LP, Newgard CB, Purnell JQ, Grambow SC, et al. Altered distribution of adiponectin isoforms in children with Prader-Willi syndrome (PWS): association with insulin sensitivity and circulating satiety peptide hormones. Clinical Endocrinology. 2007;67(6):944-51.
63. Ogawa Y, Kikuchi T, Nagasaki K, Hiura M, Tanaka Y, Uchiyama M. Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese Japanese children. Hypertension Research : Official Journal of the Japanese Society of Hypertension. 2005;28(1):51-7.
64. Carnier J, Sanches Pde L, da Silva PL, de Piano A, Tock L, Campos RM, et al. Obese adolescents with eating disorders: analysis of metabolic and inflammatory states. Physiology & Behavior. 2012;105(2):175-80.
65. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059-61.
66. Nascimento H, Costa E, Rocha-Pereira P, Rego C, Mansilha HF, Quintanilha A, et al. Cardiovascular risk factors in portuguese obese children and adolescents: impact of small reductions in body mass index imposed by lifestyle modifications. The Open Biochemistry Journal. 2012;6:43-50.
67. Ozkol M, Ersoy B, Kasirga E, Taneli F, Bostanci IE, Ozhan B. Metabolic predictors for early identification of fatty liver using doppler and B-mode ultrasonography in overweight and obese adolescents. European Journal of Pediatrics. 2010;169(11):1345-52.
68. Rank M, Siegrist M, Wilks DC, Langhof H, Wolfarth B, Haller B, et al. The Cardio-Metabolic Risk of Moderate and Severe Obesity in Children and Adolescents. The Journal of Pediatrics. 2013.
69. Siegrist M, Rank M, Wolfarth B, Langhof H, Haller B, Koenig W, et al. Leptin, adiponectin, and short-term and long-term weight loss after a lifestyle intervention in obese children. Nutrition. 2013.
70. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, et al. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. The Journal of Clinical Endocrinology and Metabolism. 2008;93(8):3051-7.
71. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation. 2003;107(5):671-4.
72. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001;50(9):2094-9.
73. Lopez-Alarcon M, Martinez-Coronado A, Velarde-Castro O, Rendon-Macias E, Fernandez J. Supplementation of n3 long-chain polyunsaturated fatty acid synergistically decreases insulin resistance with weight loss of obese prepubertal and pubertal children. Archives of Medical Research. 2011;42(6):502-8.
74. Romeo J, Martinez-Gomez D, Diaz LE, Gomez-Martinez S, Marti A, Martin-Matillas M, et al. Changes in cardiometabolic risk factors, appetite-controlling hormones and cytokines after a treatment program in overweight adolescents: preliminary findings from the EVASYON study. Pediatric Diabetes. 2011;12(4 Pt 2):372-80.
75. Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. The Journal of Clinical Endocrinology and Metabolism. 2003;88(3):1055-8.
76. Kanety H, Hemi R, Ginsberg S, Pariente C, Yissachar E, Barhod E, et al. Total and high molecular weight adiponectin are elevated in patients with Laron syndrome despite marked obesity. European Journal of Endocrinology / European Federation of Endocrine Societies. 2009;161(6):837-44.
77. Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, et al. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. Biochemical and Biophysical Research Communications. 2001;288(5):1102-7.
78. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. The Journal of clinical investigation. 2003;112(1):91-100.
79. Neumeier M, Sigruener A, Eggenhofer E, Weigert J, Weiss TS, Schaeffler A, et al. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. Biochemical and Biophysical Research Communications. 2007;352(2):543-8.
80. Kato H, Kashiwagi H, Shiraga M, Tadokoro S, Kamae T, Ujiie H, et al. Adiponectin acts as an endogenous antithrombotic factor. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006;26(1):224-30.
81. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. Circulation. 2002;105(24):2893-8.
82. Wang Y, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, et al. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. The Journal of Biological Chemistry. 2005;280(18):18341-7.
83. Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, et al. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. The Journal of Clinical Endocrinology and Metabolism. 2005;90(8):4792-6.
84. Litwin M, Michalkiewicz J, Niemirska A, Gackowska L, Kubiszewska I, Wierzbicka A, et al. Inflammatory activation in children with primary hypertension. Pediatric Nephrology. 2010;25(9):1711-8.
85. Szmitko PE, Teoh H, Stewart DJ, Verma S. Adiponectin and cardiovascular disease: state of the art? Am J Physiol Heart Circ Physiol. 2007;292(4):H1655-63.
86. Ouedraogo R, Wu X, Xu SQ, Fuchsel L, Motoshima H, Mahadev K, et al. Adiponectin suppression of high-glucose-induced reactive oxygen species in vascular endothelial cells: evidence for involvement of a cAMP signaling pathway. Diabetes. 2006;55(6):1840-6.
87. Kelly AS, Steinberger J, Kaiser DR, Olson TP, Bank AJ, Dengel DR. Oxidative stress and adverse adipokine profile characterize the metabolic syndrome in children. Journal of the cardiometabolic syndrome. 2006;1(4):248-52.

88. Sumegova K, Nagyova Z, Waczulikova I, Zitnanova I, Durackova Z. Activity of paraoxonase 1 and lipid profile in healthy children. Physiological research / Academia Scientiarum Bohemoslovaca. 2007;56(3):351-7.
89. Koncsos P, Seres I, Harangi M, Illyes I, Jozsa L, Gonczi F, et al. Human paraoxonase-1 activity in childhood obesity and its relation to leptin and adiponectin levels. Pediatric Research. 2010;67(3):309-13.
90. Matarese G, Mantzoros C, La Cava A. Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. Current Pharmaceutical Design. 2007;13(36):3676-80.
91. Bozzola E, Meazza C, Arvigo M, Travaglino P, Pagani S, Stronati M, et al. Role of adiponectin and leptin on body development in infants during the first year of life. Italian Journal of Pediatrics. 2010;36:26.
92. Ibanez L, Lopez-Bermejo A, Diaz M, Angulo M, Sebastiani G, de Zegher F. High-molecular-weight adiponectin in children born small- or appropriate-for-gestational-age. The Journal of Pediatrics. 2009;155(5):740-2.
93. Schipper HS, Nuboer R, Prop S, van den Ham HJ, de Boer FK, Kesmir C, et al. Systemic inflammation in childhood obesity: circulating inflammatory mediators and activated CD14++ monocytes. Diabetologia. 2012;55(10):2800-10.
94. Inoue M, Maehata E, Yano M, Taniyama M, Suzuki S. Correlation between the adiponectin-leptin ratio and parameters of insulin resistance in patients with type 2 diabetes. Metabolism: Clinical and Experimental. 2005;54(3):281-6.
95. Reinehr T, Stoffel-Wagner B, Roth CL. Adipocyte fatty acid-binding protein in obese children before and after weight loss. Metabolism: Clinical and Experimental. 2007;56(12):1735-41.
96. Reinehr T, Stoffel-Wagner B, Roth CL, Andler W. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. Metabolism: Clinical and Experimental. 2005;54(9):1155-61.
97. Reinehr T, Roth CL, Alexy U, Kersting M, Kiess W, Andler W. Ghrelin levels before and after reduction of overweight due to a low-fat high-carbohydrate diet in obese children and adolescents. International Journal of Obesity. 2005;29(4):362-8.
98. de Mello MT, de Piano A, Carnier J, Sanches Pde L, Correa FA, Tock L, et al. Long-term effects of aerobic plus resistance training on the metabolic syndrome and adiponectinemia in obese adolescents. Journal of Clinical Hypertension. 2011;13(5):343-50.
99. Kaas A, Pfleger C, Hansen L, Buschard K, Schloot NC, Roep BO, et al. Association of adiponectin, interleukin (IL)-1ra, inducible protein 10, IL-6 and number of islet autoantibodies with progression patterns of type 1 diabetes the first year after diagnosis. Clinical and Experimental Immunology. 2010;161(3):444-52.

14.2. Paper II

Nascimento H, Silva L, Lourenço P, Weinfurterová R, Castro E, Rego C, Ferreira H, Guerra A, Quintanilha A, Santos-Silva A, Belo L. "Lipid profile in obese children and adolescents. Interaction of apolipoprotein E polymorphism with adiponectin levels". Archives of Pediatrics & Adolescent Medicine. 2009; 163:1030-1036.

ARTICLE

Lipid Profile in Portuguese Obese Children and Adolescents

Interaction of Apolipoprotein E Polymorphism With Adiponectin Levels

Henrique Nascimento, Licenciatura; Letícia Silva; Pedro Lourenço; Radka Weinfurterová, PharmD; Elisabeth Castro, PhD; Carla Rego, MD; Helena Ferreira, MD; António Guerra, MD, PhD; Alexandre Quintanilha, MD; Alice Santos-Silva, PhD; Luís Belo, PhD

Objective: To evaluate how the lipid profile associates with apolipoprotein (apo) E gene polymorphism, plasma adiponectin level, and body mass index (BMI) *z* score in Portuguese youth.

Design: Transversal cohort study.

Setting: Hospital de São João and Hospital de Crianças Maria Pia, Porto, Portugal, between May 2006 and March 2007.

Participants: One hundred thirty-eight obese children and adolescents (62 boys; mean age, 10.8 years [range, 4-16 years]). Participants were grouped according to (1) apo E polymorphism: presence of the apo E alleles 2 or 4 in E2 (n=11) and E4 (n=31) carriers, respectively, or as E3/E3 (n=94) (carriers of E2/E4 [n=2] were excluded from apo E analysis because they carry both alleles) and (2) BMI *z* score: group 1, BMI *z* score less than 2 (n=31); group 2, BMI *z* score of 2 or more and less than 2.5 (n=65); and group 3, BMI *z* score of 2.5 or more (n=42).

Main Outcome Measures: Lipid variable comparisons between apo E polymorphism and BMI *z* score groups and influence of BMI *z* score and adiponectin level, adjusted for apo E polymorphism, on total cholesterol to high-density lipoprotein cholesterol and apo A-I to apo B ratios.

Results: E4 carriers presented with a worse lipid profile when compared with E2 and E3/E3 carriers. There was also a clear risk of worsening for the group with the highest BMI *z* score. Apolipoprotein E polymorphism, BMI *z* score, and adiponectin level were significantly associated with total cholesterol to high-density lipoprotein cholesterol (standardized β coefficient=0.283, 0.354, and –0.292, respectively; $P<.001$ for all) and apo A-I to apo B (standardized β coefficient=–0.372, –0.284, and 0.327, respectively; $P<.001$ for all) ratios.

Conclusion: Our data suggest a more atherogenic lipid profile for some apo E genotypes and for increasing BMI *z* score, whereas adiponectin level seems to play a protective role.

Arch Pediatr Adolesc Med. 2009;163(11):1030-1036

Author Affiliations are listed at the end of this article.

OBESITY IS INCREASING ALL over the world and is a significant risk factor for cardiovascular disease (CVD). Cardiovascular morbidity and mortality of obesity are associated with classic risk factors, namely dyslipidemia, hypertension, and impaired glucose metabolism. These risk factors, known as the predictors of future CVD, make part of what is known as the metabolic syndrome.[1]

The dyslipidemia commonly observed in obese patients may be due to altered cholesterol metabolism. Obese patients present with elevated cholesterol synthesis compared with normal-weight individuals.[2] The augmented cholesterol synthesis is likely to be caused by higher activity of the enzymes participating in cholesterol synthesis and by the larger livers observed in obese individuals.[3]

The adipocyte is an important source of cytokines, namely interleukin 6 and tumor necrosis factor α, that are significantly higher in the plasma of obese patients.[4] It is known that proinflammatory cytokines (eg, interleukin 6 and tumor necrosis factor α) may promote the development of obesity-related features, namely dyslipidemia, insulin resistance, and endothelial dysfunction, all known risk factors for CVD. In contrast to other adipocytokines, adiponectin, which is adipose tissue specific, has been noted as an important antiatherogenic and antidiabetic protein and as an anti-inflammatory protein.[5] Also in contrast to other cytokines, plasma concentrations of adiponectin are decreased in obese subjects. Adiponectin level seems to be inversely related to systolic blood pressure, waist circumference, and triglyceride (TG) and 2-hour glucose levels and positively related to

high-density lipoprotein cholesterol (HDL-C) level.[6] In this way, hypoadiponectemia seems to work as an independent biomarker of the metabolic syndrome.

Apolipoprotein (apo) E plays an important role in atherosclerosis by modifying inflammatory responses, facilitating cholesterol efflux from foam cells, and regulating hepatic uptake of remnant lipoproteins through the low-density lipoprotein (LDL) receptor and LDL receptor–related protein.[7] The E2/E3/E4 apo E polymorphism results from variations in exon 4 at codon positions 112 and 158. E2 has a T allele at both positions 112 and 158; E3 has T and C alleles at positions 112 and 158, respectively; and E4 has C at both positions. These genetic variances create apo E isoforms with the following amino acid differences at positions 112 and 158, respectively: E2: Cys and Cys; E3: Cys and Arg; and E4: Arg and Arg. All combinations of the 2 isoforms are possible: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4.[8,9]

For the apo E polymorphism, subjects carrying the E2 and E4 alleles tend to have lower and higher cholesterol levels, respectively, compared with E3/E3 individuals.[8] Contradictory results are found in the literature concerning the effect of the apo E allele on TG levels. However, a meta-analysis reported that TG levels were higher in E2 carriers and E3/E4 subjects than in E3/E3 subjects.[8] Similar results were found in obese adult and child populations.[9-11] The frequencies of the apo E alleles did not seem to differ between normal-weight and obese individuals.[11]

Although atherosclerosis is a chronic disease that begins early in life, and obesity is an important risk factor for the development of CVD, few studies have addressed CVD markers in obese children and adolescents. Particularly, to date, limited investigations have examined the associations between adiponectin level and the lipid profile in children and adolescents. Also, to our knowledge, no study has assessed the concomitant influence of adiponectin level and apo E genetic polymorphism on the lipid profile in that population. Because atherogenesis begins early in life, the study of individual differences in the early onset and progression of potential initiating risk factors is important. This is of particular concern in our country because a recent study showed a very high prevalence of overweight/obesity (31.5%) in Portuguese children when compared with other European countries.[12]

The aim of our work was to evaluate how the lipid profile is influenced by the apo E gene polymorphism, adiponectin plasma levels, and body mass index (BMI) *z* score in Portuguese obese children and adolescents. We hypothesized that (1) apo E polymorphism and BMI *z* score would be significantly and independently related to the lipid profile and (2) the effect of the apo E polymorphism on the lipid profile could be influenced by plasma levels of adiponectin.

METHODS

SUBJECTS

The protocol used for all participants was approved by the ethics committees of Hospital de São João and Hospital de Crianças Maria Pia, Porto, Portugal. Obese children and adolescents, aged 4 to 16 years, were identified from medical records at the departments of Pediatrics of Hospital de São João and Hospital de Crianças Maria Pia. All children who met the inclusion criteria were invited to participate. One hundred thirty-eight obese children and adolescents (62 boys and 76 girls) participated in the study after informed and written consent by their parents. The study took place between May 2006 and March 2007.

Obesity was defined as a BMI greater than the 95th percentile for age and sex (calculated as weight in kilograms divided by height in meters squared), according to 2000 Center for Disease Control and Prevention growth charts. Because BMI is not normally distributed, we calculated BMI *z* score using a calculator based on the 2000 Center for Disease Control and Prevention growth charts. Because we also wanted to study the value of obesity as defined by BMI *z* score, we divided the obese population into 3 groups: group 1, less than the percentile 97.5, corresponding to a BMI *z* score less than 2; group 2, between the percentiles 97.5 and 99.5, corresponding to a BMI *z* score of 2 or more and less than 2.5; and group 3, more than the percentile 99.5 (10% of our obese population), corresponding to a BMI *z* score of 2.5 or more.

Clinical data regarding the sample population were collected; the development of puberty was clinically assessed in the hospitals, on the basis of Tanner stages, by the pediatricians of our team. The physical examination included the measurement of height, weight, circumferences of waist and hip, Tanner stage assessment, and the presence of skin lesions related to obesity and its comorbidity. The participants were invited to come to the research centers after an overnight fast, and after clinical examination, blood was collected for laboratory analysis. Smokers and subjects with diabetes mellitus, endocrine disorders, hereditary diseases, or inflammatory or infectious diseases or who were undergoing any therapy that could interfere with our results were excluded from the study.

PROCEDURES AND ASSAYS

Blood Samples

Blood samples were obtained on a fasting basis and processed within 2 hours of collection. Blood was obtained by venipuncture in EDTA-containing tubes and in test tubes without anticoagulant. Aliquots of plasma, buffy coat, and serum were made and immediately stored at –80°C until assayed.

DNA Analysis

Genomic DNA was extracted from white blood cells (buffy coat) by the proteinase K/salt precipitation method.[13,14] Apolipoprotein E genotyping was performed by polymerase chain reaction (PCR)–restriction fragment length polymorphism using the method of Hixson and Vernier,[15] with some modifications. A 244–base pair (bp) fragment located in exon 4 of the apo E gene was amplified using oligonucleotide primers that flank positions 112 and 158 in the referred exon (F4: 5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′ and F6: 5′-TAAGCTTGGCACGGCTGTC-CAAGGA-3′). The PCR reaction was carried out in a thermal cycler (HYBAID TouchDown; Thermo Hybaid, Franklin, Massachusetts) using 1 µL of DNA in a volume of 20 µL containing 1× PCR buffer (HotStarTaq polymerase buffer, with a final concentration of 2.0mM magnesium chloride; Qiagen, Valencia, California), 1µM of each primer, 10% (volume to volume ratio) dimethyl sulfoxide, 0.2mM deoxyribonucleotide triphosphate, and 0.5 U of HotStarTaq DNA Polymerase (Qiagen). The PCR conditions were 95°C for 15 minutes followed by 31 cycles at 95°C for 45 seconds, 60°C for 1 minute, and 72°C for 2 minutes and, fi-

Table 1. Clinical Characteristics, Genotype, and Biochemical Data in 138 Obese Children and Adolescents

	No. (%)
Clinical characteristic	
Age, y, mean (SD)	10.8 (3.0)
Tanner stage of puberty ≥2	69 (50)
Waist, cm, mean (SD)	92.9 (13.4)
Hip, cm, mean (SD)	98.8 (13.9)
Waist to hip ratio, mean (SD)	0.941 (0.051)
BMI, mean (SD)	29.69 (5.34)
BMI *z* score, mean (SD)	2.30 (0.45)
BMI *z* score <2	31 (22.5)
BMI *z* score ≥2 and <2.5	65 (47.1)
BMI *z* score ≥2.5	42 (30.4)
Genotype	
E2/E2	0
E2/E3	11 (8.0)
E2/E4	2 (1.4)
E3/E3	94 (68.1)
E3/E4	28 (20.3)
E4/E4	3 (2.2)
Biochemical data, median (IQR)	
TG level, mg/dL	77.0 (53.0-109.8)
TC level, mg/dL	161.0 (143.0-181.2)
HDL-C level, mg/dL	42.0 (35.8-48.0)
LDL-C level, mg/dL	104.0 (89.0-122.5)
apo A-I level, mg/dL	118.0 (108.4-128.6)
apo B level, mg/dL	79.0 (68.0-93.3)
TC:HDL-C ratio	3.89 (3.29-4.59)
apo A-I:apo B ratio	1.50 (1.24-1.75)
Adiponectin level, mg/L	7.92 (5.19-10.99)

Abbreviations: apo, apolipoprotein; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

SI conversion factors: To convert apo A-I and apo B to grams per liter, multiply by 0.01; HDL-C, LDL-C, and TC to millimoles per liter, multiply by 0.0259; TG to millimoles per liter, multiply by 0.0113.

nally, at 72°C for 8 minutes. The PCR products (20 µL) were digested with 10 U of *Hha*I in the recommended buffer (REact 2; Gibco BRL, Carlsbad, California) for 3 hours at 37°C. Each reaction mixture was loaded onto an 8% polyacrylamide gel in TRIS/borate/EDTA and electrophoresed. The gel was stained with ethidium bromide and DNA fragments were visualized by UV illumination. The sizes of the *Hha*I fragments were estimated by comparison with known size markers (10-bp DNA ladder; Invitrogen Life Technologies, Carlsbad). *Hha*I cuts codons for arginine residues at positions 112 and 158. If both restriction cutting sites were present in both alleles (E4/E4), the length of the fragments were 72, 19, 48, and 35 bp; if the restriction cutting site at position 158 was present in both alleles (E3/E3), the size of the fragments were 91, 48, and 35 bp; if both restriction cutting sites were absent in both alleles (E2/E2), the fragment sizes were 91 and 83 bp. The gel patterns obtained for the E2/E3, E2/E4, and E3/E4 genotypes were combinations of the homozygous fragments.[15]

Serum Analysis

Serum lipid, lipoprotein, and apo analyses were performed in an auto-analyzer (Cobas Mira S; Roche, Nutley, NJ) using commercially available kits. Serum total cholesterol (TC) and TG concentrations were determined by enzymatic colorimetric tests (CHOD-PAP and GPO-PAP methods, respectively; Roche). High-density lipoprotein cholesterol and LDL cholesterol (LDL-C) levels were measured using enzymatic colorimetric tests after selective separation of high-density lipoprotein and LDL fractions (Direct HDL Cholesterol and Direct LDL Cholesterol; Roche). Apolipoprotein A-I and apo B levels in serum were evaluated by immunoturbidimetric assays (uni-kit apo A-I– and B–specific antiserums; Roche).

Plasma Analysis

Plasma concentration of adiponectin was evaluated by using a standard commercial enzyme-linked immunoassay (Adiponectin; R&D Systems, Minneapolis, Minnesota). Intra-assay and interassay coefficients of variation were lower than 5% and 7%, respectively.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software (version 16.0 for Windows; SPSS, Chicago, Illinois). Kolmogorov-Smirnov analysis was used to test if the results were normally distributed. The results normally distributed are presented as mean (SD) and those not normally distributed are presented as median (interquartile range).

Male and female patients were compared using an unpaired *t* test or Mann-Whitney *U* test. The distribution of boys and girls with respect to genotypes and other categorical variables was analyzed using the χ^2 test and Fisher exact test.

Multiple comparisons between groups were performed by 1-way analysis of variance supplemented with the Tukey Honestly Significant Difference post hoc test, after log transformation of the variables (when necessary). Adjustment of statistical differences for confounding factors was performed using analysis of covariance. The strength of the association between the substances was estimated by Pearson correlation coefficient, after log transformation of the variables (when necessary). To evaluate the contribution of the different variables to adiponectin levels, we performed multiple regression analysis, using stepwise selection, with an entry criteria of $P<.05$. To evaluate the influence of BMI *z* score and adiponectin level, adjusted for apo E polymorphism (allele carriers), on both TC:HDL-C and apo A-I:apo B ratios, we performed multiple regression analysis using the 3 variables in the models. Significance was accepted at $P<.05$.

RESULTS

The clinical characteristics of the obese children and adolescents (n=138) are presented in **Table 1**. The mean age and BMI *z* score were 10.8 years and 2.30, respectively.

Concerning the comparison between boys and girls (data not shown), no major differences were observed in both clinical and laboratory data, except for waist circumference (mean, boys, 95.4 cm and girls, 90.9 cm; $P<.05$) and waist to hip ratio (mean, boys, 0.955 and girls, 0.930; $P<.005$), which were higher in boys, and adiponectin levels (mean, boys, 6.92 mg/L and girls, 8.35 mg/L; $P<.05$), which were lower for boys. No statistically significant differences were found in the distribution of subjects with respect to apo E genotype between boys and girls.

Apolipoprotein E polymorphism was associated with different lipid and lipoprotein status (**Table 2**). To evaluate the association of lipid and lipoprotein values with

Table 2. Lipid, Lipoprotein, and Adiponectin Levels According to apo E Polymorphism Distribution in Obese Children and Adolescents

	Mean (SD)		
	E2 Carriers[a] (n=11)	E3/E3 Carriers (n=94)	E4 Carriers[a] (n=31)
Log TG level, mg/dL	1.826 (0.248)	1.888 (0.221)	1.911 (0.248)
Log TC level, mg/dL	2.168 (0.076)	2.205 (0.073)	2.255 (0.109)[b]
Log HDL-C level, mg/dL	1.622 (0.094)	1.616 (0.094)	1.601 (0.112)
Log LDL-C level, mg/dL	1.947 (0.109)	2.006 (0.102)	2.086 (0.132)[b]
apo A-I level, mg/dL	129.4 (18.9)	118.9 (17.9)	115.0 (17.9)
apo B level, mg/dL	71.7 (16.9)	80.2 (16.0)	92.8 (28.9)[b]
Log TC:HDL-C ratio	0.546 (0.117)	0.589 (0.107)	0.653 (0.142)[b]
apo A-I:apo B ratio	1.917 (0.594)[b]	1.534 (0.368)	1.324 (0.368)[b]
Log adiponectin level, mg/L	0.776 (0.234)	0.885 (0.269)	0.881 (0.242)

Abbreviations: See Table 1.
SI conversion factors: See Table 1.
[a] E2 carriers had either the E2/E2 or E2/E3 genotype. E4 carriers had either the E3/E4 or E4/E4 genotype. The E2/E4 subjects were not included in the analysis.
[b] $P<.05$ vs other 2 groups.

Table 3. Lipid, Lipoprotein, and Adiponectin Levels According to BMI *z* Score Distribution in Obese Children and Adolescents

	Mean (SD)		
	Group 1 (BMI *z* Score <2) (n=31)	Group 2 (BMI *z* Score ≥2 and <2.5) (n=65)	Group 3 (BMI *z* Score ≥2.5) (n=42)
Log TG level, mg/dL	1.832 (0.235)	1.889 (0.227)	1.946 (0.213)
Log TC level, mg/dL	2.186 (0.065)	2.212 (0.078)	2.234 (0.104)[a]
Log HDL-C level, mg/dL	1.668 (0.096)[b]	1.612 (0.090)	1.575 (0.092)
Log LDL-C level, mg/dL	1.979 (0.090)	2.014 (0.104)	2.055 (0.138)[a]
apo A-I level, mg/dL	123.4 (22.4)	121.3 (17.0)	112.0 (14.2)[b]
apo B level, mg/dL	75.0 (14.9)	81.3 (17.5)	89.1 (25.8)[a]
Log TC:HDL-C ratio	0.517 (0.079)[b]	0.599 (0.109)	0.658 (0.126)[b]
apo A-I:apo B ratio	1.694 (0.397)	1.566 (0.432)	1.325 (0.318)[b]
Log adiponectin level, mg/L	0.903 (0.243)	0.877 (0.256)	0.849 (0.279)

Abbreviations: See Table 1.
SI conversion factors: See Table 1.
[a] $P<.05$ vs group 1.
[b] $P<.05$ vs other 2 groups.

the apo E polymorphism, we divided subjects in 3 groups: E2 carriers (E2/E2 and E2/E3), E3/E3 individuals, and E4 carriers (E3/E4 and E4/E4). The 2 E2/E4 subjects were not included in analysis involving the apo E polymorphism because they presented with both alleles, and the group was too small for an adequate statistical analysis. They were, however, included in the other analyses. The results obtained in E2 carriers and E4 carriers were mainly due to the contribution of E2/E3 and E3/E4 genotypes, respectively. E4 carriers presented with significantly higher values of TC, LDL-C, and apo B compared with E2 carriers and E3/E3 individuals (Table 2). E4 carriers also presented with the highest TG level, although it was not statistically significant, and the lowest values for HDL-C and apo A-I and, consequently, with significantly higher TC:HDL-C and lower apo A-I:apo B ratios when compared with the other 2 groups ($P<.05$ for both). No differences in adiponectin levels were found between the different apo E genotypes. No statistical significance was lost after adjustment for BMI and age.

We further analyzed our results in terms of BMI *z* score (**Table 3**) by dividing participants into 3 groups (group 1: *z* score <2, n=31; group 2: *z* score ≥2 and <2.5, n=65; group 3: *z* score ≥2.5, n=42). A clear trend for worsening of the lipid profile was found from group 1 to group 3. Group 3 presented with the lowest apo A-I value and apo A-I:apo B ratio and the highest TC:HDL-C ratio ($P<.05$ for all). In addition, group 3 presented with significantly higher values for TC, LDL-C, and apo B when compared with group 1. On the other hand, group 1 had a significantly higher value of HDL-C when compared with the other 2 groups.

When all participants were considered, no statistically significant correlations were observed between the age of the participants and the lipid values, except for apo A-I level, which was inversely correlated with age ($r=-0.200$; $P=.02$). The age of the participants was strongly positively correlated with BMI ($r=0.528$; $P<.001$).

Adiponectin levels correlated inversely and significantly with age ($r=-0.288$; $P=.001$), BMI ($r=-0.305$; $P<.001$), waist to hip ratio ($r=-0.238$; $P=.005$), TG level ($r=-0.392$; $P<.001$), and TC:HDL-C ratio ($r=-0.265$; $P=.002$) and correlated positively and significantly with HDL-C level ($r=0.267$; $P=.002$). In the multiple regression analysis, TG level, age, and waist to hip ratio were the only variables that remained statistically associated with adiponectin values (log adiponectin value = 5.683 – 0.354 log TG value – 0.023 age – 0.947 waist to hip ratio; standardized β coefficients = –0.310, –0.261, and –0.186; $P<.001$, $P=.001$, and $P=.02$, respectively).

Because apo E polymorphism, BMI *z* score, and adiponectin levels were significantly associated with changes in the TC:HDL-C and apo A-I:apo B ratios (important "atherogenic" ratios), we evaluated the combined effect of apo E genotype with both other factors on such ratios. We observed that the effect of apo E polymorphism on TC:HDL-C and apo A-I:apo B ratios seems to be influenced by BMI *z* score (**Figure 1**) and adiponectin values (**Figure 2**). Indeed, by using multiple regression analysis, and when adjusted for apo E genotype, both BMI *z* score and adiponectin level remained significantly associated with TC:HDL-C ratio (log TC:HDL-C ratio = 0.245 + 0.063 apo E polymorphism + 0.094 BMI *z*

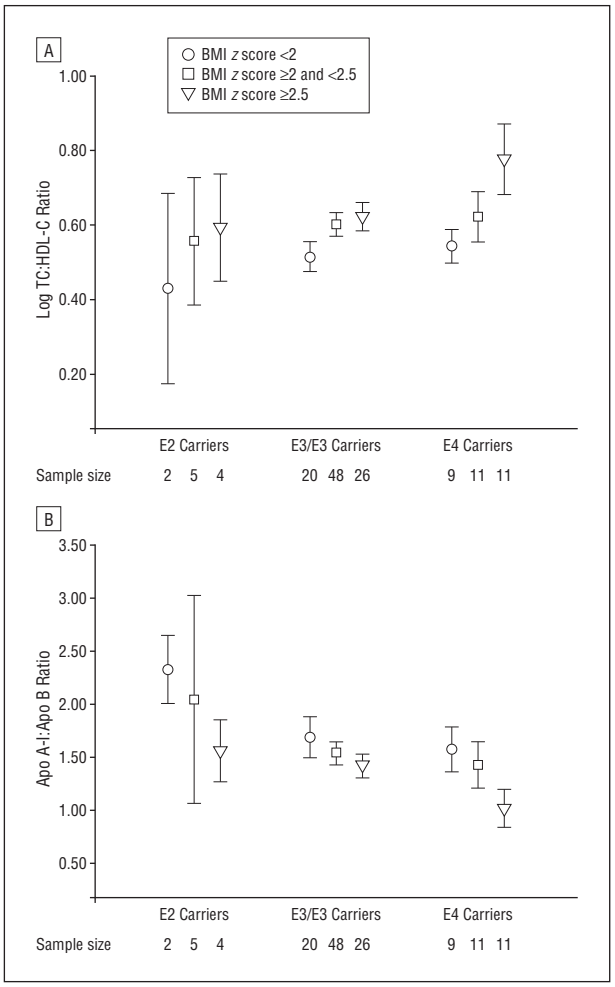


Figure 1. Effect of apolipoprotein (apo) E polymorphism on total cholesterol (TC) to high-density lipoprotein cholesterol (HDL-C) (A) and apo A-I:apo B (B) ratios, according to body mass index (BMI) *z* score. Results are presented as mean (95% confidence interval [CI]). The influence of BMI *z* score, adjusted for apo E polymorphism (allele carriers), on both the TC:HDL-C and apo A-I:apo B ratios was highly significant ($P<.001$) by multiple regression analysis.

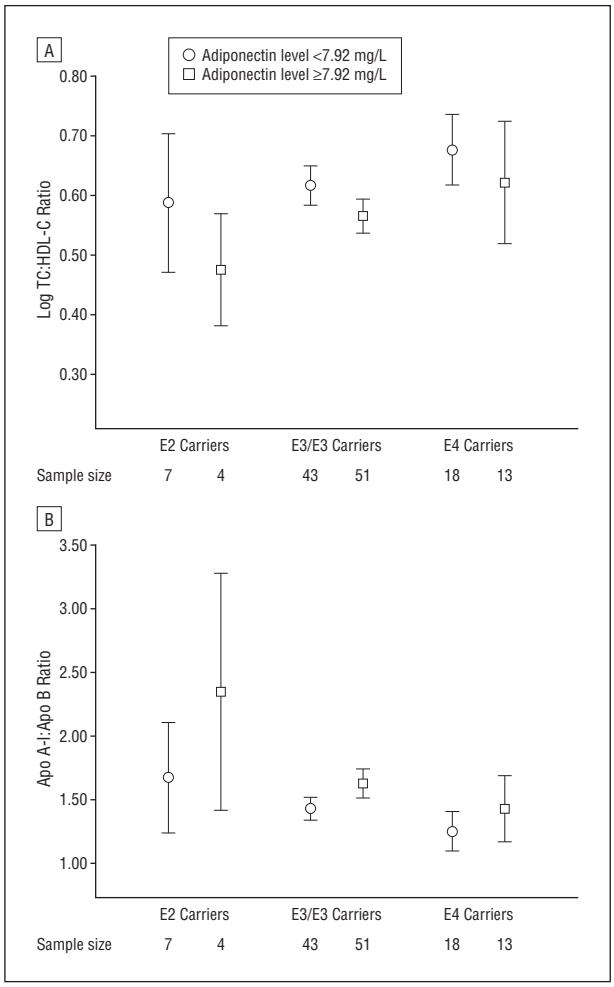


Figure 2. Effect of apolipoprotein (apo) E polymorphism on total cholesterol (TC) to high-density lipoprotein cholesterol (HDL-C) (A) and apo A-I:apo B (B) ratios, according to adiponectin level. For a better visualization of the results, we used a cutoff for adiponectin level of 7.92 mg/L, which corresponds to the median value for the entire group. Results are presented as mean (95% confidence interval [CI]). The influence of adiponectin level, adjusted for apo E polymorphism (allele carriers), on both the TC:HDL-C and apo A-I:apo B ratios was highly significant ($P<.001$) by multiple regression analysis.

score − 6.77 E-6 adiponectin; standardized β coefficients: 0.283, 0.354, and −0.292, respectively; $P<.001$ for all) and apo A-I:apo B ratio (log apo A-I:apo B ratio = 2.787 − 0.286 apo E polymorphism − 0.262 BMI *z* score + 2.62 E-5 adiponectin value; standardized β coefficients: −0.372, −0.284, and 0.327, respectively; $P<.001$ for all). For a better visualization of the results (graphically), obese participants were divided on the basis of their BMI *z* score (Figure 1) and having an adiponectin level lower than or higher than or equal to 7.92 mg/L (cutoff corresponds to the median value for the entire group) (Figure 2).

COMMENT

Obesity is associated with dyslipidemia, and both adiponectin level and apo E polymorphism are known to influence lipid values. However, as far as we know, this is the first study assessing the concomitant influence of the apo E polymorphism and adiponectin level on the lipid profile in obese children and adolescents. This is also the first study, to our knowledge, assessing adiponectin levels in Portuguese obese children and adolescents.

The adiponectin values that we describe in this article are comparable with those found in other populations.[16,17] Adiponectin levels were reported to be lower in boys than in girls, and the difference seems to be more striking at postpubertal states.[16,17] Moreover, adiponectin levels were inversely related to age and were significantly lower in pubertal compared with prepubertal obese children.[17] In our study, we also observed statistically significant differences between boys and girls, with boys presenting with lower adiponectin values. The 2 groups (boys and girls) were matched for age, BMI *z* score, and puberty stage, and therefore, the analysis of adiponectin level was not affected by these possible confounding factors. A significant inverse correlation between adiponectin levels and age was also observed for both sexes.

Adiponectin levels were lower in nonlean individuals compared with lean individuals[18] and it was reported that mean adiponectin values decreased for ev-

ery unit increase in BMI *z* score.[16] However, in obese subjects, adiponectin plasma concentrations were not correlated with BMI standard deviation score.[17] In the present study, group 3 individuals, defined as obese children and adolescents having the highest BMI *z* scores (≥2.5), presented with the lowest adiponectin values compared with the other 2 groups, but it was not statistically significant (Table 3). We also found that adiponectin levels correlated inversely with BMI and waist to hip ratio but not with BMI *z* score.

It was previously reported that adiponectin level is positively correlated with HDL-C level and negatively correlated with TG level in both lean and nonlean adolescents[18] and that these relationships strengthened with increasing adiposity. In the present study, we confirmed these relations and also that adiponectin levels correlated inversely and significantly with the TC:HDL-C ratio. As previously mentioned, adiponectin level was also inversely related to age, BMI, and waist to hip ratio. In the multiple regression analysis, TG level, waist to hip ratio, and age were the only variables that remained statistically associated with adiponectin values.

Regarding the apo E polymorphism, the distribution of subjects with respect to genotype was similar to that found in a previous work involving Portuguese obese children[11] and pregnant women.[19] Thus, and although a control group (nonobese children) was not evaluated in the present study, the frequency of the apo E genotypes is unlikely to be altered in obese children.

The association of the apo E polymorphism with changes in lipid and lipoprotein profiles is highly explored in adults, but not in children, particularly Portuguese children. In our studied population, significant allele effects of apo E genetic variability on plasma lipoprotein and apoprotein levels were observed. The results observed in carriers of E2 and E4 alleles were mainly due to the contribution of E2/E3 and E3/E4 subjects, respectively. E4 carriers presented with the highest LDL-C level, compared with those with the E3/E3 genotype and E2 carriers, in agreement with previous reports in obese children[11] and adults.[9] E4 carriers also presented with the highest TG levels and lower HDL-C and apo A-I levels, although this was not statistically significant. A previous study in pregnant women found no differences in the HDL-C levels between women with different apo E genotypes.[10] However, a meta-analysis reported HDL-C levels to be lower in E3/E4 than in E3/E3 nonpregnant subjects.[8] Furthermore, in the current study, E4 carriers presented with significantly higher TC and apo B levels ($P < .05$ for both groups); therefore, the TC:HDL-C ratio was significantly higher and apo A-I:apo B ratio significantly lower in this group when compared with the other 2. All these results remained statistically significant after adjustment for confounding factors such as age, sex, Tanner stage, and BMI *z* score.

A major finding of this study, achieved by performing multiple regression analysis, was that the effect of apo E polymorphism on the TC:HDL-C and apo A-I:apo B ratios seemed to be influenced by BMI *z* score (Figure 1) and adiponectin level (Figure 2). Individuals presenting with lower adiponectin levels or a higher BMI *z* score presented with higher TC:HDL-C and lower apo A-I:apo B ratios than those with higher adiponectin levels or lower BMI *z* scores, irrespective of their apo E polymorphism–carrying nature (Figure 1 and Figure 2). These atherogenic ratios therefore seem to worsen with lower adiponectin levels and higher BMI *z* scores, modulating early in life the effect of apo E genotype on lipids (ie, in children and adolescents). An article by Wardaningsih et al[20] reported worsening of the lipid profile for E3/E3 children with lower adiponectin levels, especially in boys.

These relations between adiponectin level, apo E polymorphism, BMI *z* score, and lipid profile that we presented in Figure 1 and Figure 2 may be because adiponectin reduces hepatic release of apo E from hepatocytes and because the hepatic secretion of apo B is also reduced by adiponectin, possibly by a genetic mechanism involving the hepatic nuclear factor 4-α.[21]

Considering that the relationships between adiponectin, HDL-C, and TG levels are strengthened with increasing adiposity, heavier adolescents seem to have a greater benefit from high levels of adiponectin than their leaner counterparts.[18] Furthermore, a recent study performed in obese children demonstrated an increase in adiponectin levels due to a significant weight loss over a 1-year period.[17] This may be of particular importance in obese individuals with certain "risk" apo E genotypes, as we demonstrated that the influence of the apo E polymorphism on lipids is influenced by BMI *z* score and adiponectin levels. Atherosclerosis is a multifactorial disease, involving the interplay of genetic and environmental factors. The improvement of the latter factors, through a healthier lifestyle, seems therefore to be particularly worthy in those obese individuals with a less favorable genetic background.

In conclusion, the lipid profile in obese children and adolescents worsens with increasing adiposity (increasing BMI *z* score) and the effect of the apo E polymorphism on the TC:HDL-C and apo A-I:apo B ratios is modulated by BMI *z* score and adiponectin levels. This may be of particular relevance because obese individuals, particularly those with risk apo E genotypes, may benefit from a closer clinical follow-up. Moreover, the implementation of lifestyle modifications (mainly by practicing regular physical activity and eating a healthy diet) should be highly encouraged in such obese children and adolescents.

Accepted for Publication: May 6, 2009.
Author Affiliations: Serviço de Bioquímica, Faculdade de Farmácia (Messrs Nascimento and Lourenço, Ms Silva, and Drs Castro, Santos-Silva, and Belo), Instituto de Biologia Molecular e Celular (Mr Nascimento and Drs Quintanilha, Santos-Silva, and Belo), Unidade de Nutrição/Serviço Pediátrico, UAG-MC, Hospital de São João E.P.E. Faculdade de Medicina (Drs Rego and Guerra), and Instituto de Ciências Biomédicas Abel Salazar (Dr Quintanilha), Universidade do Porto, and Centro Hospitalar do Porto/Departamento Pediátrico, Hospital de Crianças Maria Pia (Dr Ferreira), Porto, Portugal; and Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy, Charles University, Prague, Czech Republic (Ms Weinfurterová).

Correspondence: Henrique Nascimento, *Licenciatura*, Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal (henriqueferrao@hotmail.com).
Author Contributions: *Study concept and design:* Rego, Ferreira, Santos-Silva, and Belo. *Acquisition of data:* Nascimento, Silva, Lourenço, Weinfurterová, Rego, Ferreira, and Guerra. *Analysis and interpretation of data:* Nascimento, Castro, Quintanilha, Santos-Silva, and Belo. *Drafting of the manuscript:* Nascimento, Silva, Lourenço, Weinfurterová, and Belo. *Critical revision of the manuscript for important intellectual content:* Castro, Rego, Ferreira, Guerra, Quintanilha, Santos-Silva, and Belo. *Study supervision:* Castro, Rego, Ferreira, Guerra, Quintanilha, Santos-Silva, and Belo.
Financial Disclosure: None reported.
Funding/Support: This work was supported by Universidade do Porto grant IPG07/2007.
Additional Contributions: Technicians Amélia Ferreira, *Bacharel*, Andreia Sousa, *Bacharel*, Joana Barros, *Bacharel*, and Isabel Almeida, *Bacharel*, assisted with blood collection, and Armando Teixeira, PhD, provided statistics counseling.

REFERENCES

1. Alexander CM, Landsman PB, Grundy SM. The influence of age and body mass index on the metabolic syndrome and its components. *Diabetes Obes Metab*. 2008;10(3):246-250.
2. Miettinen TA, Gylling H. Cholesterol absorption efficiency and sterol metabolism in obesity. *Atherosclerosis*. 2000;153(1):241-248.
3. Santosa S, Varady KA, AbuMweis S, Jones PJ. Physiological and therapeutic factors affecting cholesterol metabolism: does a reciprocal relationship between cholesterol absorption and synthesis really exist? *Life Sci*. 2007;80(6):505-514.
4. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine*. 2006;29(1):81-90.
5. Ouchi N, Shibata R, Walsh K. Cardioprotection by adiponectin. *Trends Cardiovasc Med*. 2006;16(5):141-146.
6. Shaibi GQ, Cruz ML, Weigensberg MJ, et al. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. *J Clin Endocrinol Metab*. 2007;92(5):1809-1813.
7. Curtiss LK, Boisvert WA. Apolipoprotein E and atherosclerosis. *Curr Opin Lipidol*. 2000;11(3):243-251.
8. Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J Lipid Res*. 1992;33(4):447-454.
9. Larson IA, Ordovas JM, DeLuca C, Barnard JR, Feussner G, Schaefer EJ. Association of apolipoprotein (Apo)E genotype with plasma apo E levels. *Atherosclerosis*. 2000;148(2):327-335.
10. McGladdery SH, Frohlich JJ. Lipoprotein lipase and apoE polymorphisms: relationship to hypertriglyceridemia during pregnancy. *J Lipid Res*. 2001;42(11):1905-1912.
11. Guerra A, Rego C, Castro EM, Seixas S, Rocha J. Influence of apolipoprotein E polymorphism on cardiovascular risk factors in obese children. *Ann Nutr Metab*. 2003;47(2):49-54.
12. Padez C, Fernandes T, Mourão I, Moreira P, Rosado V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. *Am J Hum Biol*. 2004;16(6):670-678.
13. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*. 1992;39(5):225-235.
14. Gaffney D, Campbell RAA. PCR based method to determine the kalow allele of the cholinesterase gene: the E_1^k allele frequency and its significance in the normal population. *J Med Genet*. 1994;31(3):248-250.
15. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hha I. *J Lipid Res*. 1990;31(3):545-548.
16. Punthakee Z, Delvin EE, O'loughlin J, et al. Adiponectin, adiposity, and insulin resistance in children and adolescents. *J Clin Endocrinol Metab*. 2006;91(6):2119-2125.
17. Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. *J Clin Endocrinol Metab*. 2004;89(8):3790-3794.
18. Martin LJ, Woo JG, Daniels SR, Goodman E, Dolan LM. The relationships of adiponectin with insulin and lipids are strengthened with increasing adiposity. *J Clin Endocrinol Metab*. 2005;90(7):4255-4259.
19. Belo L, Gaffney D, Caslake M, et al. Apolipoprotein E and cholesteryl ester transfer protein polymorphisms in normal and preeclamptic pregnancies. *Eur J Obstet Gynecol Reprod Biol*. 2004;112(1):9-15.
20. Wardaningsih E, Miida T, Seino U, et al. Low adiponectin state is associated with metabolic abnormalities in obese children, particularly depending on apolipoprotein E phenotype. *Ann Clin Biochem*. 2008;45(pt 5):496-503.
21. Neumeier M, Sigruener A, Eggenhofer E, et al. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. *Biochem Biophys Res Commun*. 2007;352(2):543-548.

> Even when freshly washed and relieved of all obvious confections, children tend to be sticky.
> —Fran Lebowitz

14.3. Paper III

Nascimento H, Silva L, Lourenço P, Vieira E, Dos Santos R, Rego C, Ferreira H, Quintanilha A, Santos-Silva A, Belo L. "Lipoprotein (a) levels in obese children and adolescents: contribution of pentanucleotide repeat (TTTTA)n polymorphism in apoliporprotein (a) gene". Archives of Pediatrics & Adolescent Medicine. 2009; 163:393-394.

Lipoprotein(a) Levels in Obese Portuguese Children and Adolescents: Contribution of the Pentanucleotide Repeat (TTTTA)n Polymorphism in the Apolipoprotein(a) Gene

Lipoprotein(a) (Lp[a]) levels are known to be mainly genetically determined. A pentanucleotide repeat polymorphism, (TTTTA)n, 1.4 kilobases upstream from the gene reading frame has been studied as a possible influence in Lp(a) levels.[1] To date, repeat sequences ranging from 5 to 12 have been found, with the 8-repeat sequence the most common. Alleles containing more repeats are usually associated with lower Lp(a) levels. The influence of the pentanucleotide repeat on Lp(a) seems to be independent from other polymorphisms.[1,2] To our knowledge, this is the first study to assess the influence of this polymorphism in an obese pediatric population.

In this study, we hypothesized that the pentanucleotide repeat polymorphism influenced the Lp(a) levels in obese Portuguese children and adolescents and that the alleles with more repeats would correspond to lower Lp(a) values. To achieve this, the population was divided according to 2 criteria: individuals with Lp(a) concentrations (1) higher/equal to or lower than 19.2 mg/dL (to convert to micromoles per liter, multiply by 0.0357), the physiological cutoff that corresponds to the median of our population, or (2) higher/equal to or lower than 30 mg/dL, the traditional cutoff of an atherothrombotic serum Lp(a) concentration.

Methods. The study protocols were approved by the ethics committees of the University Hospital S. João and the Children's Hospital Maria Pia, Porto, Portugal. Obese children and adolescents aged 4 to 16 years were identified from medical records held in the hospitals' pediatrics departments. One hundred forty-seven obese children and adolescents (66 boys and 81 girls) participated in the study after informed written consent was obtained from their parents.

Obesity was defined as a body mass index greater than the 95th percentile for age and sex, according to 2000 Centers for Disease Control and Prevention growth charts. Because body mass index is not normally distributed, we used body mass index *z* scores. The sample's characteristics are presented in the **Table**.

Genomic DNA was extracted from a buffy coat by the proteinase K/salt precipitation method.[3,4] Apolipoprotein(a) genotyping was performed by polymerase chain reaction followed by electrophoresis in a polyacrylamide gel as described by Trommsdorff et al.[2] The number of pentanucleotide repeat repeats was confirmed by automated sequencing for homozygous 8/8, 9/9, and 10/10 repeats and for heterozygous 6/8, 7/8, 8/9, 8/10, 8/11, and 9/10 repeats. Serum Lp(a) was quantified by an immunoturbidimetric method in an autoanalyzer. Clinical data were compared between groups using an unpaired *t* test or a Mann-Whitney *U* test. The distribution of groups with respect to alleles and other categorical variables was analyzed using the χ^2 test and the Fisher exact test. Significance was set at $P<.05$.

Results. Groups were similar with respect to age, sex, and obesity scores (Table). The apolipoprotein(a) allele

Table. Clinical Characteristics and Apo(a) Polymorphism Allelic Distribution According to Lp(a) Levels in Obese Portuguese Children

		Physiological Cutoff			Traditional Cutoff		
		Mean (SD)			Mean (SD)		
Characteristic	Total (N = 147)	Lp(a) < 30 mg/dL (n = 92)	Lp(a) ≥ 30 mg/dL (n = 55)	*P* Value	Lp(a) < 19.2 mg/dL (n = 73)	Lp(a) ≥ 19.2 mg/dL (n = 74)	*P* Value
Age, y	10.9 (2.9)	10.9 (2.9)	11.0 (2.9)	.85	10.9 (2.9)	11.0 (2.9)	.67
Male sex, No. (%)	66 (44.9)	39 (42.4)	27 (49.1)	.49	30 (41.1)	36 (48.6)	.41
BMI	29.8 (5.4)	29.6 (5.2)	30.3 (5.9)	.53	29.4 (5.1)	30.3 (5.7)	.43
Waist to hip ratio	0.941 (0.050)	0.938 (0.051)	0.946 (0.048)	.38	0.937 (0.051)	0.945 (0.048)	.28
BMI *z* score	2.30 (0.44)	2.30 (0.48)	2.33 (0.38)	.68	2.29 (0.48)	2.32 (0.41)	.52
Lp(a), median (IQR), mg/dL	19.2 (7.26-42.8)	10.00 (4.18-17.78)	52.0 (39.0-82.0)	<.001	7.82 (2.47-10.70)	42.3 (28.08-67.50)	<.001
No. of Apo(a) alleles, No. (%)							
6	1 (0.3)	1 (0.5)	0	.001	1 (0.7)	0	.007
7	2 (0.7)	0	2 (1.8)		0	2 (1.4)	
8	209 (71.1)	121 (65.8)	88 (80.0)		93 (63.7)	116 (78.4)	
9	39 (13.3)	34 (18.5)	5 (4.5)		28 (19.2)	11 (7.4)	
10	39 (13.3)	25 (13.6)	14 (12.7)		22 (15.1)	17 (11.5)	
11	4 (1.4)	3 (1.6)	1 (0.9)		2 (1.4)	2 (1.4)	

Abbreviations: Apo(a), apolipoprotein(a); BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; Lp(a), lipoprotein(a).

SI conversion factor: To convert Lp(a) to micromoles per liter, multiply by 0.0357.

with (TTTTA) 8-repeat sequences was most prevalent (71.1%). The allele frequencies that we found are similar to those previously reported in a smaller pediatric population.[5]

The genotypic distribution between groups was somewhat different (data not shown) and reached statistical significance ($P=.03$ and $P=.02$ for the traditional and physiological cutoffs, respectively). Evaluation of the allelic distribution (Table) provided more pronounced statistically significant differences between groups, using both the traditional ($P=.001$) and the physiological ($P=.007$) cut-off values. In general, alleles with a lower number of repetitions are more frequent in the groups with higher Lp(a) concentrations.

Comment. Our results show a contribution of the pentanucleotide repeat polymorphism on serum Lp(a) concentrations in an obese pediatric Portuguese population, with alleles with more repeats corresponding to lower Lp(a) values. However, the statistical significance when analyzing the genotypes (which actually determines an individual phenotype) was not very strong, suggesting that other polymorphisms are likely to affect Lp(a) concentrations. Actually, few studies examining genetic associations have found that a single polymorphism accounts for substantial variation of a measure or outcome. A greater contribution of other polymorphisms to Lp(a) has been observed in white adults.[1,2] Additional studies are needed, especially in obese nonwhite populations.

Henrique Nascimento, Licentiate
Letícia Silva
Pedro Lourenço
Emília Vieira, MA
Rosário dos Santos, MD
Carla Rego, MD
Helena Ferreira, MD
Alexandre Quintanilha, PhD
Alice Santos-Silva, PhD
Luís Belo, PhD

Correspondence: Dr Nascimento, Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal (henriqueferrao@hotmail.com).

Author Contributions: *Study concept and design:* Ferreira, Santos-Silva, and Belo. *Acquisition of data:* Nascimento, Rego, and Ferreira. *Analysis and interpretation of data:* Nascimento, Silva, Lourenço, Vieira, dos Santos, Quintanilha, Santos-Silva, and Belo. *Drafting of the manuscript:* Nascimento, Silva, and Lourenço. *Critical revision of the manuscript for important intellectual content:* Vieira, dos Santos, Rego, Ferreira, Quintanilha, Santos-Silva, and Belo. *Statistical analysis:* dos Santos, Quintanilha, and Santos-Silva. *Study supervision:* Nascimento, Vieira, dos Santos, Rego, Ferreira, Quintanilha, Santos-Silva, and Belo.
Financial Disclosure: None reported.
Funding/Support: This work was supported by grant IPG07/2007 from the University of Porto.

1. Berglund L, Ramakrishnan R. Lipoprotein(a): an elusive cardiovascular risk factor. *Arterioscler Thromb Vasc Biol*. 2004;24(12):2219-2226.
2. Trommsdorff M, Köchl S, Lingenhel A, et al. A pentanucleotide repeat polymorphism in the 5′ control region of the apolipoprotein(a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. *J Clin Invest*. 1995;96(1):150-157.
3. Gaffney D, Campbell RAA. PCR based method to determine the kalow allele of the cholinesterase gene: the E1k allele frequency and its significance in the normal population. *J Med Genet*. 1994;31(3):248-250.
4. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*. 1992;39(5):225-235.
5. Ferreira H, Costa E, Vieira E, et al. Pentanucleotide repeat (TTTTA)n polymorphism in the 5′ control region of the apolipoprotein (a) gene and atherothrombotic serum lipoprotein (a) concentration, in a pediatric population. *Haematologica*. 2003;88(3):ELT07.

14.4. Paper IV

Belo L, Nascimento H, Kohlova M, Bronze-Rocha E, Fernandes J, Costa E, Catarino C, Aires L, Ferreira Mansilha H, Rocha-Pereira P, Quintanilha A, Rêgo C, Santos-Silva A. "Body fat percentage is a major determinant of total bilirubin levels independently of UGT1A1*28 polymorphism in obese children and adolescents". Submitted.

Body fat percentage is a major determinant of total bilirubin independently of UGT1A1*28 polymorphism in young obese

Luís Belo[1,2*¥], Henrique Nascimento[1,2¥], Michaela Kohlova[3], Elsa Bronze-Rocha[1,2], João Fernandes[1,3], Elísio Costa[1,2], Cristina Catarino[1,2], Luísa Aires[4,5] , Helena Ferreira Mansilha[6], Petronila Rocha-Pereira[7], Alexandre Quintanilha[2,8], Carla Rêgo[9], Alice Santos-Silva[1,2]

[1]Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal; [2]Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, 4150-180 Porto, Portugal; [3]IBILI, Faculdade de Medicina, Universidade de Coimbra, 3000-548 Coimbra, Portugal; [4]Research Centre in Physical Activity, Health and Leisure – CIAFEL, Faculdade de Desporto, Universidade do Porto, 4200-450 Porto, Portugal; [5]High Education Institute from Maia – ISMAI; Av. Carlos Oliveira Campos, 4475-690 S. Pedro Avioso. Portugal; [6]Departamento da Infância e Adolescência/Serviço de Pediatria do Centro Hospitalar do Porto; 4099-001 Porto, Portugal; [7]Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, 6200-506 Covilhã, Portugal: [8]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4099-003 Porto, Portugal; [9]Centro da Criança e do Adolescente. Hospital Cuf Porto. Faculdade de Medicina, Universidade do Porto; 4200-319 Porto, Portugal.

[¥]These authors contributed equally to this work.

Abstract

Objectives: Bilirubin has potential antioxidant and anti-inflammatory properties. The UGT1A1*28 polymorphism (TA repeats in the promoter region) is a major determinant of bilirubin levels and recent evidence suggest that raised adiposity may also be a contributing factor. We aimed to study the interaction between UGT1A1 polymorphism, hematological and anthropometric variables with total bilirubin levels in young individuals.

Methods: 350 obese (mean age of 11.6 years; 52% females) and 79 controls (mean age of 10.5 years; 59% females) were included. Total bilirubin and C-reactive protein (CRP) plasma levels, hemogram, anthropometric data and UGT1A1 polymorphism were determined. In a subgroup of 74 obese and 40 controls body composition was analyzed by dual-energy X-ray absorptiometry.

Results: The UGT1A1 genotype frequencies were 49.9%, 42.7% and 7.5% for 6/6, 6/7 and 7/7 genotypes, respectively. Patients with 7/7 genotype presented the highest total bilirubin levels, followed by 6/7 and 6/6 genotypes. Compared to controls, obese patients presented higher erythrocyte count, hematocrit, hemoglobin and CRP levels, but no differences in bilirubin or in UGT1A1 genotype distribution. Body fat percentage was inversely correlated with bilirubin in obese patients but not in controls. This inverse association was observed either in 6/7 or 6/6 genotype obese patients. UGT1A1 polymorphism and body fat percentage were the main factors affecting bilirubin levels within obese patients (linear regression analysis).

Conclusion: In obese children and adolescents, body fat composition and UGT1A1 polymorphism are independent determinants of total bilirubin levels. Raised inflammation and oxidative stress in obese patients may trigger the consumption of bilirubin.

Keywords: UGT1A1*28 polymorphism, body fat, bilirubin, inflammation, pediatric obesity

1. Introduction

Bilirubin is the ultimate product of the haem group catabolism and serves as a diagnostic marker of liver and blood disorders.(1) Bilirubin is a water-insoluble compound that circulates bounded to albumin and requires glucuronidation by a microsomal enzyme, the uridine diphosphate glucuronosyltransferase (UGT) 1A1, to be excreted. The UGT1A1 gene locus has been mapped to chromosome 2q37(2) and one of the most common genetic variants that affects the glucuronidation of bilirubin is a TA duplication polymorphism in the TATA box region of the gene promoter. Homozygous individuals carrying the A(TA)7TAA allele have higher levels of unconjugated bilirubin (UCB), caused by a reduction of 30% in the UGT1A1 transcription.(3) The estimated frequency of this allele is 0.35 in Caucasians, leading to a homozygous genotype in about 10% of the population, but the frequency is highly variable in different ethnicities.(4,5) Homozygosis for the TA duplication was considered as the main cause of Gilbert syndrome in Caucasian population(3,4), and justify some of the inter-individual variations in bilirubin levels.(6)

Under certain conditions bilirubin can be toxic.(7) High plasma concentrations are associated with deleterious effects in new-borns, increasing the risk of neurological dysfunction(7,8), as a result of its toxic effect on neuronal tissue. However, recent investigation has recognized that UCB exerts anti-oxidant and anti-inflammatory activities, and that mild hyperbilirubinaemia might have positive health effects. UCB inhibits lipid peroxidation(9) and suppresses inflammation in activated neonatal neutrophils(10), and population studies documented that individuals with higher circulating UCB have a reduced incidence of cardiovascular problems(11-13) and of carcinoma in general.(14) Furthermore, subjects with Gilbert syndrome seem to present low levels of oxidative stress associated with hyperbilirubinemia.(15)

Obesity, a low-grade inflammatory disease(16), is increasing all over the world and is a significant risk factor for cardiovascular diseases (CVD). This is of particular concern in our country, considering the very high prevalence of overweight/obesity (31.5%) in Portuguese children when compared to other European countries.(17) In obesity, cardiovascular morbidity and mortality are associated with classic risk factors, namely dyslipidemia, hypertension and impaired glucose metabolism. These risk factors, known as predictive of CVD, are characteristic of the metabolic syndrome (MS).(18) Moreover, serum bilirubin levels are inversely associated with the MS and systemic inflammation in adults(19-21), as well as in children and adolescents.(22) In particular, abdominal obesity per se seems to be associated with low serum bilirubin levels.(21-23) Furthermore, a recent study hypothesized that circulating bilirubin levels might be already altered in overweight asymptomatic middle-aged individuals before full development of the MS.(24)

The aim of our work was to evaluate how total bilirubin (TB) levels are influenced by UGT1A1*28 polymorphism, haematological, biochemical and anthropometric variables in Portuguese obese children and adolescents.

2. Material and methods

2.1. Subjects

Obese children and adolescents, aged 4-18 years, were identified from medical records, at the outpatient clinics of pediatric obesity in two hospitals in Porto - Portugal. A group of children from 5 primary and 2 middle and high public schools from Oporto suburban setting, were also recruited to this study, providing a control group and enlarging the obese group.

The study protocol was approved by the Committees on Ethics of the two hospitals, by the Review Committee of the Scientific Board of the Faculty of Sport of the University of Porto as well as by the Foundation of Science and Technology.

A total of 350 obese children and adolescents and 79 controls participated in the study after informed and written consent of their parents. Smokers, subjects with diabetes mellitus, endocrine disorders, hereditary diseases, inflammatory or infectious diseases or under any therapy that could interfere with our results were excluded from the study.

2.2. Procedures and assays

2.2.1 Anthropometric characterization and clinical evaluation

All participants were subjected to clinical examination. Height and weight were measured. Obesity was defined as body mass index (BMI) z-score greater than +1.65 for age and gender, according to 2000 Centre for Disease Control and Prevention (CDC) growth charts. Body composition was evaluated by dual-energy X-ray absorptiometry (DEXA) in a subgroup of participants (74 obese and 40 controls).

2.2.2. Blood samples

Blood was collected by venipuncture in EDTA containing tubes, after overnight fasting (10-12h) and processed within 2h of collection. Aliquots of buffy-coat and plasma were made, and immediately stored at -80^{o}C until assayed.

2.2.3. Haematological data

Red blood cell (RBC) count, haematocrit (Ht), haemoglobin (Hb) concentration and haematimetric indices [mean cell volume (MCV), mean cell Hb (MCH) and mean cell Hb concentration (MCHC)] were measured by using an automatic blood cell counter (ABX Micros 60-OT).

2.2.4. DNA analysis

Genomic DNA was extracted from buffy-coat) by proteinase K/salt precipitation method.(25,26) Genotyping TA duplication in the TATA box of the UGT1A1 promoter was performed by polymerase chain reaction (PCR) (forward: 5´-TAACTTGGTGTATCGATTGGTTTTTG-3´; reverse: 5´-ACAGCCATGGCGCCTTTGCT-3´). PCR was followed by electrophoresis in 15% polyacrylamide gel in a Tris/Borate/EDTA buffer. The gel was stained with silver nitrate, photographed and samples were classified.

2.2.5. Plasma analysis

The plasma levels of C-reactive protein (CRP) were determined by immunoturbidimetry [CRP (latex) High-Sensitivity, Roche Diagnostics] and quantification of TB was performed by a colorimetric test (diazotized sulfanilic acid reaction, Roche Diagnostics).

The determination of circulating levels of glucose and insulin was performed by using routine automated technology (ABX Diagnostics). Homeostasis model assessment of insulin resistance ($HOMA_{IR}$) was calculated.(27)

2.3. Statistical analysis

The distributions of continuous variables were analysed using Kolmogorov-Smirnov tests. Normally distributed variables are presented as mean ± SD and those non-normally distributed are presented as median (interquartile range). Comparisons between two groups were performed using Student's unpaired t-test or Mann-Whitney U test. Adjustment for confounding factors was performed using ANCOVA. The association between categorical variables was analysed using chi-squared (χ^2) test and Fisher's exact test.

The strength of the association between the variables was estimated by Pearson correlation coefficient, after log transformation of the variables (whenever necessary). To evaluate the contribution of the different variables to TB levels, multiple regression analysis was performed, using stepwise selection, with an entry criteria of $P < 0.05$.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0 (IBM, Armonk, NY, USA). Statistical significance was accepted at p less than 0.05.

3. Results

The anthropometric data, UGT1A1 genotypes and haematological parameters of the obese children and adolescents (n=350) and controls (n=79), according to gender, are presented in Table 1.

Table 1. Anthropometric data, UGT1A1*28 polymorphism, haematological and biochemical parameters of the participants in the study

	Controls (*n* =79)			Obese patients (*n* =350)				
	Females	Males	*p*	Females	Males	*p*	$p^{\dagger}$	$p^{\dagger\dagger}$
Number of participants	47	32		182	168			
Age (years)	10.5 ± 4.0	10.7 ± 3.6	0.830	11.6 ± 2.9	11.7 ± 2.9	0.559	0.083	0.113
Height (cm)	139.7 ± 17.9	143.8 ± 17.6	0.317	151.3 ± 13.2	155.4 ± 15.4	0.008	<0.001	<0.001
Weight (kg)	37.0 ± 14.6	39.7 ± 15.8	0.440	72.1 ± 22.5	76.2 ± 27.4	0.128	<0.001	<0.001
BMI (kg/m²)	18.1 ± 2.9	18.3 ± 2.9	0.691	30.7 ± 5.8	30.5 ± 6.4	0.762	<0.001	<0.001
BMI z-score	0.17 ± 0.65	0.24 ± 0.77	0.636	2.22 ± 0.34	2.30 ± 0.40	0.046	<0.001	<0.001
Body fat (%)	30.8[a] ± 4.1	25.4[b] ± 5.2	0.001	43.5[c] ± 4.1	39.8[d] ± 6.6	0.156	<0.001	<0.001
Trunk fat (%)	25.6[a] ± 4.8	21.9[b] ± 6.0	0.045	41.1[c] ± 8.9	37.8[d] ± 7.9	0.107	<0.001	<0.001
***UGT1A1* genotype**								
6/6, *n* (%)	21 (44.7%)	12 (37.5%)	0.298	92 (50.6%)	89 (53.0%)	0.433	0.455	0.085
6/7, *n* (%)	21 (44.7%)	19 (59.4%)		79 (43.4%)	64 (38.1%)			
7/7, *n* (%)	5 (10.6%)	1 (3.1%)		11 (6.0%)	15 (8.9%)			
RBC ($x10^{12}$/L)	4.62 ± 0.29	4.77 ± 0.29	0.031	4.78 ± 0.32	5.03 ± 0.39	<0.001	0.003	<0.001
Hb (g/dL)	13.1 ± 0.9	13.6 ± 1.2	0.029	13.6 ± 0.8	14.2 ± 1.2	<0.001	0.001	0.017
Ht (L/L)	0.39 ± 0.03	0.40 ± 0.04	0.263	0.40 ± 0.02	0.42 ± 0.03	<0.001	0.033	0.003
MCV (fL)	84.9 ± 4.6	84.0 ± 6.1	0.486	84.2 ± 5.1	83.8 ± 4.7	0.454	0.432	0.846
MCH (pg)	28.4 ± 1.7	28.6 ± 2.0	0.684	28.5 ± 1.7	28.2 ± 1.6	0.185	0.909	0.239
MCHC (g/dL)	33.4 ± 1.2	34.0 ± 1.1	0.025	33.8 ± 1.0	33.7 ± 1.1	0.271	0.027	0.081
Total bilirubin (µmol/l)	8.89 (5.47-13.34)	7.52 (5.30-11.54)	0.463	8.89 (6.16-11.63)	9.23 (6.84-12.65)	0.232	0.919	0.079
Acute phase protein								
CRP (mg/L)	0.26 (0.20-0.73)	0.36 (0.26-0.83)	0.121	1.83 (0.85-3.73)	1.64 (0.85-3.54)	0.527	<0.001	<0.001
Glucose metabolism								
Glucose (mg/dl)	85.3 ± 9.3	87.0 ± 6.5	0.365	84.0 ± 8.9	85.8 ± 12.6	0.121	0.384	0.419
Insulin (µU/ml)	6.8 (5.0-9.9)	5.3 (4.1-8.5)	0.051	16.6 (11.7-23.2)	12.8 (9.1-20.0)	0.001	<0.001	<0.001
$HOMA_{IR}$	1.41 (1.06-2.05)	1.14 (0.82-1.83)	0.130	3.39 (2.21-4.87)	2.75 (1.88-4.06)	0.006	<0.001	<0.001

Values are given as mean ± SD or median (interquartile range), unless otherwise indicated.BMI, body mass index; RBC, red blood cells; Hb, haemoglobin; Ht, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; CRP, C-reactive protein; $HOMA_{IR}$, homeostasis model assessment insulin resistance.a, n=25; b, n=15; c, n=34; d, n=40. † Controls versus obese patients (females); †† Controls versus obese patients (males)

Comparing males and females within the control group, body fat and trunk fat percentages were significantly lower for boys, whereas RBC count, Hb levels and MCHC values were significantly higher. Within obese patients, RBC count, Hb levels and Ht values were significantly higher for boys, whereas insulin levels and $HOMA_{IR}$ values were lower. No

statistical significant differences were found in the distribution of subjects with respect to UGT1A1 genotypes or in TB levels between boys and girls, within both groups.

Compared to controls (independently of gender), obese patients presented significantly higher height, weight, BMI, BMI z-score, body fat and trunk fat percentages, erythrocyte count, Ht and $HOMA_{IR}$ values and Hb, insulin and CRP levels, but no significant differences in TB levels or in UGT1A1 genotype distribution.

The UGT1A1 genotype frequencies in all studied individuals were 49.9%, 42.7% and 7.5% for 6/6, 6/7 and 7/7 genotypes, respectively. UGT1A1*28 polymorphism was associated with different TB levels (Figure 1A); patients with 7/7 genotype presented the highest TB levels, followed by 6/7 and 6/6 genotypes (p<0.01 between all groups). No significant differences were observed between obese and control individuals, for the different UGT1A1 genotypes (Figure 1B).

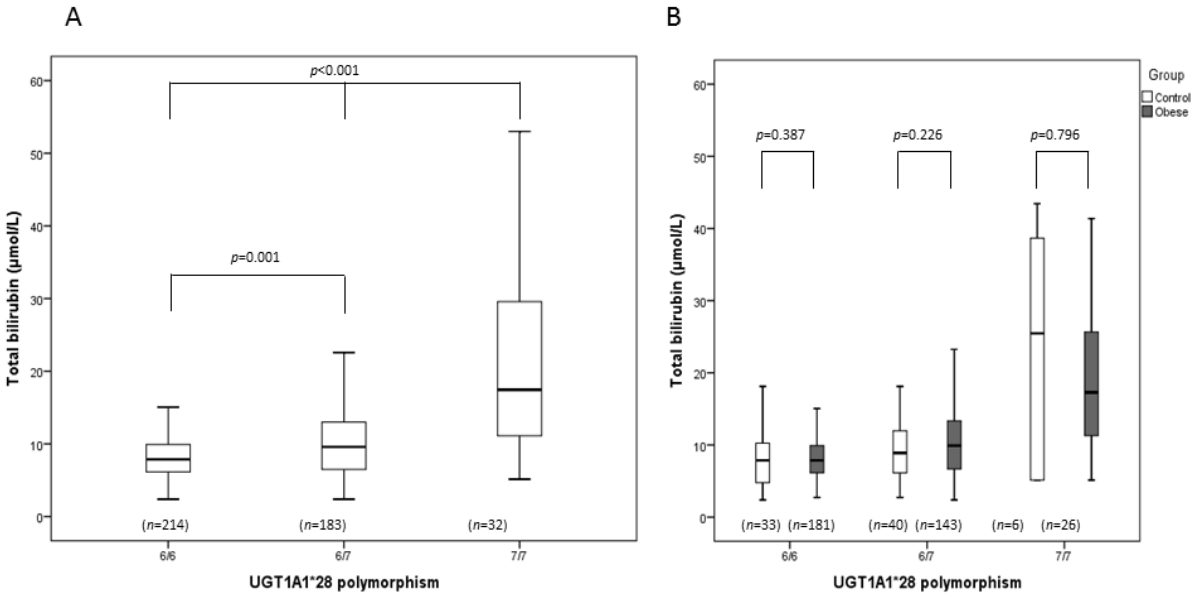


Figure 1. **Total bilirubin levels in all participants according to the number of TA repeats in the promoter region of UGT1A1 gene (A) and also according to group (B), control and obese**

The boxes represent the interquartile range (IQR), with the upper and lower edges of the boxes representing the 75th and 25th percentiles, respectively. The central horizontal lines within the boxes represent median levels for each group. The vertical whiskers above and below the boxes represent the range of outlying data points up to 1.5 times the IQR.

Within the control group (n=79), TB levels correlated positively and significantly with age (r=0.304, p=0.007), height (r=0.360, p=0.001), weight (r=0.390, p<0.001), BMI (r=0.370, p=0.001), Ht (r=0.247, p=0.028), MCV (r=0.292, p=0.009), and correlated negatively and significantly with MCHC (r=-0.258, p=0.022). Within the obese group (n=350), TB levels correlated positively and significantly with age (r=0.284, p<0.001), height (r=0.285,

p<0.001), weight (r=0.219, p<0.001), BMI (r=0.123, p=0.021), Hb (r=0.305, p<0.001), Ht (r=0.352, p<0.001), MCV (r=0.394, p<0.001), MCH (r=0.301, p<0.001) and correlated negatively and significantly with BMI z-score (r=-0.131, p=0.014), MCHC (r=-0.149, p=0.006) and CRP (r =-0.178, p=0.001).

The characteristics of obese patients that evaluated their body composition by DEXA (n=74) are presented in Table 2. These obese patients were divided in two groups according on having a body fat lower or higher/equal than 42.5% (cut-off that corresponds to the median value for the obese group). The two groups of obese patients were matched for gender and UGT1A1 genotype distribution, but not for age. Patients presenting higher body fat had lower bilirubin and higher CRP levels (Table 2). These differences were similar to both sexes (Figure 2) and remained statistically significant after adjustment for age. No significant differences in $HOMA_{IR}$ values were found between the two groups.

Associations between body and trunk fat were only accessed in participants that evaluated their body composition by DEXA (74 obese and 40 controls). Body fat and trunk fat percentages were negatively and significantly related with TB levels in obese patients (r =-0.287, p=0.013 and r=-0.245, p=0.038) but not within controls (r=0.012, p=0.941 and r=0.014, p=0.002).

Table 2. Anthropometric data, UGT1A1*28 polymorphism, haematological and biochemical parameters of obese patients according to body fat percentage (n=74) lower or higher/equal than 42.5% (median value for the obese group)

	Obese patients (*n* =74)		
	Body fat ≤ 42.5%	Body fat > 42.5%	*p*
Number of participants	37	37	
Female, *n* (%)	13 (35.1%)	21 (56.8%)	0.102
Age (years)	11.0 ± 3.0	9.5 ± 2.5	0.022
Height (cm)	149.2 ± 14.3	144.0 ± 14.2	0.126
Weight (kg)	59.8 ± 18.9	61.4 ± 23.9	0.749
BMI (kg/m²)	26.0 ± 4.0	28.4 ± 5.5	0.041
BMI z-score	1.98 ± 0.24	2.31 ± 0.26	<0.001
Body fat (%)	36.8 ± 4.3	46.1 ± 2.6	<0.001
Trunk fat (%)	34.5 ± 5.5	45.3 ± 3.4	<0.001
***UGT1A1* genotype**			
6/6, *n* (%)	21 (56.8%)	19 (51.4%)	0.359
6/7, *n* (%)	14 (37.8%)	18 (48.6%)	
7/7, *n* (%)	2 (5.4%)	0 (0%)	
RBC ($x10^{12}$/L)	4.83 ± 0.38	4.91 ± 0.34	0.389
Hb (g/dL)	13.9 ± 1.0	13.6 ± 0.8	0.268
Ht (L/L)	0.41 ± 0.03	0.41 ± 0.02	0.798
MCV (fL)	85.4 ± 4.9	83.8 ± 4.6	0.156
MCH (pg)	28.7 ± 1.7	27.8 ± 1.5	0.017
MCHC (g/dL)	33.6 ± 0.8	33.2 ± 0.9	0.020
Total bilirubin (µmol/l)	11.29 (8.72-14.36)	8.89 (7.69-11.63)	0.013
Acute phase protein			
CRP (mg/L)	1.31 (0.84-2.30)	2.00 (1.43-3.54)	0.017
Glucose metabolism			
Glucose (mg/dl)	83.5 ± 7.6	81.0 ± 8.6	0.191
Insulin (µU/ml)	11.6 (8.9-14.6)	15.3 (7.5-22.9)	0.272
$HOMA_{IR}$	2.25 (1.91-3.01)	3.15 (1.57-4.56)	0.361

Values are given as mean ± SD or median (interquartile range), unless otherwise indicated. BMI, body mass index; RBC, red blood cells; Hb, haemoglobin; Ht, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; CRP, C-reactive protein; $HOMA_{IR}$, homeostasis model assessment insulin resistance.

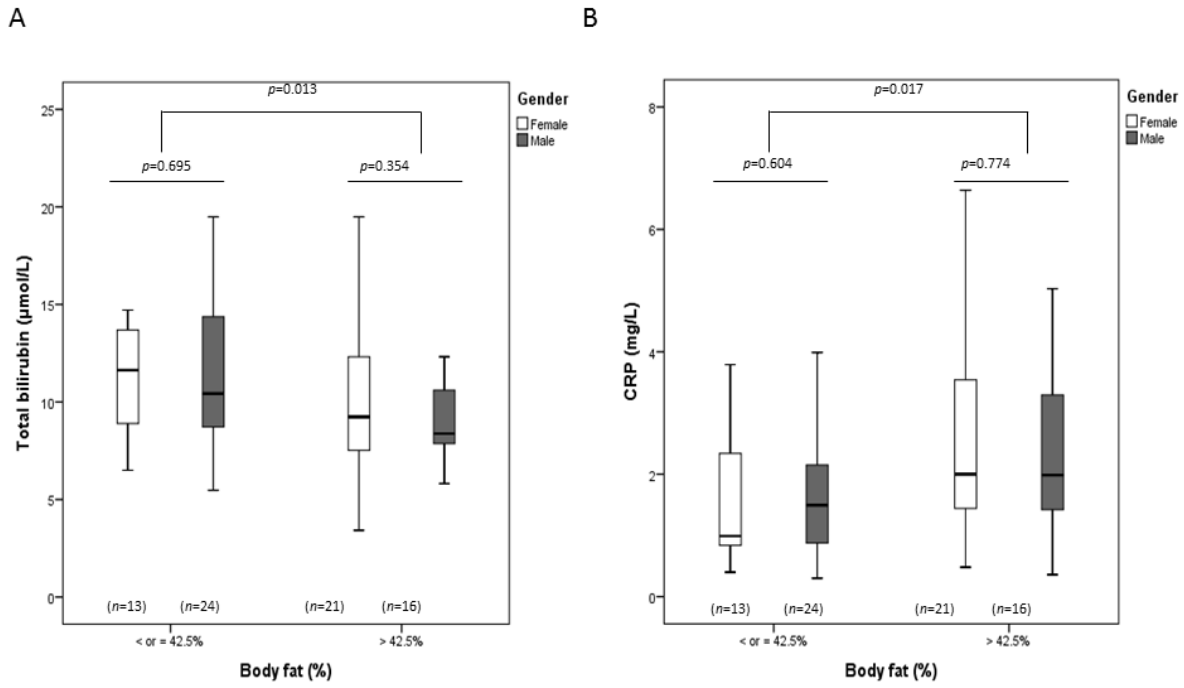


Figure 2. Total bilirubin (A) and C-reactive protein (B) levels in obese participants according to gender and to body fat percentage (n=74), using the cut-off value of 42.5 % (cut-off that corresponds to the median value for the obese group)

The boxes represent the interquartile range (IQR), with the upper and lower edges of the boxes representing the 75th and 25th percentiles, respectively. The central horizontal lines within the boxes represent median levels for each group. The vertical whiskers above and below the boxes represent the range of outlying data points up to 1.5 times the IQR.

By linear regression analysis, the UGT1A1*28 polymorphism and body weight were the only factors associated to bilirubin levels within controls (Ln TB= 1.143 +0.462 UGT1A1*28 polymorphism +0.014 weight; standardised Beta: 0.598 and 0.490; p<0.001 and p=0.001, respectively). Within obese patients, the UGT1A1 polymorphism and body fat percentage were the main determinant factors of bilirubin levels (Ln TB= 2.761 +0.251 UGT1A1*28 polymorphism -0.020 body fat; standardised Beta: 0.348, -0.291; p=0.002 and p=0.009, respectively). For a better visualization of the results (graphically), obese participants were divided on the basis of their UGT1A1 genotype and on having a body fat lower or higher/equal than 42.5% (cut-off that corresponds to the median value for the obese group; Figure 3).

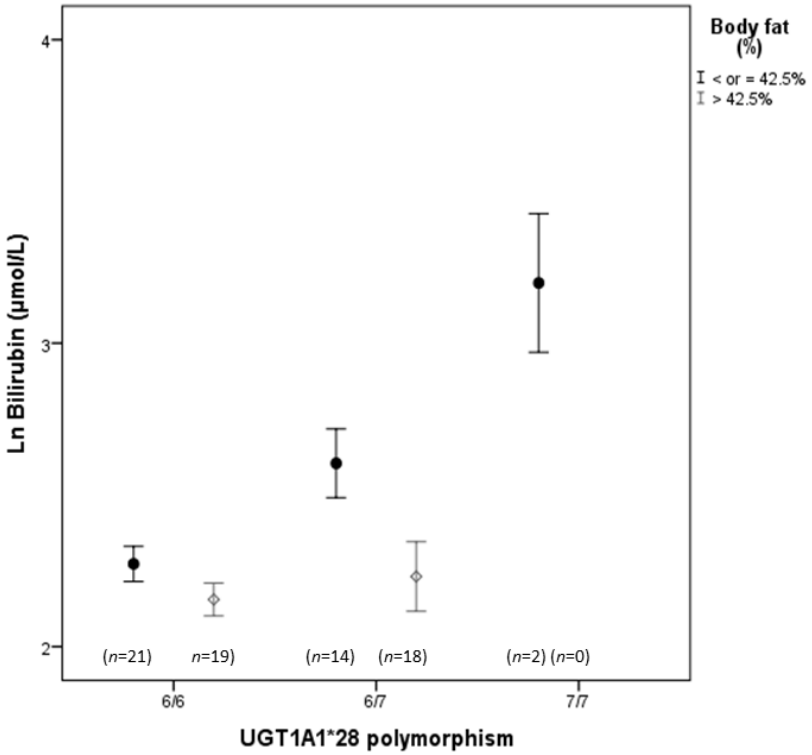


Figure 3. **Effect of body fat percentage on total bilirubin levels according to UGT1A1*28 polymorphism on obese patients**

For a better visualization of the results we used for body fat percentage a cut-off of 42.5% (cut-off that corresponds to the median value for the obese group). Results are presented as mean ± standard error of mean. The influence of body fat percentage, adjusted for UGT1A1 polymorphism, on total bilirubin levels, was highly significant (p = 0.009), by multiple regression analysis.

4. Discussion

As far as we know, this is the first report assessing the concomitant influence of UGT1A1*28 polymorphism and adiposity markers on bilirubin levels in obese children and adolescents. We demonstrated that body fat percentage is a major determinant of TB levels independently of UGT1A1*28 polymorphism in obese children and adolescents.

It is known that UGT1A1 polymorphisms are associated with bilirubin levels and our data is in agreement with previous reports in young patients and adults.(15,28-31) Patients and controls with 7/7 genotype presented the highest TB levels, followed by 6/7 and 6/6 genotypes (Figure 1A and 1B).

The frequency of 7/7 homozygotes (7.5% in the whole population) was lower than that observed in other works, namely in healthy Greek(29) and Slovenian(30) populations, with frequencies of 14.8% and 13.6%, respectively. However, the distribution of subjects with respect to UGT1A1 genotypes was similar to that found in previous studies involving Portuguese children with Hereditary spherocytosis, with a 7/7 frequency of 8.8%(28), as well as Portuguese healthy subjects, with frequencies observed in two studies of 6.3 and 9.9%.(28,31) Thus, it seems reasonable to assume that the frequency of 7/7 homozygotes in the Portuguese population may be lower than that observed in other Caucasian populations.

Other potential variables could influence TB levels. Within both controls and obese patients TB levels were positively and significantly correlated with age, height, weight, BMI, and Ht. However, BMI z-score, body fat and trunk fat percentages were negatively and significantly related with TB levels in obese patients, but not within controls. In multiple regression analysis, the UGT1A1*28 polymorphism and body weight were the only factors associated to bilirubin levels within controls, whereas the UGT1A1*28 polymorphism and body fat percentage were the main determinant factors of bilirubin levels within obese patients.

In the present study, the evaluation of body composition by DEXA was performed in a subgroup of participants. Despite the lower number of participants in this sub-analysis, the negative relation between bilirubin and body fat percentage was highly statistically significant and independent of the effect of UGT1A1*28 polymorphism. Furthermore, this negative relation is in agreement with a previous study involving 41 lean and obese adult men and women.(23)

Bilirubin derives mainly from the haem present in Hb, released during breakdown of senescent erythrocytes.(1) Thus, in healthy conditions, it would be assumed that increases in Hb levels are generally associated with increases in TB. This explains our positive association between the age of the participants and TB, as in young individuals there is a physiological increase in Hb levels with age. It is well known that Hb and Ht

increase substantially during childhood, whereas RBC count remains almost constant.(32) Differences according to gender become prominent in the second decade of life; with menstruation, these three variables tend to be lower in females. The inclusion in our study of subjects with a range of age between 4 and 18 years old justifies the higher values of RBC and Hb observed in males within both controls and obese patients (Table 1). The differences were particularly evident in obese patients, not only because of the large number of subjects included in this group but also due to the enhanced puberty development in obesity. Actually, the increasing prevalence of obesity in children worldwide is a major cause of precocious pubertal maturation, verified during the past decades.(33)

Total and direct bilirubin levels were reported to be higher in normal weight adult males than in females(24,34,35), but similar within overweight patients.(24) In our study, we observed no statistical significant differences between boys and girls regarding TB levels, either in controls or in obese patients. Within these two groups, males and females were matched for age, weight, BMI and distribution of UGT1A1 genotypes (Table 1) and, therefore, the analysis of TB was not greatly affected by these factors.

In the present manuscript, obese patients and controls were matched for age and distribution of UGT1A1 genotypes, allowing the comparison of groups. Independently of gender, obese patients presented higher RBC count, Hb levels and Ht values compared with controls (Table 1). The higher weight and BMI in obese patients trigger the stimulation of erythropoiesis in order to supply adequate oxygenation to increased body tissues. However, TB levels were similar between groups. This may be explained by the fact that obesity is associated with increased inflammation(16,36,37) and oxidative stress(38,39), and that bilirubin, presenting antioxidant and anti-inflammatory properties(9,10), may be somewhat consumed. In fact, oxidative stress increases with increasing BMI and age.(34) In line with this, we found that bilirubin levels are negatively correlated with body and trunk fat percentages and CRP levels within obese patients. Moreover, when obese patients were divided in two groups according to the median value of body fat presented by this group (42.5%), patients presenting higher body fat presented lower bilirubin and higher CRP levels (Table 2). The negative relation that we found between bilirubin and CRP levels is in line with the bilirubin's anti-inflammatory activity, as previously reported.(40-43)

In obese patients, insulin resistance may also underlie the association between lower bilirubin levels and higher body fat percentages. Indeed, it seems that the activity of heme oxygenase-1, the rate-limiting enzyme of bilirubin production, is impaired in insulin resistant states.(44,45) Also, the up-regulation of heme oxygenase-1 in adipocyte by insulin was recently demonstrated.(46) In this work obese patients presented with higher

$HOMA_{IR}$ values compared to controls (Table 1). Obese patients with body fat percentages higher than 42.5% also presented with higher $HOMA_{IR}$ values, although without statistical significance. However, no significant correlation was obtained between $HOMA_{IR}$ and bilirubin. Thus, association between insulin resistance and bilirubin might not be so clear in paediatric populations.

A previous work from our group demonstrated that BMI z-score is significantly and independently related to the lipid profile in obese children and adolescent.(47) However, in the present study BMI z-score was poorly related with TB levels in obese patients and it was not an independent predictor of bilirubin plasma concentration. This suggests that body fat percentage is a better indirect marker of oxidative stress, rather than BMI z-score. Actually BMI z-score is calculated using the BMI of patients, adjusted to age and gender, but it may not necessarily express the degree of obesity.

Individuals with a higher physical fitness index (which serves as an aerobic assessment) seem to present with higher bilirubin levels(24) and a study performed in overweight and obese adult patients demonstrated an increase in bilirubin levels due to short-term weight loss.(35) It seems that high doses of exercise training are necessary to significantly increase bilirubin levels in overweight and obese women.(48) The fact that bilirubin levels increase as a function of weight loss may be of particular importance in obese individuals with UGT1A1 genotypes associated to lower bilirubin levels, as we here demonstrated effects on TB by body fat composition in addition to the UGT1A1*28 polymorphism. It is important to keep in mind that atherosclerosis is a multifactorial disease that initiates early in life, involving the interplay of genetic and environmental factors. The lifestyle improvement is conditioned by environmental factors (such as nutritional behaviour and practice of physical activity) and may be particularly worthy in obese individuals with a less favourable genetic background.

Despite the new data reported here, this work presented some limitations. Obesity was defined according to CDC although a novel criteria is now recommended for the Portuguese population, causing us to have probably underestimated the degree of obesity. Nevertheless, at the beginning of this study the criteria recommend by the Portuguese Ministry of Health was that of CDC. Also, the evaluation of body composition by DEXA was performed only in a subgroup of participants due to logistical constraints and equipment availability. Furthermore, we did not evaluate the association between bilirubin and the MS as a large proportion of our obese patients were under the age of 10, not allowing their classification according to the International Diabetes Federation (IDF) definition.

In conclusion, body fat percentage is a major determinant of TB levels independently of UGT1A1*28 polymorphism in obese children and adolescents. This may have a particular

relevance, as obese individuals, particularly those with 6/6 UGT1A1 genotype and higher body fat mass, may benefit from a closer clinical follow-up, considering their increased risk for other comorbidities. Moreover, lifestyle modifications at low ages, when good habits can be created, should be highly encouraged in such obese children and adolescents.

Acknowledgements

The authors wish to thank the technician team from Porto Hospital Centre and Hospital São João for their expert assistance on blood collection. This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-028613 (PTDC/DTP-DES/0393/2012). A PhD grant was attributed to H. Nascimento by FCT (SFRH/BD/48060/2008).

References

1. Fevery J. Bilirubin in clinical practice: a review. Liver International. 2008; 28:592–605.
2. Gong QH, Cho JW, Huang T, et al. Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics. 2011;11:357–368.
3. Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert`s syndrome. New England Journal of Medicine. 1995; 333:1171–1185.
4. Beutler E, Gelbart T, Bemina A. Racial variability in the UDPglucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proceedings of the National Academy of Sciences USA. 1998; 95:8170–8174.
5. Maruo Y, D'Addario C, Mori A, et al. Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide. Drug Metabolism Disposition. 2005; 33:458–465.
6. Maruo Y, D'Addario C, Mori A, et al. Inheritance of hyperbilirubinemia: evidence for a major autosomal recessive gene. Digestive and Liver Disease. 2007; 39:351–355.
7. Tiribelli C, Ostrow JD. The molecular basis of bilirubin encephalopathy and toxicity: report of an EASL Single TopicvConference. Journal of Hepatology. 2005; 43:156–6.
8. Gourley GR. Bilirubin metabolism and kernicterus. Advances in Pediatrics. 1997; 44:173–229.
9. Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochemical Pharmacology. 1996; 51:859–62.
10. Weinberger B, Archer FE, Kathiravan S, et al. Effects of bilirubin on neutrophil responses in newborn infants. Neonatology. 2013; 103:105–11.
11. Novotny L, Vitek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. Experimental Biology and Medicine. 2003; 228:568–71.
12. Lin JP, O'Donnell CJ, Schwaiger JP, et al. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. Circulation. 2006; 114:1476–81.
13. Horsfall LJ, Nazareth I, Petersen I. Serum bilirubin level measured before a statin prescription to assess liver function is an independent risk factor for CVD and death in both men and women. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. Circulation. 2012; 126:2556–64.
14. Temme EH, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. Cancer Causes and Control. 2001; 12:887–94.
15. Maruhashi T, Soga J, Fujimura N, et al. Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. Circulation. 2012; 126:598–603.
16. Nascimento H, Costa E, Rocha-Pereira P, et al. Cardiovascular risk factors in portuguese obese children and adolescents: impact of small reductions in body mass index imposed by lifestyle modifications. The Open Biochemical Journal. 2012; 6:43–50.
17. Padez C, Fernandes T, Mourão I, Moreira P, Rosado V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. American Journal of Human Biology. 2004; 16:670–8.
18. Alexander CM, Landsman PB, Grundy SM. The influence of age and body mass index on the metabolic syndrome and its components. Diabetes, Obesity and Metabolism. 2008; 10:246–50.
19. Hwang H-J, Kim S-H. Inverse relationship between fasting direct bilirubin and metabolic syndrome in Korean adults. Clinical Chimica Acta. 2010; 411:1496–501.
20. Jo J, Yun JE, Lee H, et al. Total, direct, and indirect serum bilirubin concentrations and metabolic syndrome among the Korean population. Endocrine. 2010; 39:182–9.
21. Wu Y, Li M, Xu M, et al. Low serum total bilirubin concentrations are associated with increased prevalence of metabolic syndrome in Chinese. Journal of Diabetes. 2011; 3:217–24.
22. Lin L-Y, Kuo H-K, Hwang J-J, et al. Serum bilirubin is inversely associated with insulin resistance and metabolic syndrome among children and adolescents. Atherosclerosis. 2009; 203:563–8.
23. Devries MC, Samjoo IA, Hamadeh MJ, Tarnopolsky MA. Effect of endurance exercise on hepatic lipid content, enzymes, and adiposity in men and women. Obesity (Silver Spring). 2008; 16:2281–8.
24. Jenko-Pražnikar Z, Petelin A, Jurdana M, Ziberna L. Serum bilirubin levels are lower in overweight asymptomatic middle-aged adults: An early indicator of metabolic syndrome? Metabolism. 2013; 62(7:976–85.
25. Olerup O, and Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens. 1992; 39:225–235.
26. Gaffney D, Campbell RA. A PCR based method to determine the kalow allele of the cholinesterase gene: the E_1^k allele frequency and its significance in the normal population. Journal of Medical Genetics. 1994; 31:248–250.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28:412–9.
28. Rocha S, Costa E, Ferreira F, et al. Hereditary spherocytosis and the (TA)nTAA polymorphism of UGT1A1 gene promoter region - a comparison of the bilirubin plasmatic levels in the different clinical forms. Blood Cells and Molecular Diseases. 2010; 44:117–9.
29. Karatzas A, Giannatou E, Tzortzis V, et al. Genetic polymorphisms in the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene and prostate cancer risk in Caucasian men. Cancer Epidemiology. 2010; 34:345–9.
30. Ostanek B, Furlan D, Mavec T, Lukac-Bajalo J. UGT1A1(TA)n promoter polymorphism--a new case of a (TA)8 allele in Caucasians. Blood Cells and Molecular Diseases. 2007 Mar-Apr;38(2):78–82.
31. Rodrigues C, Vieira E, Santos R, et al. Impact of UGT1A1 gene variants on total bilirubin levels in Gilbert syndrome patients and in healthy subjects. Blood Cells Molecular Diseases. 2012 ;48:166–72.
32. Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr 1984; 39:427–36.
33. Kim SH, Park MJ. Childhood obesity and pubertal development. Pediatric Gastroenterology Hepatol Nutr. 2012; 15:151–159.
34. Wonisch W, Falk A, Sundl I, Winklhofer-Roob BM, Lindschinger M. Oxidative stress increases continuously with BMI and age with unfavourable profiles in males. The Aging Male. 2012; 15:159–65.
35. Andersson C, Weeke P, Fosbøl EL, et al. Acute effect of weight loss on levels of total bilirubin in obese, cardiovascular high-risk patients: an analysis from the lead-in period of the Sibutramine Cardiovascular Outcome trial. Metabolism: Clinical and Experimental. 2009; 58:1109–15.

36. Taube A, Schlich R, Sell H, Eckardt K, Eckel J. Inflammation and metabolic dysfunction: links to cardiovascular disease. American Journal Physiology Heart Circulation Physiology 2012; 302:H2148–65.
37. Greco EA, Francomano D, Fornari R, et al. Negative association between trunk fat, insulin resistance and skeleton in obese women. World Journal of Diabetes. 2013; 4:31–9.
38. Bondia-Pons I, Ryan L, Martinez JA. Oxidative stress and inflammation interactions in human obesity. Journal of Physiology and Biochemistry. 2012; 68:701–11.
39. D'Archivio M, Annuzzi G, Varì R, et al. Predominant role of obesity/insulin resistance in oxidative stress development. European Journal of Clinical Investigation. 2012; 42:70–8.
40. Hwang H-J, Lee S-W, Kim S-H. Relationship between bilirubin and C-reactive protein. Clinical Chemistry and Laboratory Medicine: CCLM/FESCC 2011; 49:1823–8.
41. Ohnaka K, Kono S, Inoguchi T, et al. Inverse associations of serum bilirubin with high sensitivity C-reactive protein, glycated hemoglobin, and prevalence of type 2 diabetes in middle-aged and elderly Japanese men and women. Diabetes Research and Clinical Practice. 2010; 88:103–10.
42. Yoshino S, Hamasaki S, Ishida S, et al. Relationship between bilirubin concentration, coronary endothelial function, and inflammatory stress in overweight patients. Journal of Atherosclerosis and Thrombosis. 2011; 18:403–12.
43. Yu K, Kim C, Sung E, et al. Association of serum total bilirubin with serum high sensitivity C-reactive protein in middle-aged men. Korean Journal of Family Medicine. 2011; 32:327–33.
44. Abraham NG. Heme oxygenase: a target gene for anti-diabetic and obesity. Current Pharmaceutical Design. 2008; 14:412–21.
45. Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 Diabetes. Diabetes. 2003; 52:2338–45.
46. Chang CL, Au LC, Huang SW, Fai Kwok C, Ho LT, Juan CC. Insulin up-regulates heme oxygenase-1 expression in 3T3-L1 adipocytes via PI3-kinase- and PKC-dependent pathways and heme oxygenase-1-associated microRNA downregulation. Endocrinology. 2011; 152:384–93.
47. Nascimento H, Silva L, Lourenço P, et al. Lipid profile in Portuguese obese children and adolescents: interaction of apolipoprotein E polymorphism with adiponectin levels. Archives Pediatric & Adolescent Medicine. 2009; 163:1030–6.
48. Swift DL, Johannsen NM, Earnest CP, Blair SN, Church TS. Effect of different doses of aerobic exercise training on total bilirubin levels. Medicine & Science in Sports & Exercise. 2012; 44:569–74.

14.5. Paper V

Nascimento H, Rocha S, Rego C, Mansilha HF, Quintanilha A, Santos-Silva A, Belo L. "Leukocyte count versus C-reactive protein levels in obese Portuguese patients aged 6-12 years old". The Open Biochemistry Journal. 2010; 4:72-76.

Leukocyte Count *versus* C-Reactive Protein Levels in Obese Portuguese Patients Aged 6-12 Years Old

Henrique Nascimento*[,1,2], Susana Rocha[1,2], Carla Rego[3], Helena Ferreira Mansilha[4], Alexandre Quintanilha[5], Alice Santos-Silva[1,2] and Luís Belo*[,1,2]

[1]*Department of Biochemistry, Faculty of Pharmacy, University of Porto, 4050-047 Porto, Portugal*

[2]*Institute for Molecular and Cell Biology (IBMC), University of Porto, 4150-180 Porto, Portugal*

[3]*Nutrition Unit / Paediatric Service. UAG-MC. Hospital de S. João E.P.E. Faculty of Medicine of Porto University; 4200-319 Porto, Portugal*

[4]*Paediatric Department of Maria Pia Children's Hospital; 4050-111, Porto, Portugal*

[5]*Institute of Biomedical Sciences of Abel Salazar (ICBAS), University of Porto, 4099-003 Porto, Portugal*

Abstract: *Objectives*: to evaluate whether total and differential WBC counts are altered in young obese patients (aged 6-12 years) and if a relationship exists between WBC counts and the severity of obesity as well as with CRP level.

Materials and Methods: a group of 77 obese patients [32 males and 45 females] and 19 controls [7 males and 12 females] were studied. Total WBC count was performed by using an automatic blood cell counter. Blood cell morphology and WBC differential count were evaluated in Wright stained blood films. The plasma levels of CRP were evaluated by immunoturbidimetry.

Results: obese participants presented with a statistically significant higher neutrophil percentage and CRP levels when compared to controls; the median CRP value was about 5 times higher than that observed in controls. Absolute neutrophil count and neutrophil/lymphocyte ratio were also higher in patients, though without statistical significance. The parameters that were statistically significant related with adiposity markers were neutrophil count and CRP levels. The neutrophil count was positively and statistically correlated with body mass index (BMI), BMI z-score, waist circumference and waist/height ratio, and also with CRP levels. In multiple regression analysis, the only variable that remained statistically associated with neutrophil count was CRP (neutrophil count = 2.612 + 0.439lnCRP; standardised coefficient/beta: 0.384, P=0.001). When performing multiple regression without CRP, the only variable that remained statistically associated with neutrophil count was BMI.

Conclusions: our results demonstrated in obese patients aged 6-12 years, a significant change in the differential leukocyte count towards neutrophilia, together with a significant higher CRP concentration, and that absolute neutrophil count correlates with obesity markers and with CRP levels. Our data also indicate that neutrophil count, a current clinically used low-cost parameter, may be used as an obesity-related inflammatory marker in young obese patients.

Keywords: Leukocytes, C-reactive protein, children obesity.

1. INTRODUCTION

Obesity is increasing all over the world, particularly in industrialised countries, and is a significant risk factor for cardiovascular disease (CVD). This is of particular concern in our country, as demonstrated by the high prevalence of overweight/obesity (31.5%) in Portuguese children, compared to other European countries [1]. Furthermore, Portugal exhibits a high rate of CVD, particularly of cerebrovascular disease.

Cardiovascular morbidity and mortality of obesity is associated with classic risk factors, namely dyslipidemia, hypertension and impaired glucose metabolism [2, 3]. Recently, it was shown that obesity may be regarded as a state of low-grade inflammation [3, 4] and atherosclerosis is accepted as an inflammatory process [5]. The adipocyte is an important source of cytokines, namely interleukin (IL)-6 and tumour necrosis factor (TNF)-alpha, and their levels are significantly higher in the plasma of obese patients [4, 6]. The rise in these cytokines, especially in IL-6, triggers an increased synthesis of C-reactive protein (CRP), one of the most sensitive makers of inflammation [7]. However, and although obesity is recognized as a possible cause for reactive leukocytosis [8], the contribution of leukocytes to the inflammatory process in obese patients is not fully understood.

*Address correspondence to these authors at the Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal; Tel: +351-222078906; Fax: +351-222003977;
E-mail: henriqueferrao@hotmail.com
Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal; Tel: +351-222078906; Fax: +351-222003977; E-mail: luisbelo@ff.up.pt

1874-091X/10

To date, limited investigations have examined the associations between white blood cell (WBC) count and obesity in children and adolescents. A National Health and Nutrition Examination Survey of the US population demonstrated that overweight is associated with higher WBC count in children [9]. Furthermore, a recent study performed in female obese adolescents showed that WBC count is positively related to body mass index (BMI), waist circumference, and total adipose tissue (TAT) and subcutaneous adipose tissue (SAT) areas [10]. After adjustment for metabolic risk factors, only neutrophil counts were positively related to BMI, waist circumference, and TAT; lymphocyte and basophil counts were negatively related to BMI and waist circumference [10]. This study was performed only in female adolescents (aged 10-19 years) and no control group was used.

It is still uncertain which WBC subfractions are more altered in obese children and adolescents, and how prematurely these changes occur in obesity. Thus, the aim of our work was to evaluate whether total and differential WBC counts are altered in young obese patients (aged 6-12 years) and if a relationship exists between WBC counts and the severity of obesity (according to BMI z-score, waist circumference and waist/height ratio) as well as with CRP level, a known sensitive marker of inflammation.

2. MATERIAL AND METHODS

2.1. Subjects

The protocol used for all participants was approved by the Committees on Ethics of the University Hospital S. João and of the Children's Hospital Maria Pia, Porto. Obese patients, aged 6-12 years, were identified from medical records at the Departments of Pediatrics of Hospital Maria Pia and Hospital S. João, Porto. All children that reached inclusion criteria were invited to participate in the study. Seventy seven obese children participated in the study after informed and written consent of their parents. The study took place between May 2006 and March 2007.

Healthy control subjects, age and sex matched with obese patients, were recruited from the general population.

Obesity was defined as BMI greater than the 95th percentile for age and gender, according to 2000 Centre for Disease Control and Prevention (CDC) growth charts. Because BMI is not normally distributed, we used body-mass index z-score (BMI z-sc); BMI z-sc values were achieved by using a calculator that has per basis the 2000 CDC growth charts.

Clinical data regarding the sample population was collected. The physical examination included the measurement of height, weight, waist circumference, and the presence of skin lesions related with obesity and its co-morbidities.

The participants were invited to the research centers after an overnight fast and, after clinical examination, blood was collected for laboratory analysis.

Smokers, subjects with diabetes *mellitus*, endocrine disorders, hereditary diseases, inflammatory or infectious diseases or under any therapy that could interfere with our results were excluded from the study.

2.2. Procedures and Assays

2.2.1. Blood Samples

Fasted blood samples were obtained and processed within 2h of collection. Blood was obtained by venipuncture in EDTA containing tubes. Plasma aliquots were made and immediately stored at – 70^{o}C until assayed.

2.2.2. Laboratory Analysis

Total WBC count was performed by using an automatic blood cell counter (ABX Micros 60-OT). Blood cell morphology and WBC differential count were evaluated in Wright stained blood films. The plasma levels of CRP were evaluated by immunoturbidimetry [CRP (latex) High-Sensitivity, Roche Diagnostics].

2.3. Statistical Analysis

Statistic analysis was performed using the Statistical Package for Social Sciences (SPSS, version 17.0) for Windows. Kolmogorov-Smirnov analysis was used to test if the results were normally distributed. The results normally distributed are presented as mean ± SD and those not-normally distributed are presented as median (interquartile range).

Controls and obese patients were compared using Student's unpaired *t* test or Mann-Whitney *U* test. The distribution of males and females with respect to categorical variables was analysed using chi-squared (χ^2) test and Fisher's exact test.

The strength of the association between the substances was estimated by Pearson correlation coefficient, after logarithmic transformation of the variables (when necessary). To evaluate the contribution of the different variables to neutrophil count, we performed multiple regression analysis, using stepwise selection, with an entry criteria of $P < 0.05$. Significance was accepted at P less than 0.05.

3. RESULTS

The demographic and clinical characteristics of the obese participants (n=77) and controls (n=19) are presented in Table **1**. The groups were matched for age and gender; as expected, BMI and BMI z-sc values were significantly higher in patients.

Obese participants presented with a statistically significant higher neutrophil percentage and CRP levels (Table **2**). Absolute neutrophil count and neutrophil/l ymphocyte ratio were also higher in patients, though without statistical significance.

Concerning the comparison between boys and girls, no statistical significant differences were observed within both groups (data not shown).

The correlations between CRP levels and the absolute count for each type of leukocyte with adiposity markers are presented in Table **3**. The only parameters that were statistically significant related with adiposity markers were neutrophil count and CRP levels. The neutrophil count was positively and statistically correlated with BMI, BMI z-sc, waist circumference and waist/height ratio, and also with CRP levels. In the multiple regression analysis, the only

Table 1. Characteristics of the Studied Subjects

	Controls (*n* = 19)	Obese patients (*n* = 77)	*P*
Age (years)	8.5 ± 1.6	9.1 ± 1.6	0.133
Gender male (*n*)	7 (36.8%)	32 (41.6%)	0.708
BMI (kg/m^2)	16.27 ± 1.54	27.48 ± 3.73	<0.001
BMI z-sc	0.005 ± 0.820	2.331 ± 0.317	<0.002
Waist circumference (cm)	-	86.9 ± 10.2	-
Waist/Height ratio	-	0.609 ± 0.054	-

Values are given as mean ± SD, unless otherwise indicated.

Table 2. Total and Differential Leukocyte Counts and CRP Levels in Controls and Obese Patients Aged 6-12 Years

	Controls (*n* = 19)	Obese patients (*n* = 77)	*P*
WBC (x 10^9/l)	7.13 ± 1.94	7.37 ± 1.75	0.597
Neutrophils (%)	47.61 ± 9.59	51.86 ± 7.91	0.048
Eosinophils (%)	5.26 ± 5.02	3.65 ±2.20	0.186
Basophils (%)	0.47 ± 0.34	0.53 ± 0.31	0.453
Lymphocytes (%)	41.18 ± 9.49	38.39 ± 7.50	0.172
Monocytes (%)	5.47 ± 1.64	5.57 ± 1.69	0.814
Neutrophils (x 10^9/l)	3.43 ± 1.31	3.87 ± 1.29	0.186
Eosinophils (x 10^9/l)	0.42 ± 0.48	0.26 ± 0.16	0.176
Basophils (x 10^9/l)	0.04 ± 0.03	0.04 ± 0.02	0.462
Lymphocytes (x 10^9/l)	2.85 ± 0.75	2.79 ± 0.71	0.739
Monocytes (x 10^9/l)	0.39 ± 0.13	0.40 ± 0.12	0.664
Neutrophil/lymphocyte ratio	1.28 ± 0.53	1.44 ± 0.49	0.193
CRP (mg/l)	0.32 (0.19-0.89)	1.73 (0.82-3.67)	<0.001

Values are given as mean ± SD or median (interquartile range).

Table 3. Correlations Between CRP Level and the Absolute Count for Each Type of Leukocytes with Adiposity Markers, in Obese Patients Aged 6-12 Years

	Neutrophils (x10^9/l)	Lymphocytes (x10^9/l)	Monocytes (x10^9/l)	Eosiniphols (x10^9.l)	Basophils (x10^9.l)	Ln PCR
BMI (kg/m^2)	0.289*	0.046	0.076	0.089	0.058	0.261*
BMI z-sc	0.249*	0.120	0.058	0.064	0.113	0.313**
Waist circumference (cm)	0.225*	0.089	0.101	0.019	-0.017	0.188
Waist/height ratio	0.230*	0.178	0.099	0.175	0.097	0.317**
Ln CRP	0.384**	0.075	0.295*	0.014	0.051	-

*P<0.05; **P<0.01.

variable that remained statistically associated with neutrophil count was CRP (neutrophil count = 2.612 + 0.439lnCRP; standardised coefficient/beta: 0.384, P=0.001); BMI was excluded from this model but its partial correlation almost reached statistical significance (0.212, P=0.067). When performing multiple regression without CRP, the only

variable that remained statistically associated with neutrophil count was BMI (neutrophil count = 1.117 + 0.100BMI; standardised coefficient/beta: 0.289, *P*=0.011).

4. DISCUSSION

Leukocytosis is often associated with atherosclerotic disease and is accepted as a risk factor for CVD [11-13]. The association between leukocyte count and risk of atherosclerotic disease is plausible because leukocytes give a major contribution to the rheologic properties of blood, alter their own adhesive properties under stress and participate also in endothelial injury [11]. Moreover, the recruitment of monocytes and lymphocytes to the artery wall is characteristic of atherosclerosis [14].

Atherosclerosis is a chronic disease that begins early in life. Obesity is an important risk factor for the development of CVD, but, to date, limited investigations have examined the associations between WBC count and obesity in children and adolescents.

A study on subjects who were referred for further evaluation of leukocytosis by their family physicians demonstrated that obesity is the second most common cause of leukocytosis, being smoking the commonest cause [8]. Furthermore, in a previous large cross-sectional epidemiological study, performed in adults, an increased BMI was a statistically significant independent predictor of a higher peripheral blood leukocyte count [15]. Leptin, IL-1, IL-6 and TNF-α, all produced by the human adipose tissue, have been implicated in this leukocyte rise [8, 14, 16, 17]. However, as far as we know, this is the first study assessing the association of leukocyte count with obesity and its associated inflammation, assessed by CRP levels, in obese patients and (lean) controls aged 6-12 years.

As leukocyte count changes with age and is influenced by gender [15], we matched controls and patients for gender and age. We chose participants aged 6-12 years, as in this age range it is not normally observable significant changes in leukocyte counts. In our study, we also did not find any significant correlation between total and differential leukocyte counts with age.

The median CRP value was about 5 times higher than that observed in controls, demonstrating an inflammatory process in such young obese patients (Table **2**). The total leukocyte count did not show a statistically significant change in obesity, but a different ratio between the different leukocyte types was observed, as showed by the significantly higher value in the percentage of neutrophils with obesity. Even though no significant differences in absolute neutrophil count were observed, patients presented a trend to higher values. Moreover, absolute neutrophil count correlated not only with markers of obesity but also with CRP levels. This suggests that neutrophil count may be used as a marker of obesity severity, with its associated inflammatory state. The fact that CRP levels remained statistically associated with neutrophil count by multiple regression analysis may be explained by the fact that there are cytokines, produced and released by the adipocyte, namely IL-6 and IL-1, that are able to induce both an increase in CRP synthesis and neutrophilia *via* demargination of neutrophils from the marginal "pool", acceleration of bone marrow neutrophil release or enhancement of bone marrow granulopoiesis [7, 18, 19]. It is worthy to emphasise that in our study, when CRP was removed from the model, BMI was the only variable that remained statistically associated with neutrophil count.

The significant positive correlations that we found between neutrophil count and BMI and waist circumference values are in agreement with those obtained by Kim and Park [10] in female obese adolescents aged 10-19 years. However, we were unable to find any correlation with absolute lymphocyte count. Kim and Park [10] also found that WBC count is strongly related to subcutaneous rather than visceral adiposity.

There are a lot of beneficial effects on health resulting from moderated aerobic physical exercise, namely a reduction in obesity markers (e.g. BMI) and in risk of type 2 diabetes, and an improvement in endothelial function and lipid profile, all risk factors for CVD [20-23]. Data from large study populations also suggest an inverse association between chronic physical activity and the level of some inflammatory markers (e.g. CRP and interleukin-6) [21, 24, 25]. In obese adult subjects who lost weight significantly, leukocytosis and the acute-phase reactants seem to gradually return to normal [8]. However, few data exists in literature regarding young obese subjects. It was shown that 3 months of moderate lifestyle intervention in obese adolescents seems to attenuate the inflammatory state associated with obesity, as observed by a reduction in elevated circulating concentrations of CRP, fibrinogen and IL-6, non-traditional risk factors for CVD [16]. However, and despite the consistency of the results, the small sample size of this study (n = 8 in the intervention group) calls for attention in strengthening these results with further studies. Reinehr *et al.* [26], by evaluating 16 obese children who lost weight over a 1-year period, found a significant decrease in CRP but no significant changes in TNF-alpha levels. If future research supports the idea that, at young ages, the protection provided by regular physical activity against obesity-mediated inflammation is relevant, this would encourage even more its practice. Moreover, at these young ages, this would also be a good opportunity to implement healthy lifestyles.

In conclusion, our results demonstrated a significant change in the differential leukocyte count towards neutrophilia, together with a significant higher CRP concentration, in obese patients aged 6-12 years, and that absolute neutrophil count correlates with obesity markers, especially BMI, and with CRP levels. Our data also indicate that neutrophil count, a current clinically used low-cost parameter, may be used as an obesity-related inflammatory marker in young obese patients. However, more studies are needed to confirm our findings, involving larger number of cases. Furthermore, young obese patients may deserve from physical activity programs.

ACKNOWLEDGEMENTS

The authors wish to thank the technicians Amélia Ferreira, Andreia Sousa, Joana Barros and Isabel Almeida for expert assistance on blood collection, and to the University of Porto (IPG07/2007) for financial support.

REFERENCES

[1] Padez, C.; Fernandes, T.; Mourão, I.; Moreira, P.; Rosado, V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. *Am. J. Hum. Biol*., **2004**, *16*(6), 670-8.

[2] Alexander, C.M.; Landsman, P.B.; Grundy, S.M. The influence of age and body mass index on the metabolic syndrome and its components. *Diabetes Obes. Metab*., **2008**, *10*(3), 246-50.

[3] Shah, A.; Mehta, N.; Reilly, M.P. Adipose inflammation, insulin resistance, and cardiovascular disease. *JPEN J. Parenter. Enteral. Nutr*., **2008**, *32*(6), 638-44.

[4] Berg, A.H.; Scherer, P.E. Adipose tissue, inflammation, and cardiovascular disease. *Circ. Res*. **2005**, *96*(9), 939-49.

[5] Ross, R. Atherosclerosis - an inflammatory disease. *N. Engl. J. Med*. **1999**, *340*(2),115-26.

[6] Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine*, **2006**, *29*(1), 81-90.

[7] Kushner, I.; Rzewnicki, D. Acute phase response. In: Gallin JI, Snyderman R, Fearon DT, Haynes BF, Nathan C, Eds. *Inflammation: Basic Principles and Clinical Correlates*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999, pp. 317-29.

[8] Herishanu, Y.; Rogowski, O.; Polliack, A.; Marilus, R. Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. *Eur. J. Haematol*., **2006**, *76*, 516-20.

[9] Visser, M.; Bouter, L.M.; McQuillan, G.M.; Wener, M.H.; Harris, T.B. Low-grade systemic inflammation in overweight children. *Pediatrics*, **2001**, *107*(1), E13.

[10] Kim, J.A.; Park, H.S. White blood cell count and abdominal fat distribution in female obese adolescents. *Metabolism*, **2008**, *57*(10), 1375-9.

[11] Ernst, E.; Hammerschmidt, D.E.; Bagge, U.; Matrai, A.; Dormandy, J.A. Leukocytes and the risk of ischemic diseases. *JAMA*, **1987**, *257*(17), 2318-24.

[12] Danesh, J.; Collins, R.; Appleby, P.; Peto, R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA*, **1998**, *279*(18), 1477-82.

[13] Danesh, J.; Whincup, P.; Walker, M.; Lennon, L.; Thomson, A.; Appleby, P.; Gallimore, J.R.; Pepys, M.B. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *BMJ*, **2000**, *321*(7255), 199-204.

[14] Lusis, A.J. Atherosclerosis. *Nature*, **2000**, *407*(6801), 233-41.

[15] Schwartz, J.; Weiss, S.T. Host and environmental factors influencing the peripheral blood leukocyte count. *Am. J. Epidemiol*., **1991**, *134*(12), 1402-1409.

[16] Balagopal, P.; George, D.; Patton, N.; Yarandi, H.; Roberts, W.L.; Bayne, E.; Gidding S. Lifestyle-only intervention attenuates the inflammatory state associated with obesity: a randomized controlled study in adolescents. *J. Pediatr*., **2005**, *146*(3), 342-8.

[17] Stewart, R.A.; White, H.D.; Kirby, A.C.; Heritier, S.R.; Simes, R.J.; Nestel, P.J.; West, M.J.; Colquhoun, D.M.; Tonkin, A.M.; Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) Study Investigators. White blood cell count predicts reduction in coronary heart disease mortality with pravastatin. *Circulation*, **2005**, *111*(14), 1756-62.

[18] Suwa, T.; Hogg, J.C.; English, D.; Van-Eeden, S.F. Interleukin-6 induces demargination of intravascular neutrophils and shortens their transit in marrow. *Am. J. Physiol. Heart Circ. Physiol*. **2000**, *279*(6), H2954-60.

[19] Veltri, S.; Smith-2nd J.W. Interleukin 1 trials in cancer patients: a review of the toxicity, antitumor and hematopoietic effects. *Stem Cells*, **1996**, *14*(2), 164-76.

[20] Tsai, A.C.; Sandretto, A.; Chung, Y.C. Dieting is more effective in reducing weight but exercise is more effective in reducing fat during the early phase of a weight-reducing program in healthy humans. *J. Nutr. Biochem*., **2003**, *14*(9), 541-9.

[21] Verdaet, D.; Dendale, P.; De-Bacquer, D.; Delanghe, J.; Block, P.; De-Backer, G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis*, **2004**, *176*(2), 303-10.

[22] Ignarro, L.J.; Balestrieri, M.L.; Napoli, C. Nutrition, physical activity, and cardiovascular disease: an update. *Cardiovasc. Res*., **2007**, *73*(2), 326-40.

[23] Smith, J.K.; Dykes, R.; Douglas, J.E.; Krishnaswamy, G.; Berk, S. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA*, **1999**, *281*(18), 1722-7.

[24] Pitsavos, C.; Chrysohoou, C.; Panagiotakos, D.B.; Skoumas, J.; Zeimbekis, A.; Kokkinos, P.; Stefanadis, C.; Toutouzas, P.K. Association of leisure-time physical activity on inflammation markers (C-reactive protein, white cell blood count, serum amyloid A, and fibrinogen) in healthy subjects (from the ATTICA study). *Am. J. Cardiol*., **2003**, *91*(3), 368-70.

[25] Reuben, D.B.; Judd-Hamilton, L.; Harris, T.B.; Seeman, T.E. MacArthur_Studies_of_Successful_Aging. The associations between physical activity and inflammatory markers in high-functioning older persons: MacArthur Studies of Successful Aging. *J. Am. Geriatr. Soc*. **2003**, *51*(8), 1125-30.

[26] Reinehr, T.; Stoffel-Wagner, B.; Roth, C.L.; Andler, W. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. *Metabolism*, **2005**, *54*(9), 1155-61.

Received: January 10, 2010 Revised: February 26, 2010 Accepted: March 04, 2010

14.6. Paper VI

Nascimento H, Costa E, Rocha-Pereira P, Rego C, Mansilha HF, Quintanilha A, Santos-Silva A, Belo L. “Cardiovascular risk factors in Portuguese obese children and adolescents: impact of small reductions in body mass index imposed by lifestyle modifications”. The Open Biochemistry Journal. 2012; 6:43-50.

Cardiovascular Risk Factors in Portuguese Obese Children and Adolescents: Impact of Small Reductions in Body Mass Index Imposed by Lifestyle Modifications

Henrique Nascimento[1,2], Elísio Costa[3], Petronila Rocha-Pereira[4], Carla Rego[5], Helena Ferreira Mansilha[6], Alexandre Quintanilha[2,7], Alice Santos-Silva[1,2] and Luís Belo*[,1,2]

[1]*Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal*

[2]*Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, 4150-180 Porto, Portugal*

[3]*Instituto de Ciências da Saúde, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal*

[4]*Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior, 6200-506 Covilhã, Portugal*

[5]*Unidade de Nutrição / Serviço de Pediatria. UAG-MC. Hospital de S. João E.P.E. Faculdade de Medicina, Universidade do Porto; 4200-319 Porto, Portugal*

[6]*Hospital Crianças Maria Pia - Centro Hospitalar do Porto; 4050-111 Porto, Portugal*

[7]*Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4099-003 Porto, Portugal*

Abstract: *Objectives*: Evaluate cardiovascular risk factors in Portuguese obese children and adolescents and the long-term effects of lifestyle modifications on such risk factors.

Design: Transversal cohort study and longitudinal study.

Setting: University Hospital S. João and Children's Hospital Maria Pia, Porto.

Patients/Participants: 148 obese children and adolescents [81 females (54.7%); mean age of 11.0 years] and 33 controls (sex and age matched) participated in a cross-sectional study. Sixty obese patients agreed to participate in an one year longitudinal study after medical and nutritionist appointments to improve lifestyle modification; a substantial body mass index (BMI) reduction was defined by a decrease in BMI z-score (BMI z-sc) of 0.3 or more over the studied period.

Main Outcome measures: Lipid profile (triglycerides, cholesterol, HDLc, LDLc, lipoprotein (a), apolipoproteins A and B) and circulating levels of C-reactive protein (CRP), adiponectin, glucose, and insulin.

Results: Compared with the lean children, obese patients demonstrated statistically significantly higher insulin resistance index [Homeostasis model assessment (HOMA)], and triglycerides, LDLc, apolipoprotein (apo) B, insulin and CRP concentrations, whereas their HDLc and apo A levels were significantly lower (cross-sectional study). In the longitudinal study (n=60), a substantial BMI reduction occurred in 17 (28.3%) obese patients which led to a significant reduction in triglycerides, cholesterol, LDLc, apo B, glucose and insulin levels and in HOMA. The ΔBMI values over the studied period correlated inversely and significantly with BMI (P<0.001) and HOMA (P=0.026) values observed at baseline. In multiple linear regression analysis, BMI at baseline remained associated to changes in BMI over the studied period (standardised Beta: -0.271, P=0.05).

Conclusion: Our data demonstrates that small reductions in BMI-zc, imposed by lifestyle modifications in obese children and adolescents, improve the cardiovascular risk profile of such patients. Furthermore, patients with higher BMI and/or insulin resistance seem to experience a greater relative reduction in their BMI after lifestyle improvements.

Keywords: Lipid profile, insulin resistance, inflammation, childhood obesity, lifestyle modifications.

1. INTRODUCTION

Obesity is increasing all over the world both in developed and underdeveloped countries [1, 2] and is known to be associated with inflammatory changes, insulin resistance and with a hyperlipidemic state [3]. The adipocyte is an important source of cytokines, namely plasminogen activator inhibitor type 1 (PAI)-1, leptin, interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha, and their levels are significantly higher in the plasma of obese patients [4]. Moreover, some inflammatory markers, such as C-reactive protein (CRP),

*Address correspondence to this author at the Departamento de Bioquímica, Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal; Tel: +351-222078906; Fax: +351-222003977; E-mail: luisbelo@ff.up.pt

1874-091X/12 2012 Bentham Open

show an independent positive association with body mass index (BMI) [5].

Cardiovascular (CV) morbidity and mortality is associated with the classic risk factors, namely dyslipidemia, hypertension and impaired glucose metabolism. However, more recently, inflammatory mechanisms were recognized as playing a vital role in initiation, maintenance and progression of atherosclerotic vascular disease. Atherosclerosis is an inflammatory disease with the participation of leukocytes and several cytokines. Among several inflammatory markers that have been shown to predict CV events [namely IL-6, tumor necrosis factor-alpha and soluble intercellular adhesion molecule (ICAM)-1] CRP levels have emerged as the most powerful marker [6].

Regular physical activity is known to reduce obesity-related markers and risk of type 2 diabetes (physical activity is associated with improved insulin sensitivity) and to improve endothelial function, lipid profile (e.g., exercise decreases plasma triglycerides (TG) and increases high density lipoprotein (HDL), as well as several oxidative stress/inflammatory markers, all risk factors for CV disease (CVD) [7-10]. Diet restriction *per se* may reduce fat mass, total cholesterol (Chol) and low density lipoprotein cholesterol (LDLc) without apparent effect in HDL and limited effect on insulin resistance. The effect of both diet and physical exercise in obese individuals appear to be additive with greater impact in the improvement of lipid profile and insulin resistance [11]. However, few data exists in literature regarding the long-term effects of physical activity on young obese subjects. Moreover, in most studies, children obey to a restricted physical exercise program or a food intake program, but the effect of "natural" lifestyle modifications on CV risk factors has been poorly explored [12-14].

Higher cardiorespiratory fitness (CRF) has been associated to a decrease in CVD risk markers, CVD morbidity and mortality [15]. Besides physical activity, the initial CRF of an individual appears to be an independent predictor of weight variation. Individuals with lower CRF are more prone to gain weight when compared to those with higher CRF [16]. Actually, children with lower CRF usually present diminished levels of physical activity and increased time expended in sedentary behaviours. Despite the strong genetic influence, CRF status can be modulated through physical activity, which is a main determinant of CRF in pediatric populations [17].

The physical activity performed by children and adolescents in southern European countries is lower than in countries from centre and northern Europe. This fact may explain the geographical differences in obese prevalence found in Europe [17]. In fact, recent studies showed a very high prevalence of overweight/obesity (about 30%) in Portuguese children when compared to other European countries [18, 19]. Thus, the study of obesity is of particular importance in our country.

The aim of this study was to evaluate the short-term effects of lifestyle modifications, through a decrease in body mass index z-score (BMI z-sc), on CV risk factors (circulating levels of C-reactive protein, glucose, insulin and lipid profile), in Portuguese obese children and adolescents.

2. MATERIAL AND METHODS

2.1. Subjects

The protocol used for all participants was approved by the Committees on Ethics of the University Hospital S. João and of the Children's Hospital Maria Pia, Porto. Obese children and adolescents, aged 5-18 years, were identified from medical records, at the Departments of Pediatrics of the referred hospitals.

Obesity was defined as BMI greater than the 95th percentile for age and gender, according to 2000 Centers for Disease Control and Prevention (CDC) growth charts. Because BMI is not normally distributed and not adjusted for sex and age we used BMI z-sc.

One hundred and forty eight (n=148) obese children and adolescents participated in the study after informed and written consent from their parents. Thirty three healthy control subjects, age and sex matched with obese patients, were recruited from the general population. The BMIs of the controls were lower than the 85th percentile adjusted for sex and age. Obese patients were evaluated at the beginning of the study and compared with controls (cross-sectional study).

All obese individuals (n=148) were motivated to change their lifestyle habits (conventional weight loss programs based on dietary counseling and encouragement to exercise), but only sixty patients agreed to participate in a longitudinal study (one year of follow up). A substantial BMI reduction was defined by a decrease in BMI z-sc of 0.3 or more over the studied period. One year after the beginning of lifestyle modification intervention, obese children/adolescents were classified as: 1) those who have achieved a substantial BMI reduction or 2) those who have not achieved a substantial BMI reduction. In the longitudinal study, clinical and biochemical data were obtained at the beginning as well as at the conclusion, after one year of follow-up.

Criteria of exclusion were: smokers, subjects under regular medication or with diabetes mellitus, endocrinologic disorders, and hereditary, inflammatory or infectious diseases.

2.2. Procedures and Assays

2.2.1. Clinical Examination

The participants were invited to the research centers after an overnight fast and clinical data regarding the sample population was collected. The development of puberty was clinically assessed on the basis of Tanner stages. The physical examination included the measurement of height, weight and the observation of the presence of cutaneous markers related with obesity and its comorbidity.

2.2.2. Blood Samples

Blood samples were obtained on a fasting basis and processed within 2h of collection. Blood was obtained by venipuncture in ethylenediaminetetraacetic acid (EDTA) containing tubes. Plasma aliquots were made and immediately stored at - 70^{o}C until assayed.

2.2.3. Laboratory Analysis

The determination of circulating levels of glucose, insulin, lipids, and lipoproteins was performed by using

Table 1. Characteristics of the Participants in the Cross-Sectional Study

	Controls (*n*=33)	Obese (*n*=148)	*p*
Age (years)	10.2 ± 3.6	11.0 ± 3.0	0.173
Sex (female)	20 (60,6%)	81 (54,7%)	0.852
Weight (kg)	34.7 ± 12.8	69.0 ± 22.8	<0.001
BMI (kg/m²)	17.4 ± 2.2	29.8 ± 5.4	<0.001
BMI z-score	0.09 ± 0.68	2.30 ± 0.44	<0.001

BMI, body mass index.
Values are given as mean ± SD, unless otherwise indicated.

routine automated technology (Glucose, Insulin, Cholesterol, Triglycerides, HDL Cholesterol Direct, LDL Cholesterol Direct, Apolipoprotein (apo) A1 and apo B, ABX Diagnostics). Lipoprotein (a) ((Lp(a)) plasma concentration was determinated by an immunoturbidimetric method.

Homeostasis model assessment (HOMA), used to detect the degree of insulin resistance [20], was calculated by using the following formula: resistance (HOMA) = (fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5).

Plasma levels of CRP were evaluated by immunoturbidimetry [CRP (latex) High-Sensitivity, Roche Diagnostics].

Plasma concentration of adiponectin was evaluated by using a standard commercial enzyme-linked immunoassay (Adiponectin, R&D Systems).

2.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 17.0) for Windows. Kolmogorov-Smirnov analysis was used to test if the results were normally distributed. Results with normal distribution were presented as mean ± standard deviation, whilst irregular distributions were presented as median (interquartile range).

To compare obese and control groups, Student's unpaired *t*-test was used for parameters presenting a Gaussian distribution and the Mann-Whitney *U*-test in the case of a non-Gaussian distribution. To evaluate the differences occurring along the longitudinal study, we used the paired Student *t* test for parametric and Wilcoxon Signed Ranks Test for non-parametric variables.

Spearman's rank correlation coefficient was used to evaluate relationships between sets of data.

To evaluate the contribution of the different variables to BMI reduction over the studied period, we performed multiple regression analysis, using stepwise selection with an entry criteria of P <0.05, after log transformation of the variables (when necessary).

Significance was accepted at P less than 0.05.

3. RESULTS

The characteristics of the participants in the cross-sectional study are presented in Table **1**. Obese patients and controls were matched for age and gender. Compared with the lean children, obese patients demonstrated significantly higher insulin resistance index (HOMA), and TG, LDLc, apo B, insulin and CRP concentrations, whereas their HDLc and apo A levels were significantly lower (Table **2**). The participants in the cross-sectional study had BMI which correlated positively and significantly to TG (r = 0.300, P < 0.001), LDLc (r = 0.235, P = 0.001), insulin (r = 0.652, P < 0.001), HOMA (r = 0.646, P < 0.001), and CRP (r = 0.451, P < 0.001) values, and negatively with HDLc (r = -0.462, P < 0.001). Adiponectin levels (n=153) correlated inversely and significantly with age (r = -0.299, P < 0.001), BMI (r = -0.220, P = 0.006), TG (r = -0.390, P < 0.001) and total cholesterol/HDLc (Chol/HDLc) ratio (r = -0.270, P = 0.001), insulin (r = -0.211, P = 0.009) and HOMA (r = -0.223, P = 0.006), and correlated positively and significantly with apo A (r = 0.214, P = 0.008) and HDLc (r = 0.270, P = 0.001).

Sixty obese patients participated in the longitudinal study and, after one year of follow up, 17 (28.3%) achieved a substantial BMI z-sc reduction (mean ΔBMI z-sc = -0.46 ± 0.16), while 43 individuals did not reach the cut-off value (mean ΔBMI z-sc = 0.01 ± 0.26) (Table **3**). At baseline (T1), no significant differences were observed in age, sex, BMI, BMI z-sc, and in any markers of the metabolic syndrome or inflammatory markers between obese children who presented with and without substantial BMI z-sc reduction, at the end of the study (T2).

A substantial decrease in BMI z-sc in 17 children led to a significant reduction in TG, cholesterol, LDLc, apo B, glucose, insulin levels and in HOMA (Table **4**). In the 43 children without change of BMI z-sc, a reduction in adiponectin and in glucose values occurred as well as a significantly increase in Lp(a) values (Table **4**).

The ΔBMI values over the studied period (longitudinal study, n=60) correlated inversely and significantly with BMI at baseline (r = -0.487, P<0.001, Fig. **1A**), and HOMA at baseline (r = -0.291, P=0.026, Fig. **1B**). In multiple linear regression analysis, BMI values at baseline remained associated to changes in BMI over the studied period (standardised Beta: -0.271, P=0.05).

4. DISCUSSION

The present study was able to demonstrate in young obese patients that small reductions in BMI z-sc are associated with significant improvements in lipid profile and in insulin resistance index.

Table 2. Biochemical Data of the Participants in the Cross-Sectional Study

	Controls (*n*=33)	Obese (*n*=148)	*p*
Lipid profile			
TG (mmol/l)	0.72 (0.57-0.85)	0.86 (0.60-1.25)	0.017
Chol (mmol/l)	4.29 (3.82-4.81)	4.11 (3.68-4.60)	0.241
HDLc (mmol/l)	1.25 (1.12-1.33)	1.09 (0.92-1.24)	<0.001
LDLc (mmol/l)	2.31 (1.85-2.65)	2.63 (2.20-3.10)	0.001
Lpa (mg/dl)	28.6 (18.1-50.3)	25.8 (10.6-46.5)	0.194
Apo A (mg/dl)	132.5 ± 9.7	118.5 ± 18.2	<0.001
Apo B (mg/dl)	73.8 ± 15.8	81.3 ± 20.3	0.042
Chol/HDLc	3.53 (3.12-3.91)	3.86 (3.26-4.56)	0.017
Apo A/Apo B	1.89 ± 0.50	1.53 ± 0.41	<0.001
Glucose metabolism			
Glucose (mmol/l)	4.90 (4.68-5.17)	5.00 (4.80-5.20)	0.174
Insulin (mmol/l)	5.28 (4.11-6.82)	12.95 (8.92-18.88)	<0.001
HOMA	1.14 (0.84-1.47)	2.90 (1.94-4.24)	<0.001
Inflammatoy markers			
Adiponectin (mg/l)	7.83 (5.15-10.01)*	7.99 (5.19-11.12)**	0.884
CRP (mg/l)	0.25 (0.18-0.68)	1.64 (0.80-3.66)	<0.001

*, *n*=14; **, *n*=139 ; Values are given as mean ± SD or median (interquartile range).
TG, triglycerides; Chol, cholesterol; HDLc, High Density Lipoprotein cholesterol; LDLc, Low Density Lipoprotein cholesterol; Lp(a), lipoprotein (a); apo, apolipoprotein; HOMA, Homeostasis Model Assessment (insulin resistance index); CRP, C-reactive protein.

Table 3. Characteristics of the Participants in the Longitudinal Study (One Year of Follow-Up)

	Δ BMI z-sc < 0,3 (*n*=43)			Δ BMI z-sc ≥ 0,3 (*n*=17)		
	T1	T2	*p*	T1	T2	*p*
Age (years)	11.5 ± 2.21	12.6 ± 2.2	<0.001	11.5 ± 3.0	12.6 ± 3.0	<0.001
Weight (kg)	70.2 ± 19.1	78.0 ± 18.0	<0.001	69.9 ± 16.7	67.5 ± 13.4	0.191
Waist/Hip	0.928 ± 0.054	0.920 ± 0.065	0.347	0.912 ± 0.028	0.905 ± 0.059	0.630
Waist/Height	0.609 ± 0.059	0.616 ± 0.058	0.354	0.628 ± 0.045	0.569 ± 0.057	<0.001
BMI (kg/m²)	29.2 ± 4.8	30.6 ± 4.8	0.005	30.2 ± 4.0	27.1 ± 3.1	<0.001
BMI z-score	2.14 ± 0.36	2.15 ± 0.32	0.845	2.36 ± 0.68	1.90 ± 0.65	<0.001

BMI, body mass index Values are given as mean ± SD.

In a first analysis (cross-sectional study) we compared obese patients with a lean control group. As some of the studied CV risk factors change with age and are influenced by gender, we matched controls and patients for gender and age. The results obtained confirm obesity as an inflammatory disease. Indeed, the median CRP value observed in obese children and adolescents was about 6 times higher than that observed in controls, demonstrating an inflammatory process in such young patients (Table **2**). We also observed that obese patients presented with an altered lipid profile and raised markers of insulin resistance when compared with controls (Table **2**).

There are few studies assessing the long-term changes of lifestyle modifications in obese children and adolescents, particularly on CV risk factors. In the present longitudinal study, obese patients (children and adolescents) were asked to improve their lifestyle habits (conventional weight loss programs based on dietary counselling and encouragement to

Table 4. Biochemical Data of the Participants in the Longitudinal Study (One Year of Follow-Up)

	Δ BMI z-sc < 0,3 (*n*=43)			Δ BMI z-sc ≥ 0,3 (*n*=17)		
	T1	**T2**	***p***	**T1**	**T2**	***p***
Lipid profile						
TG (mmol/l)	0.77 (0.50-1.02)	0.65 (0.51-0.95)	0.795	0.87 (0.64-1.30)	0.63 (0.50-0.90)	0.003
Chol (mmol/l)	3.93 (3.50-4.68)	4.10 (3.51-4.50)	0.929	4.24 (3.80-4.70)	4.00 (3.58-4.24)	0.009
HDLc (mmol/l)	1.14 (0.98-1.32)	1.13 (0.93-1.31)	0.960	1.13 (0.98-1.26)	1.17 (0.92-1.32)	0.819
LDLc (mmol/l)	2.59 (2.20-3.00)	2.47 (2.21-2.90)	0.534	2.69 (2.30-3.24)	2.42 (2.06-2.69)	0.007
Lpa (mg/dl)	19.0 (10.0-43.0)	28.0 (12.6-48.6)	0.011	22.0 (14.0-49.1)	30.60 (8.29-51.55)	0.403
Apo A (mg/dl)	118.5 ± 18.5	114.1 ± 18.0	0.052	118.3 ± 22.4	109.8 ± 17.4	0.060
Apo B (mg/dl)	75.9 ± 16.0	73.8 ± 17.2	0.498	79.8 ± 14.7	72.9 ± 14.1	0.013
Chol/HDLc	3.68 (3.17-4.16)	3.60 (3.10-4.50)	0.808	3.91 (3.34-4.24)	3.50 (3.05-4.20)	0.416
Apo A/Apo B	1.62 ± 0.40	1.60 ± 0.33	0.780	1.54 ± 0.44	1.55 ± 0.37	0.803
Glucose metabolism						
Glucose (mmol/l)	5.00 (4.83-5.22)	4.80 (4.50-5.10)	0.014	5.00 (4.64 - 5.28)	4.57 (4.04-4.98)	0.002
Insulin (mmol/l)	12.70 (9.50-15.70)	11.40 (9.54-16.60)	0.395	14.40 (11.60 - 17.80)	10.90 (7.6-14.38)	0.015
HOMA	2.80 (2.14-3.56)	2.33 (1.82-3.31)	0.255	3.01 (2.41 - 4.24)	1.98 (1.51-2.89)	0.007
Inflammatoy markers						
Adiponectin (mg/l)	8.58 (5.56-11.66)	6.04 (4.74-9.13)	0.004	9.28 (7.37 - 15.02)	10.17 (6.63-14.22)	0.675
CRP (mg/l)	1.61 (0.82-4.62)	1.67 (0.78-4.12)	0.402	0.94 (0.43 - 2.02)	1.07 (0.54-2.38)	0.213

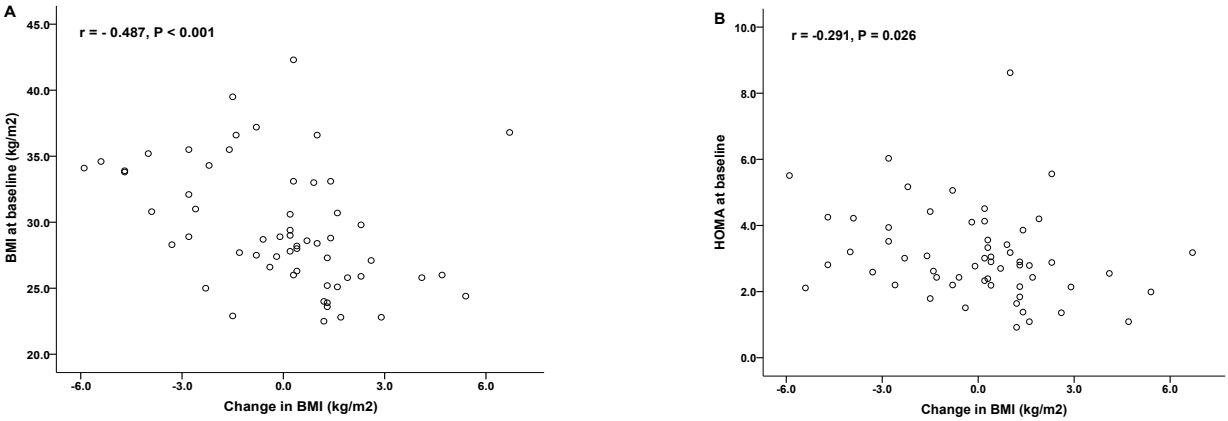


Fig. (1). Associations between change in body mass index (BMI) over the studied period (one year of follow-up) and BMI and insulin resistance index (Homeostasis model assessment; HOMA) values presented by participants at baseline. Spearman's rank correlation coefficient values are shown.

exercise) and an objective measure (BMI z-sc reduction) was used to observe which children achieved the objective after one year of follow-up. We observed that 17 patients (28.3%) presented a significant reduction in BMI z-sc (0.3 or more ($\Delta BMI \leq -0.3$)) over the studied period and that changes in BMI over the studied period were independently associated with BMI values presented at baseline. It seems that patients with higher BMI experience a greater relative reduction in their BMI (Fig. **1**). Also, the correlation between HOMA in the beginning of the study and relative BMI reduction points to a predisposition for children who are more insulin resistant to lose more weight. These associations can be related to a change in body physiology towards weight loss.

In our longitudinal study, children did not obey to a restricted physical exercise program or a food intake programme. Also, it was not controlled the initial CRF of our population. Thus, it is impossible to know the possible impact of CRF in BMI z-sc reduction. It is true that the lack of standardisation on plans on intervention may mitigate the

interpretation of the results that we observed. On the other hand, the counseling to improve lifestyle habits may be a "more natural" approach, and is useful in clinical practice, as participants can directly observe the real benefits of changing their lifestyle habits. Furthermore, the reduction in BMI-zc that we observed is likely to express a real change in lifestyle habits.

It was shown that 3 months of moderate lifestyle intervention (45 min of physical activity 3 times per week) in obese adolescents seems to attenuate the inflammatory state associated with obesity, as observed by a reduction in elevated circulating concentrations of CRP, fibrinogen and IL-6, non-traditional risk factors for CVD [12, 14]. Reinehr *et al.*, by evaluating 16 [14] and 10 [13] obese children who lost weight over a 1-year period, found a significant decrease in CRP but no significant changes in TNF-alpha levels. In the studies performed by Reinehr *et al.*, obese children participated in a 1-year obesity intervention program and the cut-off value used for BMI z-sc reduction was 0.5. As we used a smaller cut-off value (0.3), this may explain why we did not observe significant changes in CRP levels. Indeed, CRP shows a positive association with BMI, and our cut-off value may be insufficient to observe clear improvements in CRP. Even thus, it is important to emphasize that in our study, involving a larger number of participants, 64.3% of the patients that reduced their BMI z-sc presented a decrease in circulating CRP levels, in contrast with the group without change of BMI z-sc (47.6%). Furthermore, we were able to demonstrate that smaller reductions in BMI z-sc are associated with significant improvements in lipid profile and in insulin resistance index HOMA. Previous studies suggested that the improvement of insulin sensitivity and CV risk factors in obese children only occurs if BMI z-sc decreases by at least 0.5 over a 1-year period [21], but our data suggests that such improvements may be observable with smaller reductions in BMI-zc. Again, our results are probably explained by the fact that the reductions that we observed in BMI z-sc are likely to express a continuous daily motivation in changing lifestyle habits. Nevertheless, although there was no change in HDLc, the lower values of HOMA together with the reduction in TG seen in the group that reached the cut-off values leads to the probable influence of concomitant changes in diet and in the levels of physical exercise in this group to be linked with the improvement seen in the global CV risk profile.

The improvement in insulin sensitivity with BMI z-sc reduction observed in our study is of particular importance, as insulin resistance represents a major underlying abnormality driving CVD [22]. It is important to emphasize that insulin resistance associates with increased TG and that one of the most striking findings in the longitudinal analysis of the present study was the reduction in TG levels. Although hypercholesterolemia is a major risk factor for the initiation and progression of atherosclerosis [23], hypertriglyceridemia and elevated levels of triglyceride (TG)-rich lipoproteins have recently re-emerged as risk factors for atherosclerosis [24]. TG-rich particles may directly damage the endothelium principally *via* oxidative mechanisms. Also, an important consequence of hypertriglyceridemia, relating to increased atherosclerotic risk, is a shift in the spectrum of LDL subfractions towards smaller, denser species, which are believed to be more atherogenic [25]. Moreover, raised TG levels are frequently associated with reduced HDL levels. In our study, we only observed a slight (non-significant) increase in HDLc levels, which seems to have a protective role in the development of atherosclerosis [26-28]. Reinehr *et al.* observed a trend to increase HDLc levels in patients who achieved a substantial BMI z-sc reduction [14].

Plasma Lp(a) levels are known to be mainly genetically determined [29, 30]. However, it appears that plasma Lp(a) concentration increases with age. In our study, Lp(a) levels were similar betweens controls and obese individuals but, in the longitudinal study an increase in this lipoprotein was observed (Table **4**). In fact, this increase achieved statistically significance in the group without a substantial reduction in BMI z-sc. Thus, our data is in agreement with the idea that ageing elevates plasma Lp(a) concentration, and that this increment might be modulated be lifestyle modifications.

Adiponectin is an adipocytokine that has been noted as an important antiantherogenic, antidiabetic and as an anti-inflammatory protein [31]. Adiponectin seems to be inversely related to systolic blood pressure, waist circumference, triglycerides, and 2-hour glucose levels, while powsitively related to HDL [32]. These associations are in agreement with the results obtained in our cross-sectional study. However, and although it was suggested that adolescent obesity is associated with low plasma adiponectin levels [33], we were unable to find statistically significant differences in plasma levels of adiponectin between obese children and controls. This may be explained by the fact that we defined controls as having a BMI lower than the 85^{th} percentile adjusted for sex and age, in contrast with other work where control group was defined as a group of adolescents who had a BMI between the 50^{th} and 75^{th} percentiles [33].

Regarding our longitudinal study, it is important to highlight that adiponectin levels decreased significantly in the group without BMI z-sc reduction, in contrast with a moderate (non-significant) increase in the group that presented a significant decrease in BMI z-sc. It was previously reported that a significant weight loss in children, defined as BMI z-sc reduction of 0.5 or more, is associated with a significant increase in adiponectin [34]. Considering the protective effects of adiponectin, it seems reasonable to hypothesize that the increase in adiponectin is likely to explain, at least in part, some of modifications in the lipid profile (namely the reduction in triglycerides) and in glucose metabolism associated with BMI z-sc reduction.

The concern with childhood obesity must begin in overweight children and not only in severe obese children as it is easier to intervene in an early phase. Besides, there are studies that point that the majority of the diseases appear in individuals with moderated elevated risk factors [35].

Further studies involving a standardized physical activity and diet programs together with the assessment of important variables as CRF, at baseline and through the study period, could help to better understand the process involved in weight loss differences found among children and, also, the relation between the changes in adiposity and variations in CVD risk markers.

In summary, our data demonstrates that lifestyle modifications in obese children and adolescents, even associated with small reductions in BMI z-sc, improve the CV risk profile, namely lipid profile and insulin resistance. This may be important in motivating patients as health changes can appear earlier than the esthetical ones. Furthermore, patients with higher BMI and/or insulin resistance seem to experience a greater relative reduction in their BMI after lifestyle improvements, probably due to a physiologic adaptation favoring weight loss.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

The authors wish to thank the technicians Amélia Ferreira, Andreia Sousa, Joana Barros and Isabel Almeida for expert assistance on blood collection, and University of Porto and "Fundação para a Ciência e a Tecnologia" (FCT) (SFRH/BD/61407/2009) for financial support.

REFERENCES

[1] Duncan, S.; Duncan, E.K.; Fernandes, R.A.; Buonani, C.; Bastos, K.D.; Segatto, A.F.; Codogno, J.S.; Gomes, I.C.; Freitas, I.F., Jr., Modifiable risk factors for overweight and obesity in children and adolescents from Sao Paulo, Brazil. *BMC Public Health,* **2011,** *11*, 585.

[2] Ko, G.T.; Chan, J.C. Burden of obesity--lessons learnt from Hong Kong Chinese. *Obes. Rev.*, **2008,** *9*(Suppl 1), 35-40.

[3] Alexander, C.M.; Landsman, P.B.; Grundy, S.M. The influence of age and body mass index on the metabolic syndrome and its components. *Diabetes Obes. Metab.*, **2008,** *10*(3), 246-250.

[4] Rondinone, C.M. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine*, **2006,** *29*(1), 81-90.

[5] Folsom, A.R.; Pankow, J.S.; Tracy, R.P.; Arnett, D.K.; Peacock, J.M.; Hong, Y.; Djousse, L.; Eckfeldt, J. H. Association of C-reactive protein with markers of prevalent atherosclerotic disease. *Am. J. Cardiol.*, **2001,** *88*(2), 112-117.

[6] Blake, G.J.; Ridker, P.M. Novel clinical markers of vascular wall inflammation. *Circ. Res.*, **2001**, *89*(9), 763-771.

[7] Ignarro, L.J.; Balestrieri, M.L.; Napoli, C. Nutrition, physical activity, and cardiovascular disease: an update. *Cardiovasc. Res.*, **2007**, *73*(2), 326-340.

[8] Smith, J.K.; Dykes, R.; Douglas, J.E.; Krishnaswamy, G.; Berk, S. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA*, **1999**, *281* (18), 1722-1727.

[9] Tsai, A.C.; Sandretto, A.; Chung, Y.C. Dieting is more effective in reducing weight but exercise is more effective in reducing fat during the early phase of a weight-reducing program in healthy humans. *J. Nutr. Biochem.,* **2003**, *14*(9), 541-549.

[10] Verdaet, D.; Dendale, P.; De Bacquer, D.; Delanghe, J.; Block, P.; De Backer, G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis*, **2004**, *176*(2), 303-310.

[11] Ben Ounis, O.; Elloumi, M.; Ben Chiekh, I.; Zbidi, A.; Amri, M.; Lac, G.; Tabka, Z. Effects of two-month physical-endurance and diet-restriction programmes on lipid profiles and insulin resistance in obese adolescent boys. *Diabetes Metab.*, **2008**, *34*(6 Pt 1), 595-600.

[12] Balagopal, P.; George, D.; Patton, N.; Yarandi, H.; Roberts, W.L.; Bayne, E.; Gidding, S. Lifestyle-only intervention attenuates the inflammatory state associated with obesity: a randomized controlled study in adolescents. *J. Pediatr.*, **2005**, *146*(3), 342-348.

[13] Reinehr, T.; Stoffel-Wagner, B.; Roth, C.L. Adipocyte fatty acid-binding protein in obese children before and after weight loss. *Metab. Clin. Exp.*, **2007**, *56*(12), 1735-1741.

[14] Reinehr, T.; Stoffel-Wagner, B.; Roth, C.L.; Andler, W. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. *Metabolism*, **2005**, *54*(9), 1155-1161.

[15] Solbraa, A.K.; Mamen, A.; Resaland, G.K.; Johannessen, J.S.; Ylvisåker, E.; Holme, I.M.; Anderssen, S.M. Level of physical activity, cardiorespiratory fitness and cardiovascular disease risk factors in a rural adult population in Sogn og Fjordane. *Nor. Epidemiol.*, **2011**, *20*(2), 179-188.

[16] McGavock, J.M.; Torrance, B.D.; McGuire, K.A.; Wozny, P.D.; Lewanczuk, R.Z. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. *Obesity (Silver Spring),* **2009**, *1*(9), 1802-1807.

[17] Ruiz, J.R.; Ortega, F.B.; Martinez-Gomez, D.; Labayen, I.; Moreno, L.A.; De Bourdeaudhuij, I.; Manios, Y.; Gonzalez-Gross, M.; Mauro, B.; Molnar, D.; Widhalm, K.; Marcos, A.; Beghin, L.; Castillo, M.J.; Sjostrom, M. Objectively measured physical activity and sedentary time in European adolescents: the HELENA study. *Am. J. Epidemiol.*, **2011**, *174*(2), 173-184.

[18] *COSI*, European Childhood Obesity Surveillance Initiative - Saúde, M.d., Ed. **2009**.

[19] Padez, C.; Fernandes, T.; Mourao, I.; Moreira, P.; Rosado, V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. *Am. J. Hum. Biol.*, **2004**, *16*(6), 670-678.

[20] Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **1985**, *28*(7), 412-419.

[21] Reinehr, T.; Andler, W. Changes in the atherogenic risk factor profile according to degree of weight loss. *Arch. Dis. Child.*, **2004**, *89*(5), 419-422.

[22] Ginsberg, H.N. Insulin resistance and cardiovascular disease. *J. Clin. Investig.*, **2000,** *106*(4), 453-458.

[23] Witztum, J.L. Susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Med.*, **1993**, *94*(4), 347-349.

[24] Byrne, C.D. Triglyceride-rich lipoproteins: are links with atherosclerosis mediated by a procoagulant and proinflammatory phenotype? *Atherosclerosis*, **1999**, *145*(1), 1-15.

[25] Packard, C.; Caslake, M.; Shepherd, J. The role of small, dense low density lipoprotein (LDL): a new look. *Intern. J. Cardiol.*, **2000**, *74*(1), S17-S22.

[26] Lusis, A.J. Atherosclerosis. *Nature*, **2000**, *407*(6801), 233-241.

[27] Mertens, A.; Holvoet, P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J.*, **2001**, *15* (12), 2073-2084.

[28] Nofer, J.R.; Kehrel, B.; Fobker, M.; Levkau, B.; Assmann, G.; von Eckardstein, A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis*, **2002**, *161*(1), 1-16.

[29] Berglund, L.; Ramakrishnan, R. Lipoprotein(a): an elusive cardiovascular risk factor. *Arterioscler. Thromb. Vasc. Biol.*, **2004**, *24*(12), 2219-2226.

[30] Trommsdorff, M.; Kochl, S.; Lingenhel, A.; Kronenberg, F.; Delport, R.; Vermaak, H.; Lemming, L.; Klausen, I.C.; Faergeman, O.; Utermann, G.; et al., A pentanucleotide repeat polymorphism in the 5' control region of the apolipoprotein(a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. *J. Clin. Investig.*, **1995**, *96*(1), 150-157.

[31] Ouchi, N.; Shibata, R.; Walsh, K. Cardioprotection by adiponectin. *Trends Cardiovasc. Med.*, **2006,** *16* (5), 141-146.

[32] Shaibi, G.Q.; Cruz, M.L.; Weigensberg, M.J.; Toledo-Corral, C.M.; Lane, C.J.; Kelly, L.A.; Davis, J. N.; Koebnick, C.; Ventura, E.E.; Roberts, C.K.; Goran, M.I. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. *J. Clin. Endocrinol. Metab.*, **2007**, *92*(5), 1809-1813.

[33] Weiss, R.; Dufour, S.; Groszmann, A.; Petersen, K.; Dziura, J.; Taksali, S. E.; Shulman, G.; Caprio, S., Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. *J. Clin. Endocrinol. Metab*., **2003,** *88*(5), 2014-2018.

[34] Reinehr, T.; Roth, C.; Menke, T.; Andler, W. Adiponectin before and after weight loss in obese children. *J. Clin. Endocrinol. Metab*., **2004**, *89*(8), 3790-3794.

[35] Rodgers, A.; Ezzati, M.; Vander Hoorn, S.; Lopez, A.D.; Lin, R.B.; Murray, C.J. Distribution of major health risks: findings from the Global Burden of Disease study. *PLoS Med*., **2004**, *1*(1), e27.

Received: September 10, 2011 Revised: October 12, 2011 Accepted: October 30, 2011

14.7. Paper VII

Nascimento H, Costa E, Rocha S, Lucena C, Rocha-Pereira P, Rêgo C, Ferreira Mansilha H, Quintanilha A, Aires L, Mota J, Santos-Silva A, Belo L. “Adiponectin and markers of metabolic syndrome in obese children and adolescents: Impact of 8-month regular physical exercise program”. Submitted.

Adiponectin and markers of metabolic syndrome in obese children and adolescents: Impact of 8-month regular physical exercise program

Henrique Nascimento[1,2], Elísio Costa[1,2], Susana Rocha[1,2], Clarice Lucena[3,4], Petronila Rocha-Pereira[5], Carla Rêgo[6], Helena Ferreira Mansilha[7], Alexandre Quintanilha[2,8], Luísa Aires[3,4], Jorge Mota[3], Alice Santos-Silva[1,2], Luís Belo[1,2*]

[1]Biological Science Department, Faculty of Pharmacy, University of Oporto, 4050-313 Oporto, Portugal; [2]Institute for Molecular and Cell Biology (IBMC), University of Oporto, 4150-180 Oporto, Portugal; [3]Research Centre in Physical activity, health and leisure – CIAFEL, Faculty of Sports, University of Oporto, 4200-450 Oporto, Portugal; [4]High Education Institute from Maia – ISMAI, 4475-690 Maia, Portugal; [5]Health Science Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal; [6]Children and Adolescent Centre, CUF Hospital. Faculty of Medicine, University of Oporto; 4200-319 Oporto, Portugal; [7]Childhood and Adolescence Department / Paediatric Service of Oporto Hospital Centre; 4099-001 Oporto, Portugal; [8]Abel Salazar Biomedical Sciences Institute (ICBAS), University of Oporto, 4050-313 Oporto, Portugal.

Abstract

Background: Adiponectin circulates as low, medium and high molecular weight multimers (LMW, MMW and HMW) and influences lipid profile and insulin resistance (IR), being HMW considered the most biological-active form. We aimed to study the relation between adiponectin and markers of metabolic syndrome, in paediatric obesity, and the impact of physical exercise.

Methods: The study consisted of a cross-sectional part and an 8-months physical exercise program. Lipid profile, insulin, glucose, C-reactive protein (CRP), total adiponectin (TA), and homeostasis model assessment insulin resistance (HOMA) were measured. Adiponectin multimers were studied in a pre-pubertal group.

Results: Obesity associated with increased dyslipidaemia, IR and inflammation. TA correlated inversely with adiposity, triglycerides, HOMA and CRP, and positively with HDLc/Total Cholesterol (TC) ratio. HMW mimicked TA associations. The intervention program led to a reduction of TC, LDLc, insulin, HOMA and trunk percentage of fat, and an increase of HDLc/TC ratio, in the obese group. BMI improvements prevented adiponectin reduction and correlated with increments in HMW and MMW.

Conclusions: Obesity-related increase in MS features might be linked to lower adiponectin. HMW and MMW were the multimers that most explained MS features. The intervention program improved the lipid profile and IR, and prevented the reduction of adiponectin.

Keywords: childhood obesity, Metabolic Syndrome, intervention program, adiponectin, cardiovascular risk.

1. Introduction

Adiponectin is mainly secreted by adipocytes in humans,(1, 2) but total adiponectin (TA) levels are paradoxically lower in obese children, and even lower in obese individuals with metabolic syndrome (MS).(3, 4)

Adiponectin improves lipid metabolism by lowering the synthesis of free fatty acid and stimulating β-oxidation.(5) Furthermore, it is inversely related with triglyceride (TG) levels and positively related with high-density lipoprotein cholesterol (HDLc) concentration. A study from our group showed that adiponectin modulates the effect of apo E genotype on lipid profile. (6) Adiponectin also presents an anti-inflammatory action, e.g. it inhibits pro-inflammatory tumour necrosis factor α (TNF-α) secretion by macrophages. This activity might have an important role in insulin resistance (IR), as TNF-α inhibits insulin signalling,(7, 8) while adiponectin increases insulin sensitivity.(9)

In pediatric ages adiponectin levels correlate negatively with age.(6) The difference between genders is well established, with women presenting hig her adiponectin concentrations.(6, 10) Sexual hormones might underlie sex-related changes as before puberty no differences are found between genders.(11)

The body fat distribution influences adiponectin levels, being abdominal obesity associated with lower concentrations.(4, 12) The association between central adiposity and reduced adiponectin was seen with visceral fat accumulation in children,(13) and adults.(14)

Adiponectin circulates as 3 complexes: trimer, hexamers (two trimmers), and larger structures of trimers – 12 to 18mers being referred as low, medium and high molecular weight adiponectin, respectively (LMW, MMW, and HMW).(15)

The different multimers are suggested to present different biological functions, but results are still inconclusive. HMW was described as a better marker of metabolic abnormalities in obese children than TA or the other multimers,(16) and to be particularly related to improved insulin sensitivity(17) and lipid profile.(18)

The decrease of TA in obese children appears to be due mainly to the reduction of the HMW multimer.(16) Controversially, another study found no difference between obese and lean pre-pubertal boys regarding total and HMW adiponectin.(19)

Diet-induced weight-loss in obese adults associated with an improvement in adiponectin levels, with no further impact from exercise.(20) Contrarily, another study found no impact on TA levels after a nutritional counselling program in pre-pubertal Portuguese children, despite the improvement in BMI z-score (BMIzsc) and blood lipids.(21) Different results were found in a study involving obese adolescent boys, as improvements in adiponectin levels were obtained both by energy restriction and by exercise separately, moreover, cumulative effects were present when both approaches were combined.(22)

The aims of this study were: 1)Clarify changes in circulating TA and related multimers in obese children and adolescents; 2)Evaluate adiponectin relation with markers of MS; 3)Study the impact of regular physical exercise (PE) in adiponectin and in MS features.

2. Material and methods

2.1. Subjects

The study consisted of a cross-sectional and a longitudinal part.

The cross-sectional study involved a 104 children and adolescents (59 females), aged 5-18 years, after informed and written consent of their parents. The population was recruited in: 1)Two paediatric obesity outpatient clinics in Oporto; 2)A school-based PE program in 5 primary and 2 middle and high public schools from Oporto suburban setting.

The longitudinal study consisted of an 8-month PE program. Fifty-seven individuals completed the intervention (27 females).

2.2. Procedures and assays

2.2.1. Anthropometric and clinical evaluation

Height and weight were evaluated. Waist circumference was measured. at the superior border of the iliac crest.(23) Obesity was defined as BMIzsc greater than +1.65 for age and gender, according to 2000 Centre for Disease Control and Prevention (CDC) growth charts. Body composition was evaluated by dual-energy X-ray absorptiometry (DEXA).

Development of puberty was assessed by Tanner stages.

2.2.2. Physical activity program

Participants were involved in an 8-month PE program (October 2011 to May 2012). Five hours per week of moderate-to-vigorous PE were lectured divided in 3 regular physical education classes, and 2 PE program classes. The PE aimed to increase moderate-vigorous PE intensities. All activities were done in indoor schools´ sports facilities and taught by Physical Education teachers after school time. Procedures were approved by the Scientific Board of the Faculty of Sports, University of Oporto.

2.2.3. Blood samples

After an overnight fast, blood was obtained by venepuncture in EDTA containing tubes and processed within 2 hours of collection. Aliquots of plasma were made and stored at -80^{o}C until assayed.

2.2.4. Biochemical Analysis

Total cholesterol (TC), TG, HDLc, glucose, insulin and C-reactive protein (CRP) were measured using automated technology, as described elsewhere.(24) LDLc-cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were calculated using

Friedewald formula.(25) Homeostasis model assessment of insulin resistance (HOMA) was determinate according to Matthews.(26)

2.2.5. Adiponectin

Total adiponectin (TA)

Plasma concentration of TA was evaluated by a commercial enzyme-linked immunoassay (ELISA) (e-Bioscence, San Diego, CA).

Adiponectin multimers

Absolute and relative plasma concentration of adiponectin circulating multimers (HMW, MMW and LMW) was determined using a commercial available kit (Alpaco, Salem, NH).

2.3. Exclusion criteria and ethical approval

Smokers, subjects under regular medication or with diabetes mellitus, endocrinology disorders, hereditary, inflammatory or infectious diseases were excluded from the study.

The protocol was approved by the Hospitals Committees of Ethics and the Regional Education Board, and schools agreed to participate.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 20). Kolmogorov-Smirnov analysis was used to test if the results were normally distributed. Results normally distributed are presented as mean±standard deviation, otherwise are presented as median (interquartile range). Variables which distribution differed from normal were transformed for better fitting.

Distributions were analysed using chi-squared test and Fisher's exact test.

Differences between groups were assessed using Student's t-test. The strength of the associations was estimated by Spearman correlation coefficient.

Significance was accepted at P less than 0.05.

3. Results

3.1. Cross-sectional study

The control and obese groups were matched for sex, age and tanner stage. The obese individuals presented a lipid profile with significant cardiovascular risk changes, namely, higher TG, LDLc and VLDLc, and lower HDLc and HDLc/TC ratio; they also presented increased IR (measured by HOMA) and insulin, although showing normal levels of glucose. A pro-inflammatory status was present in obese individuals, with lower values of adiponectin and CRP median being almost 5 times higher than that of the control group (supplemental Table 1).

Supplemental Table 1. Anthropometric, nutritional and biochemical variables characterization of obese and control groups at the beginning of the study (cross-sectional study)

	Control (41)			Obese (63)			*P*
Sex (female(%))	25 (61.0)			34 (54.0)			0.546
Tanner (pre-pub (%))	19 (46.3)			23 (36.5)			0.414
Age (years)	10.6	±	3.7	10.2	±	3.0	0.783
Height (cm)	143.6	±	17.2	145.9	±	13.7	0.458
Weight (Kg)	40.4	±	16.3	59.6	±	20.8	<0.001
BMI (kg/m2)	18.6	±	3.3	27.0	±	4.8	<0.001
BMI z-score	0.29	±	0.73	2.13	±	0.29	<0.001
Waist (cm)	67.0	±	12.2	88.8	±	13.5	<0.001
Waist/Height	46.5	±	4.7	60.7	±	6.0	<0.001
Total % Fat[1)]	28.9	±	5.1	41.7	±	5.9	<0.001
Trunk % Fat[1)]	24.3	±	5.5	40.1	±	7.2	<0.001
Lipid Profile							
TG (mg/dl)	59.0		(46.5-73.5)	71.0		(49.0-103.0)	0.031
TC (mg/dl)	154.0		(132.0-169.0)	164.0		(142.0-189.0)	0.116
HDLc (mg/dl)	50.1		(42.6-60.0)	47.0		(38.8-53.7)	0.007
LDLc (mg/dl)	88.0		(71.5-103.0)	102.0		(79.0-117.0)	0.013
VLDLc (mg/dl)	11.8		(9.4-14.7)	14.2		(9.8-20.6)	0.030
HDLc/TC	0.335		(0.307-0.382)	0.283		(0.236-0.337)	<0.001
Glucose Metabolism							
Glicose (mg/dl)	85.0		(79.0-91.0)	81.0		(76.0-87.0)	0.014
Insulina (µU/ml)	7.4		(5.2-11.7)	12.0		(8.1-19.0)	<0.001
HOMA	1.60		(1.06-2.52)	2.46		(1.60-3.85)	<0.001
Inflammatory Markers							
CRP (mg/l)	0.35		(0.24-0.78)	1.71		(0.95-3.23)	<0.001
Adiponectin (µg/ml)	5.16		(3.50-6.70)	4.14		(2.88-5.94)	0.004

Results are presented mean ± standard deviation or median (interquartile range). [1)], Control n=37, Obese n=61. Pre-pub, pre-pubertal. BMI, Body Mass Index; CRP, C Reactive Protein; HDLc, High Density Lipoprotein Cholesterol; HOMA, Homeotasis Model Assesment Insulin Resistance; LDLc, Low Density Lipoprotein Cholesterol; TC, Total Cholesterol; TG, triglycerides; VLDLc, Very Low Density Lipoprotein Cholesterol;

3.2. Longitudinal study

The variation of anthropometric variables during the studied period for obese and controls is presented in Table 1. The groups did not differ regarding sex and tanner stage (data not shown). At the end of the intervention program both groups presented a significant decrease in BMIzsc but only the obese group presented a significant reduction of the trunk percentage of fat.

The weight loss in the obese group, as shown by the reduction in BMIzsc, was accompanied by changes in the studied biochemical variables. Obese individuals improved the lipid profile, with a significant reduction of TC and LDLc concentrations, and an increase in the HDLc/TC ratio; IR was also improved, with a decrease in insulin levels and in HOMA.

On their turn, controls did not present any significant changes in lipid profile or IR markers, despite the variation of BMIzsc.

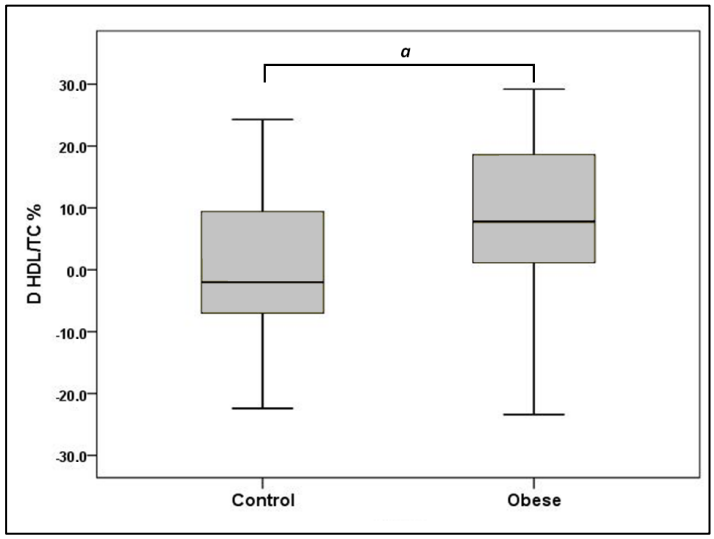
Levels of adiponectin reduced in both groups during the studied period.

Obese and control groups were also compared for the absolute and relative variations of the anthropometric and biochemical variables (data not shown). No difference between the two groups was found when comparing changes in anthropometric data. Conversely, an increase in ΔHDLc/TC% and a trend for the lowering of HOMA was seen in obese (supplemental figures 1 and 2).

Table 1. Variation of anthropometric, nutritional and biochemical characterization during the intervention program in control and obese groups (longitudinal study)

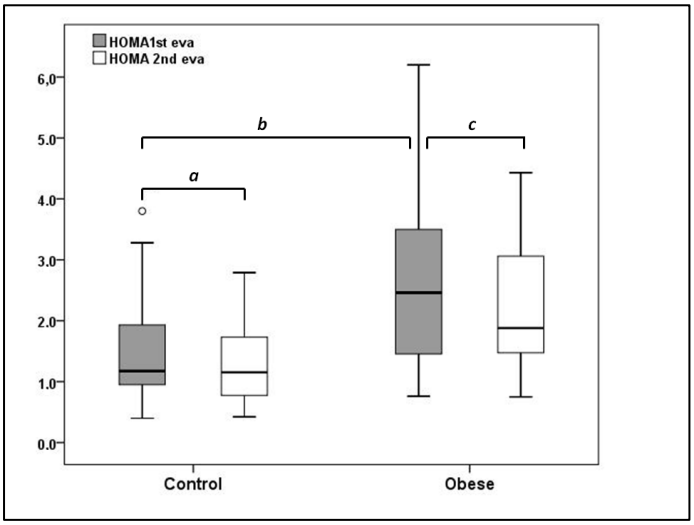
	Control (22)							Obese(35)						
	1st Evaluation			2nd Evaluation			*P*	1st Evaluation			2nd Evaluation			*P*
Height (cm)	140.9	±	17.5	142.4	±	16.8	<0.001	145.7	±	15.1	148.0	±	14.5	<0.001
Weight (Kg)	38.6	±	16.5	39.1	±	16.9	0.101	60.0	±	23.3	61.0	±	23.2	0.099
BMI (kg/m2)	18.4	±	3.4	18.3	±	3.8	0.350	27.1	±	5.4	26.7	±	5.6	0.206
BMI z-score	0.21	±	0.79	-0.04	±	0.96	0.002	2.1	±	0.3	1.9	±	0.5	0.001
Waist (cm)	66.9	±	12.7	66.7	±	12.4	0.856	88.6	±	14.9	90.2	±	15.0	0.093
Waist/Height	0.472	±	0.048	0.467	±	0.054	0.494	0.607	±	0.065	0.608	±	0.063	0.886
Total % Fat[1)]	28.5	±	4.6	28.9	±	5.6	0.460	40.9	±	6.7	40.7	±	6.0	0.663
Trunk % Fat[1)]	24.1	±	4.8	23.5	±	6.2	0.429	39.8	±	8.2	38.1	±	7.2	0.013
Lipid Profile														
TG (mg/dl)			(46.0-72.8)	68.5		(57.5-80.2)	0.147	71.0		(52.0-108.0)	66.0		(53.0-102.0)	0.257
TC (mg/dl)	152.0		(132.8-179.0)	155.0		(141.0-171.8)	0.874	173.0		(151.0-194.0)	157.0		(135.0-186.0)	0.022
HDLc (mg/dl)	50.1		(43.2-60.8)	51.0		(44.1-60.5)	0.957	49.0		(39.0-53.7)	48.0		(41.0-53.6)	0.471
LDLc (mg/dl)	91.5		(75.8-108.5)	89.0		(76.8-102.4)	0.778	108.0		(89.0-120.0)	91.0		(77.0-117.0)	0.006
VLDLc (mg/dl)	11.6		(9.2-14.6)	13.7		(11.6-16.0)	0.145	14.2		(10.4-21.5)	13.2		(10.6-20.4)	0.255
HDLc/TC	0.329		(0.307-0.374)	0.326		(0.294-0.392)	0.934	0.293		(0.231-0.327)	0.319		(0.251-0.348)	0.004
Glucose Metabolism														
Glicose (mg/dl)	84.5		(77.0-90.2)	74.5		(70.0-83.2)	0.002	81.0		(76.0-85.0)	77.0		(72.0-81.0)	0.001
Insulina (µU/ml)	6.1		(4.5-9.8)	6.5		(3.8-9.8)	0.636	12.0		(7.0-17.3)	10.7		(8.1-15.1)	0.048
HOMA	1.18		(0.94-1.94)	1.15		(0.74-1.74)	0.796	2.46		(1.38-3.85)	1.88		(1.40-3.09)	0.014
Inflammatory Markers														
CRP (mg/l)	0.32		(0.24-0.88)	0.60		(0.21-2.19)	0.083	2.00		(1.12-3.41)	1.42		(0.96-3.44)	0.688
Adiponectin (µg/ml)	4.68		(3.39-5.98)	3.60		(2.78-4.63)	<0.001	3.87		(2.68-4.49)	3.00		(2.16-4.03)	<0.001

Results are presented mean ± standard deviation or median (interquartile range). [1)], Control n=19, obese n=31. BMI, Body Mass Index. Results are presented. BMI, Body Mass Index; CRP, C Reactive Protein; HDLc, High Density Lipoprotein Cholesterol; HOMA, Homeotasis Model Assesment Insulin Resistance; LDLc, Low Density Lipoprotein Cholesterol; TC, Total Cholesterol; TG, triglycerides; VLDLc, Very Low Density Lipoprotein Cholesterol.



Supplemental Figure 1. HDL/TC relative variation in obese and control groups

D, Delta; HDLc, High Density Lipoprotein Cholesterol; TC, Total Cholesterol. a, *p*=0.028.



Supplemental Figure 2. HOMA in obese and control groups before and after the intervention program

HOMA, Homeostasis Model Assessment. HOMA, Homeostasis Model Assessment; eva, evaluation. a, *p*=0.796 ; b, *p*=0.001 ; c, *p*=0.014.

3.3. Analysis of adiponectin multimers

To better understand the function of adiponectin isoforms and to verify any difference between obese and controls we selected a pre-pubertal population, in order to minimize the variations caused by other factors rather than obesity (e.g. sexual hormones). The control (n=10) and obese (n=13) groups were adjusted for gender (50% females vs 46.2% females, respectively) and age (mean 7.3±1.4 vs 7.9±1.4, respectively).

The differences in anthropometric and biochemical variables observed between obese and controls in the larger population tend to be kept although TG, HDLc, LDLc and VLDLc, became of borderline statistical significance (data not shown), and adiponectin lost significance.

No differences were observed between obese and controls regarding the absolute and relative multimers concentrations, before and after the PE program (data not shown).

We further analysed the impact of the PE program in TA and in its multimers by dividing the obese group based on having achieved, or not, a BMIzsc reduction of 0.2 (Table 2). The control group did not present significant changes in adiponectin during the intervention period. Regarding the obese participants, the group that reached the cut-off value also did not present any significant reduction of adiponectin. Conversely, obese individuals who did not reach the cut-off value presented a general reduction of TA and its multimers absolute concentration. The relative percentage of the different isoforms was maintained in all groups.

To clarify the functions of the different adiponectin multimers several correlations were performed. First, we analysed associations between TA and the other studied variables using the entire studied population (cross-sectional study; n=104), and then we performed sub-analysis between changes in studied variables over the interventional study (n=57 for TA; n=23 for adiponectin multimers). The levels of TA (n=104) correlated inversely with BMI, total percentage of body fat, TG, insulin, HOMA and CRP, and positively with HDLc and HDLc/TC ratio (Table 3). Regarding the longitudinal analysis (n=57), changes in TA were inversely correlated with changes in markers of adiposity and in TG levels. The multimer that most mimic TA associations at the baseline, was the HMW isoform; even though, statistical significance was lost, probably due to the lower number of cases (n=23).

Table 2. Total adiponectin and adiponectin isoforms in the control and obese groups variation with the intervention program (longitudinal study)

								Obese													
	Control (10)							BMI z-score reduction < 0.2 (8)							BMI z-score reduction ≥ 0.2 (5)						
	1st Evaluation			2nd Evaluation			*P*	1st Evaluation			2nd Evaluation			*P*	1st Evaluation			2nd Evaluation			*P*
Adipo (µg/ml)	4.36	±	1.79	3.87	±	1.25	0.185	4.18	±	1.41	3.31	±	1.34	0.008	4.05	±	1.33	3.58	±	0.94	0.133
HMW (µg/ml)	2.37	±	1.34	2.16	±	0.98	0.442	2.15	±	0.86	1.74	±	0.95	0.015	2.00	±	0.99	1.88	±	0.58	0.648
MMW (µg/ml)	1.03	±	0.40	0.94	±	0.25	0.484	1.11	±	0.33	0.93	±	0.28	0.140	1.07	±	0.38	0.99	±	0.27	0.512
LMW (µg/ml)	0.96	±	0.40	0.76	±	0.16	0.146	0.93	±	0.30	0.64	±	0.26	0.023	0.98	±	0.22	0.72	±	0.22	0.149
% HMW	52.1	±	7.7	53.5	±	9.8	0.476	50.6	±	3.9	50.3	±	7.7	0.897	48.1	±	8.1	51.8	±	4.0	0.372
% MMW	24.4	±	5.5	25.0	±	3.4	0.700	26.8	±	3.2	29.2	±	4.6	0.155	26.2	±	6.0	27.8	±	3.1	0.708
% LMW	23.5	±	7.8	21.5	±	7.9	0.452	22.6	±	4.7	20.5	±	7.1	0.455	25.6	±	7.4	20.4	±	4.6	0.233

Results are presented mean ± standard deviation. BMI, Body Mass Index; Adipo, adiponectin; HMW, High Molecular Weight adiponectin; MMW, Medium Molecular Weight adiponectin; LMW, Low Molecular Weight adiponectin.

Table 3. Associations between total adiponectin and relative adiponectin isoforms with biochemical and anthropometric data, at the beginning of the study and respective variation after the intervention program

Cross-sectional Study				
	Adipo[3)]	**% HMW[4)]**	**% MMW[4)]**	**% LMW[4)]**
Age	-0.071	-0.239	-0.198	0.465*
BMI	-0.322**	-0.240	0.105	0.085
BMI z-score	-0.306**	-0.144	0.163	-0.109
Total % fat[1)]	-0.200*	-0.186	0.129	-0.010
Trunk % fat[1)]	-0.276**	-0.300	0.207	0.040
TG	-0.195*	-0.509*	0.122	0.436*
TC	-0.086	-0.159	0.508*	-0.170
HDLc	0.257**	0.055	0.186	-0.129
LDLc	-0.181	-0.145	0.497*	-0.178
HDLc/TC	0.323**	0.135	-0.166	0.000
HOMA	-0.200*	-0.308	0.206	0.076
CRP	-0.202*	-0.065	-0.105	0.175
Longitudinal Study				
	D Adipo[5)]	**D % HMW[4)]**	**D % MMW[4)]**	**D % LMW[4)]**
D BMI	-0.415**	-0.406	-0.052	0.391
D BMI z-score	-0.350**	-0.332	-0.076	0.368
D Total % fat[2)]	-0.333*	0.399	-0.269	-0.206
D Trunk % fat[2)]	-0.299	0.107	-0.179	-0.009
D TG	-0.213	-0.191	0.272	-0.040
D TC	0.070	-0.174	0.528**	-0.233
D HDLc	0.188	0.023	0.021	0.001
D LDLc	0.090	-0.140	0.476*	-0.237
D HDLc/TC	0.076	0.055	-0.371	0.257
D HOMA	0.084	0.244	-0.167	-0.171
D CRP	-0.176	0.119	-0.117	0.020

Presented associations were calculated using Spearman rank test. Adipo, adiponectin; HMW, High Molecular Weight adiponectin; MMW, Medium Molecular Weight adiponectin; LMW, Low Molecular Weight adiponectin, BMI, Body Mass Index; TG, Triglycerides; TC, Total Cholesterol; HDLc, High Density Lipoprotein Cholesterol; LDLc, Low Density Lipoprotein Cholesterol; HOMA, Homeostasis Model Assessment Insulin Resistance; CRP, C Reactive Protein. D, delta (2^{nd} evaluation – 1^{st} evaluation). **3)**, n=104; **4)**, n=23; **5)** n=57. **1)** n=98 for Adipo; **2)** n=50 for D Adipo. *, $P<0.05$; **, $P<0.01$.

Improvements in adiposity variables at pre-pubertal stages, especially BMI, correlated with increments in circulating levels of HMW and MMW forms (Figure 1). Moreover, by multiple regression analysis, the parameter that best explained changes in HMW isoform was the delta BMIzsc (R^2=0.299, standardized coefficient/beta: -0.547, *P*=0.013).

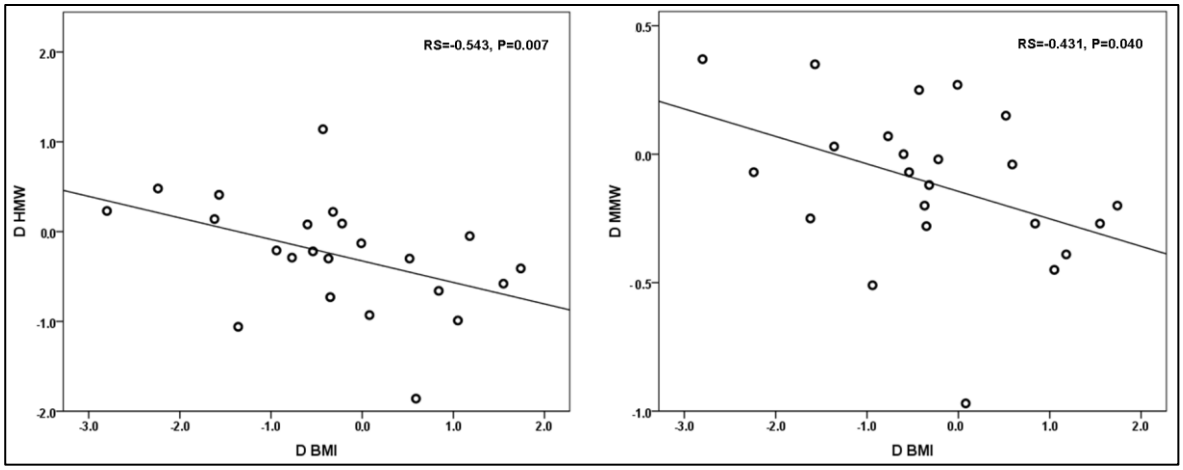


Figure 1. Variation of high and medium molecular weight adiponectin (HMW and MMW, respectively) with changes in body mass index (BMI) after the intervention program

The increase in HMW relative percentage was related with lower levels of circulating TG. In contrast, despite the negative correlation with adiposity, an increased relative percentage of MMW was associated with increased levels of TC and LDLc ($p<0.01$ and 0.05, respectively).

4. Discussion

4.1. Cross-sectional study

Childhood obesity was accompanied by worsening of MS markers(3, 4) In our study the obese group presented a worse lipid profile, increased IR (measured by HOMA) and inflammation, as seen in supplemental Table 1.

The obese group presented increased IR, although showing normal levels of glucose, pointing at an unbalanced glucose metabolism. In children, the insulin levels seem to be an early indicator of metabolic changes, appearing first than anomalies in glycaemia.

4.2. Longitudinal study

Although both obese and controls reduced BMI-score following the intervention program, only the obese group presented clear changes in the body composition, with a significant reduction of the trunk percentage of fat. The body fat distribution influences the metabolism; actually, a central accumulation of adiposity has been associated with an increase in CVD risk.(4, 12, 13) The reduction of the central adiposity in the obese group is an important indicator of the positive impact of the PE program.

Accompanying the decrease in adiposity in obese individuals, were improvements in biochemical variables, namely lipid profile and insulin. Obese individuals presented with an improved lipid profile as seen by a decrease in ΔHDLc/TC% ratio. Actually, more than 75% of the obese individuals presented a decrease in that atherogenic index, while only less than 50% of the controls. This is an important finding as this ratio is an accepted marker of atherosclerotic risk.(6) As well, the trend to an improvement in HOMA in obese patients indicated a better glucose homeostasis; a particularly relevant point as IR continues to explain most, if not all, of MS.(27) Together, these changes demonstrate a global improvement in lipid and glucose metabolisms following the intervention program.

The lack of changes seen for controls, regarding lipid profile or IR, is probably due to the fact that these variables were already at good levels and, although the practice of exercise improved the nutritional status, their metabolism was previously balanced and no further changes could be detected.

Considering that the metabolism of obese individuals is unbalanced, the BMIzsc reduction observed in this group, with a particular decrease in central adiposity, likely contributed to lead the biochemical variables to the desirable levels. In fact, changes in body fat distribution might be the key factor behind the metabolic improvement.

The PE program was unable to increase TA values in any of the groups, contrarily to other studies.(22) This might be related with a lower reduction of BMIzsc presented by our participants, despite the significant changes in anthropometric variables. Additionally,

adiponectin is negatively correlated with age in children and adolescents, meaning that it might be necessary greater BMIzsc reductions to oppose this physiological trend.(6)

4.3. Analysis of adiponectin multimers

For the study of adiponectin isoforms we selected a pre-pubertal population in order to minimize the variations caused by other factors rather than obesity. Despite the differences in anthropometric and biochemical variables between obese and controls in the larger population were, somehow, maintained, no significant changes were observed between the two groups regarding TA and absolute and relative multimers concentrations, both before and after the PE program (data not shown). Oppositely, some studies have pointed to an increase in HMW following intervention.(16, 28) The lack of significant differences might be related to a small sample size, however it can also indicate that the differences previously found could be due to confounding factors, present later in life, or, that differences in TA concentrations seen at older ages, might be caused by a cumulative effect, still not evident at early ages. So, acting early might be the best strategy to avoid obesity impact later in life.

TA and its multimers absolute concentrations did not vary both in controls and in the obese group that reached the cut-off value for BMIzsc reduction (0.2), however in the obese individuals who did not reach that cut-off, there was noted a general reduction. Interestingly the relative percentage of the different isoforms was maintained in all groups.

Other studies have reported that BMIzsc reduction is related to an increase in TA, particularly in the HMW form.(16, 28) In children, adiponectin decreases with aging (6), therefore, a small reduction of BMIzsc (0.2), might not be enough to raise the TA levels, but it seems to be enough to slow the process as age-related adiponectin reduction seems to be accelerated in obese individuals. Moreover, previous data from our group demonstrated that small reductions in BMIzsc are associated with significant improvements in lipid profile and IR, markers of MS inversely related to adiponectin levels.(24)

Increased TA, at baseline, was correlated with lower adiposity, inflammation and IR and with a better lipid profile (Table 3). Additionally, the longitudinal analysis showed that increases in TA are related to decreases in adiposity and TG. These results are in agreement with previous studies, showing that higher values of TA are associated with reduced body weight and improved lipid profile, inflammatory status and insulin sensitivity. (6, 9)

An important finding of our longitudinal study was that improvements in the adiposity variables, at pre-pubertal stages, correlated with increments in circulating HMW. In fact, when multiple regression analysis was used, variations of HMW were explained by

changes in BMIzsc (negative association). HMW is proposed to be the most biological-active form of adiponectin, being lower adiposity associated with higher circulating HMW.(16)

HMW adiponectin has been reported to correlate closer with IR than with adiposity, however, the association between TA and HMW with IR in pre-pubertal children is not so clear and is said to appear only between the ages of 2 and 6 years.(29) Despite the age of our pre-pubertal group fell outside those limits, we did not find any association of HMW with IR. Nevertheless, the increased levels of HMW relative percentage were associated with lower TG levels. HMW relation with a better lipid profile is probably explained by the influence of this multimer in liver insulin sensitivity.(18)

Thus, it seems that HMW mimic the associations of TA with adiposity and lipid profile, and is possible that this adiponectin fraction explains most of those associations.

Another multimer, MMW, presented ambiguous results. Although the increase of MMW adiponectin was associated with the improvement of body weight (Figure 1), it was also linked with a worse lipid profile. More studies are necessary to verify these relations.

The impact of the other multimers rather than HMW in obesity has been poorly explored;(16) actually we are, to the best of our knowledge, the first group to describe associations between the worsening of lipid profile and increasing MMW.

The study has some weakness: 1)The sample size is not very large and some differences might have been underestimated; 2)Adiponectin multimers were analysed only in pre-pubertal individuals, an age period when the relation between adiponectin and MS is still not clear.

Despite these limitations, the study of adiponectin multimers in pediatric patients before and after an interventional study is rare and, thus, we think our work may offer some important information. Moreover, this is, as far as we know, the first study on this subject in Portugal.

5. Conclusion

Childhood obesity is accompanied by an increase in MS features (worse lipid profile and increased IR) and a pro-inflammatory state. Lower adiponectin levels in obese patients are likely to be related with most of these deleterious changes; however, differences are not evident at pre-pubertal stages. The adiponectin multimers that mostly explained MS features at pre-pubertal stages were HMW and MMW.

Insulin is an early indicator of IR and should be used as a routine measurement in pediatric obesity.

The PE program helped to improve the lipid profile and to decrease IR in obese individuals, though with no significant variation of TA or adiponectin multimers. Nevertheless, pre-pubertal patients that reduced the BMIzsc maintained adiponectin levels, contrarily to individuals who did not.

Understanding how total adiponectin and its oligomers influence the metabolism is an important issue, particularly for children, in who an early intervention can present relevant impact in the future. Thus, our work highlights that the different circulating forms of adiponectin might present different, or even opposite effects, in MS features. On its turn, weight loss helped to sustain what appears to be a positive profile of adiponectin isoforms.

Acknowledgements

The authors wish to thank to all participants and parents for their collaboration, and the nurses and laboratory technicians of the hospitals involved for their technical support. We also want to thank the ACORDA team for all the help in the intervention program.

This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-028613 (PTDC/DTP-DES/0393/2012). A PhD grant was attributed to H. Nascimento by FCT (SFRH/BD/48060/2008).

References

1. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochemical and Biophysical Research Communications. 1996;221(2):286-9.
2. Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, et al. Organization of the gene for gelatin-binding protein (GBP28). Gene. 1999;229(1-2):67-73.
3. Calcaterra V, De Amici M, Klersy C, Torre C, Brizzi V, Scaglia F, et al. Adiponectin, IL-10 and metabolic syndrome in obese children and adolescents. Acta Bio-medica : Atenei Parmensis. 2009;80(2):117-23.
4. Yoshinaga M, Takahashi H, Shinomiya M, Miyazaki A, Kuribayashi N, Ichida F. Impact of having one cardiovascular risk factor on other cardiovascular risk factor levels in adolescents. Journal of Atherosclerosis and Thrombosis. 2010;17(11):1167-75.
5. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. The Journal of Clinical Investigation. 2003;112(1):91-100.
6. Nascimento H, Silva L, Lourenco P, Weinfurterova R, Castro E, Rego C, et al. Lipid profile in Portuguese obese children and adolescents: interaction of apolipoprotein E polymorphism with adiponectin levels. Archives of Pediatrics and Adolescent Medicine. 2009;163(11):1030-6.
7. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001;50(9):2094-9.
8. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, et al. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. American Journal of Physiology. 2003;285(3):E527-33.
9. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. The Journal of Clinical Endocrinology and Metabolism. 2009;94(10):3687-95.
10. Urbina EM, Khoury P, Martin LJ, D'Alessio D, Dolan LM. Gender differences in the relationships among obesity, adiponectin and brachial artery distensibility in adolescents and young adults. International Journal of Obesity. 2009;33(10):1118-25.
11. Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. The Journal of clinical endocrinology and metabolism. 2004;89(8):3790-4.
12. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(4):459-69.
13. Ogawa Y, Kikuchi T, Nagasaki K, Hiura M, Tanaka Y, Uchiyama M. Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese Japanese children. Hypertension Research : official journal of the Japanese Society of Hypertension. 2005;28(1):51-7.
14. Kwon K, Jung SH, Choi C, Park SH. Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. International Journal of Cardiology. 2005;101(3):385-90.
15. Schober F, Neumeier M, Weigert J, Wurm S, Wanninger J, Schaffler A, et al. Low molecular weight adiponectin negatively correlates with the waist circumference and monocytic IL-6 release. Biochemical and Biophysical Research Communications. 2007;361(4):968-73.
16. Araki S, Dobashi K, Kubo K, Asayama K, Shirahata A. High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. The Journal of Clinical Endocrinology and Metabolism. 2006;91(12):5113-6.
17. Liu Y, Retnakaran R, Hanley A, Tungtrongchitr R, Shaw C, Sweeney G. Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. The Journal of Clinical Endocrinology and Metabolism. 2007;92(11):4313-8.
18. Neumeier M, Sigruener A, Eggenhofer E, Weigert J, Weiss TS, Schaeffler A, et al. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. Biochemical and Biophysical Research Communications. 2007;352(2):543-8.
19. Murdolo G, Nowotny B, Celi F, Donati M, Bini V, Papi F, et al. Inflammatory adipokines, high molecular weight adiponectin, and insulin resistance: a population-based survey in prepubertal schoolchildren. PLoS One. 2011;6(2):e17264.
20. Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. American Journal of Physiology. 2010;298(4):E824-31.
21. Pedrosa C, Oliveira BM, Albuquerque I, Simoes-Pereira C, Vaz-de-Almeida MD, Correia F. Metabolic syndrome, adipokines and ghrelin in overweight and obese schoolchildren: results of a 1-year lifestyle intervention programme. European Journal of Pediatrics. 2011;170(4):483-92.
22. Elloumi M, Ben Ounis O, Makni E, Van Praagh E, Tabka Z, Lac G. Effect of individualized weight-loss programmes on adiponectin, leptin and resistin levels in obese adolescent boys. Acta Paediatrica. 2009;98(9):1487-93.
23. The Third National Health and Nutrition Examination Survey. The Third National Health and Nutrition Examination Survey (NHANES III 1988-94) Reference Manuals and Reports. 1996.
24. Nascimento H, Costa E, Rocha-Pereira P, Rego C, Mansilha HF, Quintanilha A, et al. Cardiovascular risk factors in portuguese obese children and adolescents: impact of small reductions in body mass index imposed by lifestyle modifications. The Open Biochemical Journal. 2012;6:43-50.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
27. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2010;375(9710):181-3.
28. Araki S, Dobashi K, Yamamoto Y, Asayama K, Kusuhara K. Increased plasma isoprostane is associated with visceral fat, high molecular weight adiponectin, and metabolic complications in obese children. European Journal of Pediatrics. 2010;169(8):965-70.
29. Ibanez L, Lopez-Bermejo A, Diaz M, Angulo M, Sebastiani G, de Zegher F. High-molecular-weight adiponectin in children born small- or appropriate-for-gestational-age. The Journal of Pediatrics. 2009;155(5):740-2.

14.8. Paper VIII

Nascimento H, Catarino C, Rêgo C, Ferreira Mansilha H, Quintanilha A,Santos-Silva A, Belo L. "*CDC BMI z-score is a better predictor of metabolic syndrome than WHO BMI z-score in Portuguese obese adolescents." Submitted.

CDC BMI z-score is a better predictor of metabolic syndrome than WHO BMI z-score in Portuguese obese adolescents

Henrique Nascimento[1,2], Cristina Catarino[1,2], Carla Rêgo[3], Helena Ferreira Mansilha[4], Alexandre Quintanilha[2,5], Alice Santos-Silva[1,2], Luís Belo[1,2*]

[1]Laboratório de Bioquímica, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, 4050-313 Porto, Portugal; [2]Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, 4150-180 Porto, Portugal; [3]Centro da Criança e do Adolescente. Hospital Cuf Porto. CINTESIS - Faculdade de Medicina, Universidade do Porto; 4200-319 Porto, Portugal; [4]Departamento da Infância e Adolescência/Serviço de Pediatria do Centro Hospitalar do Porto; 4099-001 Porto, Portugal; [5]Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4099-003 Porto, Portugal

Abstract

Introduction: Obesity, particularly central obesity, associates with dyslipidemia, insulin resistance and hypertension, to form a cluster of risk factors of cardiovascular disease called metabolic syndrome (MS). Different criteria are used to define body mass index (BMI) z-score, a variable used to evaluate the nutritional status, in children and adolescents. In this study we aimed to evaluate the differences in BMI z-scores determined using the criteria of the Center for Disease Control and Prevention (CDC), and that of the World Health Organization (WHO), and their relation with MS and its features.

Methods: 246 obese adolescents (10-18 years, 122 females) participated in the study. Anthropometric variables, systolic (SBP) and diastolic blood pressure (DBP), plasma~~tic~~ triglycerides, HDLc, glucose, insulin, $HOMA_{IR}$, adiponectin and C-reactive protein (CRP) were determined.

Results: Using the International Diabetes Federation (IDF) criteria, obese individuals with MS presented (as a group) increased adiposity markers, dyslipidaemia (increased triglycerides, and reduced HDLc), blood pressure (increased SBP and DBP) and insulin resistance (increased insulin, glucose and $HOMA_{IR}$) than those without MS. MS patients also presented lower adiponectin values ($p< 0.001$) and a trend to higher CRP values ($p=0.072$). By linear regression analysis, the factors that better predicted MS were SBP, triglycerides and HDLc. When only anthropometric data was analysed, CDC BMI z-score was an independent predictor of MS ($p< 0.001$).

Conclusion: In our adolescent population, BMI z-score determined using the CDC reference showed higher specificity to predict MS than the WHO reference. Furthermore, the measurement of SBP should be highly encouraged in routine appointments, as we found that this low-cost and non-invasive measurement has a high predictive value for MS.

Keywords: Metabolic Syndrome, CDC, WHO, obesity, cardiovascular disease

1. Introduction

Obesity is a chronic inflammatory disease that is spreading worldwide, particularly in pediatric ages. This is an important problem in Portugal, as our country has a high prevalence of weight excess, with almost one third of overweight or obese children and adolescents (1, 2). Obesity, together with dyslipidaemia, hypertension and insulin resistance (IR), form a cluster of risk factors for the development of cardiovascular disease (CVD), known as the metabolic syndrome (MS) (3).

In adults, obesity is defined by a body mass index (BMI) higher than 30 kg/m^2. During childhood, BMI evolution is not linear and must be adjusted for age and gender. The two most used criteria to define obesity are from the Centre for Disease Control and Prevention (CDC) and from the World Health Organization (WHO) (4). These two references were built based on different populations. The WHO criterion is based on a multi-ethnic population, while the CDC criterion used only values from the United States. This fact led to the construction of different anthropometric growth curves and cut-offs regarding the nutritional status in pediatrics: according to 2000 CDC growth charts obesity is defined by a BMI z-score higher than +1.65 (5), while WHO defines obesity by a BMI z-score higher than +2 (1). If the same population is studied using both criteria, different outputs are obtained: WHO reference associates with a lower prevalence of thinness and a higher prevalence of obesity and overweight, while CDC criterion gives lower prevalence of overweight and obesity and increased rates of thinness (1).

In Portugal the CDC criterion was used for a long time, as it was recommend by the Portuguese Ministry of Health. However, the current recommended criterion is that from the WHO (6). The aim of this study was to evaluate the impact of this change, by comparing the relation between the BMI z-score, calculated according to the CDC and to the WHO criteria, with MS features, in a Portuguese obese pediatric population.

2. Material and methods

2.1. Subjects

Obese adolescents, aged 10-18 years, were identified from medical records, at the outpatient clinics of pediatric obesity from two hospitals in Porto - Portugal. The study protocol was approved by the Committees on Ethics of the two hospitals.

A total of 246 obese adolescents participated in the study after informed and written consent of their parents. Smokers, subjects with diabetes mellitus, endocrine disorders, hereditary diseases, inflammatory or infectious diseases or under any therapy that could interfere with our results were excluded from the study.

2.2. Procedures and assays

2.2.1 Anthropometric characterization and clinical evaluation

All participants were subjected to clinical examination. Height, weight and waist circumference (at the level of the iliac crest) were measured. BMI z-score was determined according to CDC and WHO criteria and children were classified as normal weight, overweight or obese (7, 8). Only obese individuals entered in this study.

2.2.2. Blood samples

Blood was collected by venipuncture in ethylenediaminetetraacetic acid (EDTA) containing tubes, after overnight fasting (10-12h) and processed within 2h of collection. Aliquots of plasma were made, and immediately stored at -80ºC until assayed.

The plasma levels of C-reactive protein (CRP) were determined by immunoturbidimetry [CRP (latex) High-Sensitivity, Roche Diagnostics].

The determination of circulating levels of glucose and insulin was performed by using routine automated technology (ABX Diagnostics). Homeostasis model assessment of insulin resistance ($HOMA_{IR}$) was determined by using the following formula: $HOMA_{IR}$ = fasting insulin (μU/ml) × fasting glucose (mg/dl) / 405.

Plasma lipids and lipoprotein analysis were performed in an auto-analyser (Cobas Mira S, Roche) using commercially available kits. Triglycerides concentrations were determined by enzymatic colorimetric tests (GPO-PAP methods, Roche). HDLc was measured using an enzymatic colorimetric test (Direct HDLcholesterol, Roche).

2.2.3 Definition of metabolic syndrome

The MS was defined according to the International Diabetes Federation (IDF) worldwide criterion. Briefly, the MS is defined by the presence of at least 3 risk factors, of which the presence of abdominal obesity, as defined by a waist circumference higher than the 90th percentile, adjusted for age and sex, is mandatory. The other risk factors cut-offs are: pre-diagnosed hypertension or type 2 diabetes mellitus; triglycerides ≥ 150mg/dl; high density lipoprotein cholesterol (HDLc) <40 mg/dl (or 50mg/dl if female and older than 16 years); systolic blood pressure (SBP) ≥ 130 mmHg; diastolic blood pressure (DBP) ≥ 85 mmHg; fasting glucose ≥100 mg/dl (9).

2.3. Statistical analysis

The distributions of continuous variables were analysed using Kolmogorov-Smirnov tests to assess significant departures from normality. Normally distributed variables are presented as mean±SD. Variables non-normally distributed are presented as median (interquartile range) and were log transformed before further analyses. Comparisons between two groups were performed using Student's unpaired t-test. Adjustment of statistical differences for confounding factors was performed using ANCOVA. The association between categorical variables was analysed using chi-squared test.

The strength of the association between the variables was estimated by Pearson correlation coefficient. To evaluate the contribution of the different variables to detect MS, multiple regression analysis was performed, using stepwise selection, with an entry criteria of $p < 0.05$.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0 (IBM, Armonk, NY, USA). Statistical significance was accepted at p less than 0.05.

3. Results

The anthropometric data, blood pressure, glucose metabolism, lipid profile and inflammatory data of the obese children and adolescents, according to the presence or not of MS, are presented in Table 1.

The groups were adjusted for gender and Tanner stage of pubertal development (data not shown). Obese patients with MS presented increased adiposity, a worst lipid profile (increased triglycerides and reduced HDLc), higher blood pressure (increased SBP and DBP), increased insulin resistance (increased insulin, glucose and $HOMA_{IR}$) and lower adiponectin values. CRP levels were also higher in MS patients, though this result only reached borderline statistical significance (p=0.072).

Table 1 also presents the correlations between the different markers of metabolic risk and the BMI z-score, as determined by the WHO and CDC criteria. The correlations were similar for both criteria, however the CDC criterion presented, in general, a stronger correlation with the different variables (exception made for CRP and DBP).

To clarify the predictive value of the studied factors to identify MS, a logistic regression analysis was performed involving all the variables presented in Table 1 and the Tanner stage. The best predictive marker of MS was the SBP, followed by triglycerides and HDLc ($p<0.001$ for all). The used model presented a sensitivity of 67.4% and a specificity of 94.1%.

In order to evaluate which clinical anthropometric data were more closely associated with MS, we performed a logistic regression considering only waist circumference, weight, BMI, and BMI z-score (adjusted for gender, age and Tanner stage) calculated using the two criteria. CDC BMI z-score was the only independent predictor of MS ($p< 0.001$). The model presented a sensitivity of 19.7% and a specificity of 96.0%.

Table 1. Clinical and biochemical data of obese children and adolescents based on the presence of metabolic syndrome and the correlation with BMI z-score according to CDC or WHO criteria

			Correlatio with BMI z-score	
	Without MS	**With MS**	***p***	**WHO**	**CDC**
Number of participants (%)	185 (75.2)	61 (24.8)		-	-
Females, n (%)	97 (52.4)	25 (41.0)	0.141	-	-
Age (years)	12.7 ± 1.8	13.4 ± 2.1	0.017	0.057	0.122
Height (cm)	158.5 ± 8.9	162.8 ± 9.9	0.002	0.189**	0.277**
Weight (kg)	78.0 (68.0-89.0)	90.2 (76.0-113.5)	<0.001	0.673**	0.726**
BMI (kg/m²)	30.55 (28.15-34.08)	34.52 (30.20-39.14	<0.001	0.848**	0.865**
BMI z-score (CDC)	2.20 ± 0.29	2.43 ± 0.31	<0.001	0.933**	-
BMI z-score (WHO)	3.00 ± 0.62	3.51 ± 0.86	<0.001	-	0.933**
Waist circumference (cm)	99.0(91.8-106.5)	105.0 (96.5-118.0)	<0.001	0.736**	0.778**
Blood pressure[1)]					
Systolic (mm Hg)	116.5 ± 11.6	135.0 ± 13.2	<0.001	0.323**	0.360**
Diastolic (mm Hg)	63.4 ± 8.3	69.9 ± 12.2	0.001	0.267**	0.218**
Lipid profile					
TG (mg/dL)	71.0 (53.1-100.6	126.7 (81.5-181.5)	<0.001	0.186**	0.185**
HDLc (mg/dL)	43.0 (38.3-49.0)	35.0 (32.0-38.4)	<0.001	-0.215**	-0.223**
Glucose metabolism					
Glucose (mg/dl)	83.0 (77.0-89.6)	88.0 (80.0-93.8)	0.005	0.043	0.046
Insulin (µU/ml)	15.9 (12.2-22.1)	23.3 (14.6-33.3)	<0.001	0.421**	0.440**
$HOMA_{IR}$	3.18 (2.43-4.52)	4.94 (3.34-7.06)	<0.001	0.410**	0.429**
Inflammatory mediators					
CRP (mg/L)[2)]	1.61 (0.84-4.05)	2.30 (1.00-4.59)	0.072	0.326**	0.302**
Adiponectin[3)]	5.92 (4.14-8.36)	4.25 (2.82-5.70)	<0.001	-0.073	-0.124

*, $p<0.05$; **, $p<0.001$. 1) With MS n=45, without MS n= ; 2) With MS n=60, without MS, n=180. 3) With MS n=60, without MS, n=177. BMI, Body mass index; CDC, Center for Disease Control; C-reactive protein; TG, Triglycerides; HDLc, High Density Lipoprotein cholesterol; MS, metabolic syndrome, WHO, World Health Organization.

Figure 1 illustrates the relation between the BMI z-score obtained using the two criteria. The relation presents an exponential behaviour, with WHO criteria presenting a wider range of BMI z-score values.

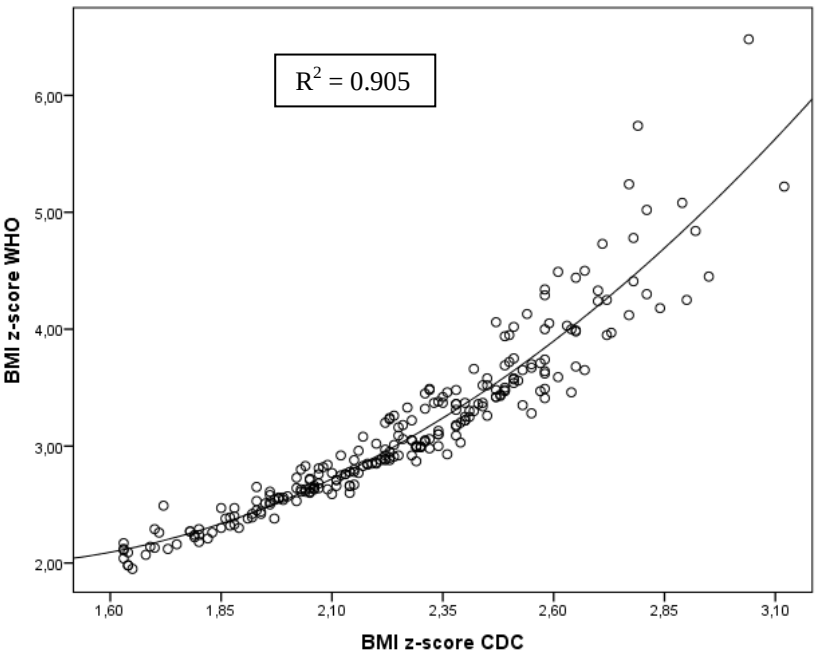


Figure 1. Distribution of BMI z-score values of obese children and adolescents using CDC and WHO criteria.
BMI, body mass index; CDC, Center for Disease Control and Prevention; WHO, World Health Organization.

4. Discussion

Obesity, particularly central obesity, is associated with a worse metabolic profile and an increased risk of presenting MS, a cluster of risk factors for CVD. The fact that almost one quarter of the subjects in our population presented MS is in accordance with other studies, reporting a high prevalence of MS for obese individuals; lean individuals presented no cases of MS (10).

In our population, the factors that better predicted MS were SBP, triglycerides and HDLc. Interestingly abdominal obesity, as defined by the waist circumference, a *sine qua non* condition for MS, according to IDF, did not enter the model (although closely associated with most of the variables (data not shown). Furthermore, when only anthropometric data was analysed by linear regression analysis, CDC BMI z-score was an independent predictor of MS. Thus, the evaluation of SBP and BMI z-score should be encouraged as they can be cost effective determinations for identifying individuals with increased metabolic derangement.

We verified that the BMI z-score calculated using the criteria of CDC had a better correlation with metabolic and anthropometric variables, when compared to WHO criteria. As a result, CDC BMI z-score was a better predictor of the presence of MS.

Although the WHO criteria is based on a multi-ethnic population, and the CDC criteria uses only values from the United States, it appears that, in what metabolic risk is concern, the CDC criteria is more sensible. Moreover, the widening of the BMI z-score scale in the WHO criteria (Figure 1), especially for higher values, does not seem to add much to the capacity of BMI z-score to identify individuals in increased metabolic risk.

In conclusion, although the BMI z-score is not the most sensible marker for MS, the values defined by the CDC criteria correlated better with other risk markers and were able to predicted (amongst all anthropometric data) the presence of MS. SBP has a high predictive value for MS for our population and, thus, this low-cost and non-invasive measurement should be used in routine appointments.

Acknowledgements

Funding by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT under the project FCOMP-01-0124-FEDER-028613 (PTDC/DTP-DES/0393/2012). A PhD grant was attributed to H. Nascimento by FCT (SFRH/BD/48060/2008).

References

1. Rito A, Wijnhoven TM, Rutter H, Carvalho MA, Paixao E, Ramos C, et al. Prevalence of obesity among Portuguese children (6-8 years old) using three definition criteria: COSI Portugal, 2008. Pediatric obesity. 2012;7(6):413-22.
2. Padez C, Fernandes T, Mourao I, Moreira P, Rosado V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. Am J Hum Biol. 2004;16(6):670-8.
3. Alexander CM, Landsman PB, Grundy SM. The influence of age and body mass index on the metabolic syndrome and its components. Diabetes, obesity & metabolism. 2008;10(3):246-50.
4. Flegal KM, Ogden CL. Childhood obesity: are we all speaking the same language? Adv Nutr. 2011;2(2):159S-66S.
5. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. Vital and health statistics Series 11, Data from the national health survey. 2002(246):1-190.
6. Programa Nacional de Saúde Infantil e Juvenil. In: Divisão de Saúde Sexual RIeJ, editor.: Direção Geral de Saúde - Ministério da Saúde; 2013.
7. Martinez-Costa C, Nunez F, Montal A, Brines J. Relationship between childhood obesity cut-offs and metabolic and vascular comorbidities: comparative analysis of three growth standards. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2013.
8. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320(7244):1240-3.
9. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059-61.
10. Papoutsakis C, Yannakoulia M, Ntalla I, Dedoussis GV. Metabolic syndrome in a Mediterranean pediatric cohort: prevalence using International Diabetes Federation-derived criteria and associations with adiponectin and leptin. Metabolism: clinical and experimental. 2012;61(2):140-5.

15. Discussion

Obesity is increasing worldwide both in adults and in children. In Portugal the prevalence of pediatric obesity is high, with almost one third of the children and adolescents presenting overweight or obesity (1, 12, 183).

Childhood obesity is a serious public health problem, as obese children have an increased prevalence of a large number of comorbidities (e.g. T2DM, dyslipidemia and hypertension), increasing the risk for CVD and for mortality later in life (5, 20, 179). Moreover, obese-related complications represent great costs to our society, both at human and financial levels.

With this work we aimed to clarify the changes in classical and new CVD risk factors in obese children and adolescents, and the impact of two different approaches in those risk factors and in obesity itself: a lifestyle modification program and an interventional program involving physical exercise. In this way we performed a cross-sectional analysis and a longitudinal analysis.

CROSS-SECTIONAL ANALYSIS

Obese Portuguese children and adolescents presented altered CV risk markers, namely worsening of MS features (glucose metabolism, lipid profile and blood pressure), inflammation and oxidative stress, when compared to normal weight controls (Papers V, VI and VII).

Glucose metabolism

IR is reported to underlie many of the CV risk changes observed in obesity. We found that obese children and adolescents present increased $HOMA_{IR}$ and insulin levels, despite the normal values of glucose (Papers VI e VII). In fact, hyperinsulinemia has been reported as the earlier marker of metabolic derangement and insipient IR in obese children (4, 25). The peripheral resistance to insulin leads to the accumulation of glucose in the blood, triggering the secretion of more insulin by the pancreas, in order to normalize glucose levels. Through this feedback mechanism, the body is able to keep normal glucose levels and enables its normal uptake and utilization by peripheral tissues. On the other hand, a

continuous exposition of organs and tissues to increased insulin concentrations would further increase IR, contributing to a vicious cycle that, if not counterbalanced, will progress until the pancreas achieves its maximum secretory capacity and, therefore, the peripheral IR is no longer compensated by increasing insulin secretion. The development of T2DM is the final step of this pathway (5, 33, 179).

Our data suggest that it is important to measure insulin in pediatric obesity, in order to detect early metabolic changes, especially in children with increased risk factors (family history of early CVD, diabetes and obesity, or personal history of obesity and comorbidities commonly associated with IR, such as dyslipidemia, hypertension and acanthosis nigrican) (197, 218). Portugal has a program for diabetes screening, however, it focus only on the importance to determine glycemia (18). The inclusion of insulin measurement, adapted for age and gender, as a part of the diabetes screening protocol, would be valuable. $HOMA_{IR}$ is a good indicator of the degree of IR, even in children, and has a positive correlation with oral glucose tolerance test (OGTT); thus, $HOMA_{IR}$ could also be useful, as a routine marker of IR, in pediatric populations (197, 218). By doing so, we could promote early detection of children at increased risk of developing diabetes and, therefore, to prevent its associated complications.

Lipid profile

Concerning the lipid profile, we found that obese children and adolescents presented risk changes in the lipid profile, with higher TG, TC, LDLc, apo B, and lower HDLc and apo A-1 levels, as compared to normal weight individuals. The ratios TC/HDLc and apo B/apo A-1, known markers of atherogenic risk, were increased as well (Paper VI and VII). A worsening of the lipid risk profile can be directly linked to an increase in IR, both at hepatic and peripheral levels (4, 25). The increase in IR causes the increase in the hepatic production of VLDL, leading to increased circulating levels of TG and LDLc. Moreover, the LpL activity is reduced in the presence of peripheral IR, further increasing the plasmatic TG, and reducing HDLc, by increasing its degradation and reducing its synthesis (4, 5, 25, 179).

In obese individuals, the atherogenic changes in blood lipids seem to be present since early in life. The chronic exposition of the vasculature to atherogenic lipids and lipoproteins leads to the deposit of lipids in the subendothelial space of the vessels walls, to the formation of foam cells and atherosclerotic plaques and to vascular remodeling.

Hyperinsulinemia will also contribute to an increase in smooth muscle cell proliferation and, thus, to atherosclerosis. These changes are cumulative and increase the risk for CVD in the adult life (197, 218).

Increased blood pressure in obese individuals is, in part, a direct effect of the atherosclerotic changes, which lead to less elastic blood vessels. Moreover, higher insulin concentration contributes to hypertension, by increasing sympathetic activity and promoting sodium retention by the kidneys (4, 25).

Inflammation and oxidative stress

Besides the study of classical markers, our work focused on inflammatory mediators as new markers of CVD. Inflammatory processes are reported to be linked to the genesis of IR. In fact, inflammatory mediators, such as TNF-α, inhibit insulin signaling, while the anti-inflammatory adipokine adiponectin increases insulin sensitivity (5, 31) (Paper I).

We found that obese children and adolescents presented an increased inflammatory condition. Actually, CRP, a sensitive marker of inflammation, was 3-5 fold higher in obese individuals (Paper VI and VII), when compared with lean controls, while adiponectin was decreased (Paper VII). CRP is known to be secreted by the liver, in response to VAT IL-6. Thus, an increase in VAT, and therefore in central obesity, is associated with an increase in inflammatory mediators (95, 123) (Paper I).

The observed increase in inflammatory markers in obese children and adolescents was closely linked to changes in other markers of CVD risk, namely increased adiposity, particularly central adiposity, increased IR and risk changes in the lipid profile (Papers II, IV, V, VI and VII).

Leukocytosis and an increase in neutrophil/lymphocyte ratio have been associated with obesity (175, 247). In our study obese individuals presented an increase in neutrophil percentage, and neutrophil count was positively correlated with adiposity, namely with central adiposity markers (WC and WC/height ratio). Moreover, a positive correlation was observed between CRP and neutrophil count (Paper V). Neutrophils are able to secrete a large number of inflammatory mediators in response to WAT adipokines. The WAT adipokines, IL-6 and IL-1, are able to induce an increase in hepatic CRP synthesis and in circulating neutrophils, via demargination of neutrophils from the marginal pool (248-250).

The increase in the neutrophil/lymphocyte ratio, in the absence of another cause for neutrophilia, suggests an unbalance towards an inflammatory response (173). Regarding this finding, the leukogram, a non-expensive routine analysis, might be used as an obese-related inflammation indicator.

Worsening of the metabolic profile was closely associated to increased adiposity. In fact, when considering only the obese individuals, those with increased BMI z-score, or with increased percentage of body fat, presented a worsening of the lipid profile and higher levels of inflammatory markers (Papers II and IV). Furthermore, the obese individuals with MS presented a general worsening of the majority of the anthropometric and biochemical risk factors (Paper VIII).

Inflammation and oxidative stress are tightly connected and we found relevant to study some oxidative stress markers. Bilirubin, a molecule with important antioxidant and anti-inflammatory properties, was inversely correlated with BMI z-score, body and trunk fat percentages and with CRP in the obese population, but not in controls (Paper IV). Although obese individuals presented increased erythrocyte count, hemoglobin concentration and hematocrit, no differences were observed in bilirubin levels between obese and controls. Moreover, we found that obese individuals with increased body fat percentage presented decreased bilirubin and increased CRP. The reduction of bilirubin in the obese individuals is likely to be caused by a “consumption” of this antioxidant factor, due to the increased inflammation and oxidative stress in obesity.

Genetic polymorphisms

Obesity is a multifactorial disease involving both environmental and genetic factors. We studied some genetic polymorphisms associated with obese-related comorbidities, namely dyslipidemia and increased oxidative stress, and verified that individuals presenting specific genotypes are at increased metabolic risk:

- Apo E polymorphism: obese individuals with the E4 allele presented a worst lipid profile, with higher TC, LDLc, apoB and TC/HDLc ratio and lower apo A-I/apo B ratio, when compared to E2 carriers and E3/3 subjects (Paper II).
- Apo (a) PNR polymorphism: obese individuals with a lower number of repeats of the apo (a) pentanucleotide (TTTAT)n polymorphism presented increased Lp(a) levels and, consequently, an increased CV risk (Paper III).

- Bilirubin UGT1A1*28 polymorphism: 6/6 homozygous obese individuals presented a worst metabolic profile, including lower levels of bilirubin, increased IR and dyslipidemia, when compared with 6/7 and 7/7 subjects (Paper IV).

Nevertheless, and because obesity is multifactorial, other factors can influence the impact of a certain polymorphism in the metabolism. In fact, the worsening of the lipid profile observed in E4 (apo E) and 6/6 (UGT1A1*28) obese individuals might benefit from BMI reductions, as leaner individuals, for the same risk genotypes, presented a lower lipid risk profile (Paper II and IV). Furthermore, adiponectin also modulated the effect of apo E polymorphism, as obese individuals presenting increased adiponectin had a better lipid profile, regardless of the apo E genotype (Paper II). Thus, considering that adiponectin concentration increases with BMI improvement, it appears particularly important to focus on the control of adiposity to manage an increased genetic risk. Although the distribution of the studied genetic polymorphisms (associated with CVD risk markers) did not differ between obese and lean controls, the use of genetic studies could help to identify individuals who are at increased metabolic risk that may, therefore, deserve a closer follow-up by health professionals.

Criteria used to classify obesity in Portugal: impact on MS diagnosis

The prevalence of MS is much greater in obese individuals when compared to lean subjects. In fact, normal weight is considered a protection against the appearance of MS (148, 179). Our obese adolescent population presented a high prevalence of MS – 24.8% (Paper VIII). This prevalence was higher than those found in Greek obese children (7.7%, 10-14 years, IDF criterion) (56) and in another Portuguese overweight and obese children cohort (16%, children 7-9 years, NCEP criterion) (148). However, it was similar to the prevalence found in German obese children (25%, 6-19 years, IDF criterion) (70). The differences in the prevalence among different studies are mainly related to the characteristics of the studied population, particularly with the degree of obesity, and with the used criterion.

MS is a cluster of CVD risk factors (4, 5, 197). In agreement, obese adolescents with MS in our population presented, as expected, with worsening in all markers of MS: increased adiposity (BMI, WC, WC/H), BP (both SBP and DBP) and IR (insulin, glucose and $HOMA_{IR}$), when compared to obese adolescents without MS. A worst inflammatory status, with decreased adiponectin and a trend to increased CRP, was also present in individuals

with MS. High BP was the most common MS feature in the obese adolescents, besides the mandatory increase in WC stipulated by the IDF criterion. In agreement, the SBP was the best predictor of MS in this population (Paper VIII).

In the present work, as we have finished our recruitment of participants in July 2012, children were still classified regarding their nutritional status according to the CDC 2000 growth charts, as recommended by the Portuguese Ministry of Health. The CDC growth charts are based in the American population (USA), a population known to have increased anthropometrics measures (height and weight) when compared to Portuguese subjects. This fact probably led to an underestimation of obesity and overweight in our study. In fact, the WHO criterion (the reference that is now recommended) is associated with lower prevalence of thinness and increased prevalence of overweight and obesity, when compared to CDC criterion (6, 17). We found that, for obese Portuguese children and adolescents, the association of CDC criterion with MS features and other markers of CV risk is stronger than those found with the WHO criterion. Consequently, it seems that the CDC criterion has a higher sensitivity to identify individuals with increased metabolic risk (Paper VIII).

LONGITUDINAL ANALYSIS

In the second part of this work, we studied the impact of two different approaches on tackling obesity: a lifestyle intervention program (Paper VI) and an exercise based program (Paper VII).

Both approaches achieved very similar results. Actually, the percentage of obese individuals presenting significant weight loss was not very different. The weight improvements with both intervention programs were not very marked, and the majority of the individuals who lost weight presented only moderate weight reductions (Papers VI and VII).

Nevertheless, these small weight reductions were already associated with significant improvements in several risk factors, namely in the lipid profile and IR. The improvements in adiposity, however, were not associated to significant changes in the inflammatory markers, such as in CRP and adiponectin (Paper VI and VII). Actually, other studies have reported that higher improvements in BMI are necessary to observe beneficial effects on inflammation (42, 234). It is important to highlight, though, that the protective changes that

we observed in lipid profile and insulin sensitivity were closely linked to weight reduction and to central adiposity in particular (WC and % trunk fat) (Paper VI and VII). Thus, the magnitude of adiposity reduction might be the limiting factor for the improvement in inflammation.

Regarding the exercise interventional program (Paper VII), we found a greater beneficial metabolic effect in obese patients than in controls. Although after the intervention program similar changes in adiposity and nutritional markers were observed in both groups, the obese group presented a higher improvement in lipid profile and IR. Some of the beneficial effects in the metabolic risk might be related to changes in the body fat distribution as the obese group reduced central adiposity, and central obesity was associated with a worst lipid profile, IR and inflammation, at baseline.

In both program studies, an increase in circulating adiponectin levels was associated to a reduction in adiposity, inflammation and IR. Moreover, an increase of total adiponectin following exercise therapy was associated with improvements in lipid profile (reduction in TG) and with a decrease in adiposity (Paper VII).

Concerning adiponectin multimers, the HMW multimer was the one that better mimic the relations of total adiponectin with other variables, at pre-pubertal stages. HMW adiponectin levels were also inversely correlated with TG levels, and with an improvement in adiposity, following the exercise program (Paper VII).

MMW adiponectin multimer presented more ambiguous results. In the longitudinal study, the increase in MMW was associated with improvements in body weight, but it was also linked to a worst lipid profile.

The changes in adiponectin before puberty are not well established, as there are controversial results (Paper I). In our study, total adiponectin and the absolute and relative concentrations of adiponectin multimers did not differ, before and after the exercise interventional program, between obese and control pre-pubertal individuals (Paper VII). Nevertheless, controls and obese children who improved their BMI z-score did not present a reduction in total adiponectin following the interventional study, as occurred with obese participants who did not improve their body weight. Regardless of that, the multimer relative percentage was maintained in all groups (Paper VII).

Studies with larger populations of both pre-pubertal and pubertal individuals are needed to clarify how adiponectin multimers vary in obese pediatric populations, their relation with other metabolic risk markers and the influence of weight reduction.

Although with both interventional programs the improvement in adiposity was not very striking (Papers VI and VII), it is important to highlight that a considerable percentage of obese children stabilized their weight, breaking the positive caloric balance and the increase of obesity. Although this is not the final target of the program, it is an important achievement for the children. Also, the fact that metabolic improvements, occur before esthetical modifications is particularly important, and must be used to motivate children, care-takers and health professionals involved in the fight against obesity.

The fact that both approaches presented similar effects must be further explored, in order to understand which variables limit, and which increase, the success rates. Moreover, some other strategies to improve the success of obesity treatment can be considered, such as:

1. An interventional approach focused on the family environment, aiming to educate parents and care takers on healthy habits and avoidance of an obesogenic environment. The inclusion of parents in the interventional programs would also be of great importance;
2. A closer participation of a nutritionist in the interventional programs. A nutritional re-education of parents and children is crucial to obtain the best results and to their maintenance;
3. The inclusion of a psychologist in the team. Personal appointments with a psychologist, and the use of questionnaires to clarify psychological and motivational issues that could block the treatment, might increase the success and prevent drop-outs;
4. To evaluate different exercise and activity protocols. An interesting option would be to randomly address to each school a different protocol;
5. Increase the number of check points, to evaluate child progress and to detect individuals that are not improving weight, or that significantly reduced the improvement rate.

16. Concluding remarks

Portuguese obese children and adolescents presented an increased metabolic risk profile, including dyslipidemia, IR, inflammation and oxidative stress, when compared to their lean counterparts.

Abdominal obesity, inflammation, IR and oxidative stress are at the basis of the metabolic derangement caused by obesity, and as they present a very close interrelation, it is difficult to find causality relations.

The use of insulin and $HOMA_{IR}$ as early markers of IR in routine clinical analyzes of pediatric obesity could help to identify individuals with increased risk of developing T2DM and other comorbidities.

Adiponectin has a central role in obesity-related metabolic changes. The different circulating forms of adiponectin might present different, or even opposite effects on MS features. Our data suggest that the HMW adiponectin might be particularly important in pre-pubertal individuals, being the adiponectin form with more beneficial effects, particularly in lipid profile and IR. BMI improvement helps to prevent adiponectin reduction in children and adolescents. Understanding how total adiponectin and its multimers influence the metabolism, before and after puberty, is important, particularly in pediatric populations.

Bilirubin, an anti-oxidant molecule, is positively correlated with BMI and inversely correlated with body and trunk fat percentages and with CRP, in obese children and adolescents. In fact, individuals who are more obese have reduced bilirubin, probably due to its consumption in increased metabolic stress. Furthermore, we demonstrated that adiposity is a major determinant of bilirubin levels, independently of UGT1A1 polymorphism, a major determinant of bilirubin levels in general population.

Neutrophil count, a routine clinical analysis, can be used as a cost effective obesity-related inflammation marker, as it is positively related with adiposity markers and CRP levels (a high sensitive marker of inflammation).

Genetic polymorphisms in the apo E, apo (a) and UGT genes were associated with increased metabolic derangement. Nevertheless, the effect of a less favorable genetic profile on risk markers can be modulated by improvements in adiposity and inflammatory mediators (e.g. adiponectin).

More studies are needed to understand the causality and the mechanistic of obese related inflammation and metabolic disturbances. The study of genetic polymorphisms of inflammatory mediators and of their influence on the plasmatic levels of those markers, and on other routine clinical parameters, could help to identify individuals at increased risk, who would benefit of an early intervention.

School and community based interventional approaches are good options to reduce weight gain and to improve metabolic status of obese children and adolescents. In fact, we verified that even small reductions in body weight are associated to improvements in CV risk profile, although the improvements in inflammatory mediators might need greater reductions. The study of different, family based, protocols with the support of a multidisciplinary team, could enhance the success of the treatment of obesity in children and adolescents.

17. References

1. OECD OFEC-OAD-. Health at a Glance 2009 - OECD Indicators: OECD Publishing 2009. p. 43-58.
2. Padez C, Fernandes T, Mourao I, Moreira P, Rosado V. Prevalence of overweight and obesity in 7-9-year-old Portuguese children: trends in body mass index from 1970-2002. American Journal of Human Biology. 2004;16(6):670-8.
3. Rito A. Estado nutricional de crianças e oferta alimentar do pré-escolar do município de Coimbra, Portugal, 2001. Rio de Janeiro: Escola Nacional de Saúde Pública Sérgio Arouca; 2004.
4. Yoshinaga M, Takahashi H, Shinomiya M, Miyazaki A, Kuribayashi N, Ichida F. Impact of having one cardiovascular risk factor on other cardiovascular risk factor levels in adolescents. Journal of Atherosclerosis and Thrombosis. 2010;17(11):1167-75.
5. Martos-Moreno GA, Argente J. Paediatric obesities: from childhood to adolescence. Anales de Pediatria. 2011;75(1):63 e1-23.
6. Martinez-Costa C, Nunez F, Montal A, Brines J. Relationship between childhood obesity cut-offs and metabolic and vascular comorbidities: comparative analysis of three growth standards. Journal of Human Nutrition and Dietetics : the Official Journal of the British Dietetic Association. 2013.
7. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259(5091):87-91.
8. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32.
9. Wijnhoven TM, van Raaij JM, Spinelli A, Rito AI, Hovengen R, Kunesova M, et al. WHO European Childhood Obesity Surveillance Initiative 2008: weight, height and body mass index in 6-9-year-old children. Pediatric Obesity. 2013;8(2):79-97.
10. Plano Nacional de Saúde 2012-2016. Ministério da Saúde; 2012.
11. Carreira H, Pereira M, Azevedo A, Lunet N. Trends of BMI and prevalence of overweight and obesity in Portugal (1995-2005): a systematic review. Public Health Nutrition. 2012;15(6):972-81.
12. Padez C, Mourao I, Moreira P, Rosado V. Prevalence and risk factors for overweight and obesity in Portuguese children. Acta Paediatrica. 2005;94(11):1550-7.
13. Gomes S, Espanca R, Gato A, Miranda C. Obesity in preschool age - too early to be too heavy!. Acta Medica Portuguesa. 2010;23(3):371-8.
14. Ferrao MM, Gama A, Marques VR, Mendes LL, Mourao I, Nogueira H, et al. Association between parental perceptions of residential neighbourhood environments and childhood obesity in Porto, Portugal. European Journal of Public Health. 2013.
15. Branco S, Jorge Mdo S, Chaves H. Childhood obesity: a health care centre reality. Acta Medica Portuguesa. 2011;24 Suppl 2:509-16.
16. Albuquerque D, Nobrega C, Samouda H, Manco L. Assessment of obesity and abdominal obesity among Portuguese children. Acta Medica Portuguesa. 2012;25(3):169-73.
17. Rito A, Wijnhoven TM, Rutter H, Carvalho MA, Paixao E, Ramos C, et al. Prevalence of obesity among Portuguese children (6-8 years old) using three definition criteria: COSI Portugal, 2008. Pediatric Obesity. 2012;7(6):413-22.
18. Programa Nacional de Saúde Infantil e Juvenil. In: Divisão de Saúde Sexual RIeJ, editor.: Direção Geral de Saúde - Ministério da Saúde; 2013.
19. Schwiebbe L, Talma H, van Mil EG, Fetter WP, Hirasing RA, Renders CM. Diagnostic procedures and treatment of childhood obesity by pediatricians: 'The Dutch Approach'. Health Policy. 2013;111(2):110-5.
20. Park MH, Falconer C, Viner RM, Kinra S. The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. Obesity Reviews. 2012;13(11):985-1000.
21. Butte NF, Cai G, Cole SA, Comuzzie AG. Viva la Familia Study: genetic and environmental contributions to childhood obesity and its comorbidities in the Hispanic population. The American Journal of Clinical Nutrition. 2006;84(3):646-54; quiz 73-4.
22. Hitze B, Bosy-Westphal A, Plachta-Danielzik S, Bielfeldt F, Hermanussen M, Muller MJ. Long-term effects of rapid weight gain in children, adolescents and young adults with appropriate birth weight for gestational age: the Kiel Obesity Prevention Study. Acta Paediatrica. 2010;99(2):256-62.
23. Ibanez L, Lopez-Bermejo A, Suarez L, Marcos MV, Diaz M, de Zegher F. Visceral adiposity without overweight in children born small for gestational age. The Journal of Clinical Endocrinology and Metabolism. 2008;93(6):2079-83.
24. Tadokoro N, Shinomiya M, Yoshinaga M, Takahashi H, Matsuoka K, Miyashita Y, et al. Visceral fat accumulation in Japanese high school students and related atherosclerotic risk factors. Journal of Atherosclerosis and Thrombosis. 2010;17(6):546-57.
25. Dalmau Serra J, Alonso Franch M, Gomez Lopez L, Martinez Costa C, Sierra Salinas C. Childhood obesity. Recommendations of the Nutrition Committee of the Spanish Association of Pediatrics. Part II. Diagnosis. Comorbidities. Treatment. Anales de Pediatria. 2007;66(3):294-304.
26. WHO. WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva. 2009.
27. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. British Medical Journal. 2000;320(7244):1240-3.
28. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. The British Journal of Nutrition. 2004;92(3):347-55.
29. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. Science (New York, NY. 2001;294(5549):2166-70.
30. Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, et al. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science (New York, NY. 1987;237(4813):402-5.
31. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. The Journal of Clinical Investigation. 1995;95(5):2409-15.
32. Gasic S, Tian B, Green A. Tumor necrosis factor alpha stimulates lipolysis in adipocytes by decreasing Gi protein concentrations. The Journal of Biological Chemistry. 1999;274(10):6770-5.
33. de Luca C, Olefsky JM. Inflammation and insulin resistance. FEBS letters. 2008;582(1):97-105.
34. Rayner DV, Trayhurn P. Regulation of leptin production: sympathetic nervous system interactions. Journal of Molecular Medicine. 2001;79(1):8-20.
35. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochemical Society Transactions. 2005;33(Pt 5):1078-81.
36. Villena JA, Cousin B, Penicaud L, Casteilla L. Adipose tissues display differential phagocytic and microbicidal

activities depending on their localization. International Journal Obesisty Related Metabolic Disorders. 2001;25(9):1275-80.
37. Wozniak SE, Gee LL, Wachtel MS, Frezza EE. Adipose Tissue: The New Endocrine Organ? A Review Article. Digestive Diseases and Sciences. 2008.
38. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, et al. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. American Journal of Physiology. 2003;285(3):E527-33.
39. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46(4):459-69.
40. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation. 2003;107(5):671-4.
41. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obesity Research. 2003;11(3):368-72.
42. Reinehr T, Roth C, Menke T, Andler W. Adiponectin before and after weight loss in obese children. The Journal of Clinical Endocrinology and Metabolism. 2004;89(8):3790-4.
43. Kwon K, Jung SH, Choi C, Park SH. Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. International Journal of Cardiology. 2005;101(3):385-90.
44. Ogawa Y, Kikuchi T, Nagasaki K, Hiura M, Tanaka Y, Uchiyama M. Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese Japanese children. Hypertension research : official journal of the Japanese Society of Hypertension. 2005;28(1):51-7.
45. Pilz S, Horejsi R, Moller R, Almer G, Scharnagl H, Stojakovic T, et al. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. The Journal of Clinical Endocrinology and Metabolism. 2005;90(8):4792-6.
46. Trujillo ME, Scherer PE. Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. Journal of Internal Medicine. 2005;257(2):167-75.
47. Aso Y, Yamamoto R, Wakabayashi S, Uchida T, Takayanagi K, Takebayashi K, et al. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes. 2006;55(7):1954-60.
48. Gilardini L, McTernan PG, Girola A, da Silva NF, Alberti L, Kumar S, et al. Adiponectin is a candidate marker of metabolic syndrome in obese children and adolescents. Atherosclerosis. 2006;189(2):401-7.
49. Liang X, Kanjanabuch T, Mao SL, Hao CM, Tang YW, Declerck PJ, et al. Plasminogen activator inhibitor-1 modulates adipocyte differentiation. American Journal of Physiology. 2006;290(1):E103-E13.
50. Ouedraogo R, Wu X, Xu SQ, Fuchsel L, Motoshima H, Mahadev K, et al. Adiponectin suppression of high-glucose-induced reactive oxygen species in vascular endothelial cells: evidence for involvement of a cAMP signaling pathway. Diabetes. 2006;55(6):1840-6.
51. Herder C, Schneitler S, Rathmann W, Haastert B, Schneitler H, Winkler H, et al. Low-grade inflammation, obesity, and insulin resistance in adolescents. The Journal of Clinical Endocrinology and Metabolism. 2007;92(12):4569-74.
52. Neumeier M, Sigruener A, Eggenhofer E, Weigert J, Weiss TS, Schaeffler A, et al. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. Biochemical and Biophysical Research Communications. 2007;352(2):543-8.
53. Shaibi GQ, Cruz ML, Weigensberg MJ, Toledo-Corral CM, Lane CJ, Kelly LA, et al. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. The Journal of Clinical Endocrinology and Metabolism. 2007;92(5):1809-13.
54. Ozkol M, Ersoy B, Kasirga E, Taneli F, Bostanci IE, Ozhan B. Metabolic predictors for early identification of fatty liver using doppler and B-mode ultrasonography in overweight and obese adolescents. European Journal Pediatrics. 2010;169(11):1345-52.
55. Romeo J, Martinez-Gomez D, Diaz LE, Gomez-Martinez S, Marti A, Martin-Matillas M, et al. Changes in cardiometabolic risk factors, appetite-controlling hormones and cytokines after a treatment program in overweight adolescents: preliminary findings from the EVASYON study. Pediatric Diabetes. 2011;12(4 Pt 2):372-80.
56. Papoutsakis C, Yannakoulia M, Ntalla I, Dedoussis GV. Metabolic syndrome in a Mediterranean pediatric cohort: prevalence using International Diabetes Federation-derived criteria and associations with adiponectin and leptin. Metabolism: Clinical and Experimental. 2012;61(2):140-5.
57. Schipper HS, Nuboer R, Prop S, van den Ham HJ, de Boer FK, Kesmir C, et al. Systemic inflammation in childhood obesity: circulating inflammatory mediators and activated CD14++ monocytes. Diabetologia. 2012;55(10):2800-10.
58. Ashwin PJ, Dilipbhai PJ. Leptin and the cardiovascular system: a review. Recent Patents on Cardiovascular Drug Discovery. 2007;2(2):100-9.
59. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nature Genetics. 1998;18(3):213-5.
60. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. Faseb Journal. 1998;12(1):57-65.
61. Ahima RS, Flier JS. Leptin. Annual Review of Physiology. 2000;62:413-37.
62. Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, et al. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes. 1998;47(1):98-103.
63. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, et al. Leptin-replacement therapy for lipodystrophy. The New England Journal of Medicine. 2002;346(8):570-8.
64. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature. 2002;415(6869):339-43.
65. Gualillo O, Eiras S, Lago F, Dieguez C, Casanueva FF. Elevated serum leptin concentrations induced by experimental acute inflammation. Life Sciences. 2000;67(20):2433-41.
66. Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. Circulation. 2004;109(18):2181-5.
67. Castracane VD, Kraemer RR, Franken MA, Kraemer GR, Gimpel T. Serum leptin concentration in women: effect of age, obesity, and estrogen administration. Fertility and Sterility. 1998;70(3):472-7.
68. Howard JK, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. Trends in Endocrinology and Metabolism. 2006;17(9):365-71.
69. Reilly MP, Iqbal N, Schutta M, Wolfe ML, Scally M, Localio AR, et al. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. The Journal of Clinical Endocrinology and Metabolism. 2004;89(8):3872-8.

70. Rank M, Siegrist M, Wilks DC, Langhof H, Wolfarth B, Haller B, et al. The Cardio-Metabolic Risk of Moderate and Severe Obesity in Children and Adolescents. The Journal of Pediatrics. 2013.
71. Gherlan I, Vladoiu S, Alexiu F, Giurcaneanu M, Oros S, Brehar A, et al. Adipocytokine profile and insulin resistance in childhood obesity. Maedica. 2012;7(3):205-13.
72. Utzschneider KM, Carr DB, Tong J, Wallace TM, Hull RL, Zraika S, et al. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. Diabetologia. 2005;48(11):2330-3.
73. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature. 2001;409(6818):307-12.
74. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochemical and Biophysical Research Communications. 2005;334(4):1092-101.
75. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, et al. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation. 2003;108(6):736-40.
76. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. Biochemical and Biophysical Research Communications. 2004;314(2):415-9.
77. Zhang J, Qin Y, Zheng X, Qiu J, Gong L, Mao H, et al. The relationship between human serum resistin level and body fat content, plasma glucose as well as blood pressure. Zhonghua Yi Xue Za Zhi. 2002;82(23):1609-12.
78. Stejskal D, Adamovska S, Bartek J, Jurakova R, Proskova J. Resistin - concentrations in persons with type 2 diabetes mellitus and in individuals with acute inflammatory disease. Biomedical papers of the Medical Faculty of the University Palacky. 2003;147(1):63-9.
79. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation. 2005;111(7):932-9.
80. Pischon T, Bamberger CM, Kratzsch J, Zyriax BC, Algenstaedt P, Boeing H, et al. Association of plasma resistin levels with coronary heart disease in women. Obesity Research. 2005;13(10):1764-71.
81. Ohmori R, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, et al. Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. Journal of the American College of Cardiology. 2005;46(2):379-80.
82. Jung HS, Park KH, Cho YM, Chung SS, Cho HJ, Cho SY, et al. Resistin is secreted from macrophages in atheromas and promotes atherosclerosis. Cardiovascular Research. 2006;69(1):76-85.
83. Bajaj M, Suraamornkul S, Hardies LJ, Pratipanawatr T, DeFronzo RA. Plasma resistin concentration, hepatic fat content, and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. International Journal of Obesity Related Metabolic Disorders. 2004;28(6):783-9.
84. Mangge H, Almer G, Haj-Yahya S, Pilz S, Gasser R, Moller R, et al. Preatherosclerosis and adiponectin subfractions in obese adolescents. Obesity (Silver Spring, Md. 2008;16(12):2578-84.
85. Xu H, Uysal KT, Becherer JD, Arner P, Hotamisligil GS. Altered tumor necrosis factor-alpha (TNF-alpha) processing in adipocytes and increased expression of transmembrane TNF-alpha in obesity. Diabetes. 2002;51(6):1876-83.
86. Tsigos C, Kyrou I, Chala E, Tsapogas P, Stavridis JC, Raptis SA, et al. Circulating tumor necrosis factor alpha concentrations are higher in abdominal versus peripheral obesity. Metabolism: Clinical and Experimental. 1999;48(10):1332-5.
87. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. The Journal of Clinical Endocrinology and Metabolism. 1997;82(12):4196-200.
88. Wang B, Trayhurn P. Acute and prolonged effects of TNF-alpha on the expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture. Pflugers Archives. 2006;452(4):418-27.
89. Stephens JM, Lee J, Pilch PF. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. The Journal of Biological Chemistry. 1997;272(2):971-6.
90. Mateo T, Naim Abu Nabah Y, Losada M, Estelles R, Company C, Bedrina B, et al. A critical role for TNFalpha in the selective attachment of mononuclear leukocytes to angiotensin-II-stimulated arterioles. Blood. 2007;110(6):1895-902.
91. Matsuki T, Horai R, Sudo K, Iwakura Y. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. The Journal of Experimental Medicine. 2003;198(6):877-88.
92. Jager J, Gremeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology. 2007;148(1):241-51.
93. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes. 2003;52(3):812-7.
94. Yang KG, Raijmakers NJ, van Arkel ER, Caron JJ, Rijk PC, Willems WJ, et al. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. Osteoarthritis and Cartilage / OARS, Osteoarthritis Research Society. 2008;16(4):498-505.
95. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis. 2000;148(2):209-14.
96. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. The Journal of Clinical Endocrinology and Metabolism. 1998;83(3):847-50.
97. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. European Cytokine Network. 2006;17(1):4-12.
98. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. The Journal of Clinical Endocrinology and Metabolism. 2000;85(9):3338-42.
99. Kopp HP, Kopp CW, Festa A, Krzyzanowska K, Kriwanek S, Minar E, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23(6):1042-7.
100. Nonogaki K, Fuller GM, Fuentes NL, Moser AH, Staprans I, Grunfeld C, et al. Interleukin-6 stimulates hepatic triglyceride secretion in rats. Endocrinology. 1995;136(5):2143-9.

101. Maachi M, Pieroni L, Bruckert E, Jardel C, Fellahi S, Hainque B, et al. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. International Journal of Obesity Related Metabolic Disorders. 2004;28(8):993-7.
102. Lagathu C, Bastard JP, Auclair M, Maachi M, Capeau J, Caron M. Chronic interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: prevention by rosiglitazone. Biochemical and Biophysical Research Communications. 2003;311(2):372-9.
103. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. Biochemical and Biophysical Research Communications. 2002;293(1):560-5.
104. Wernstedt I, Eriksson AL, Berndtsson A, Hoffstedt J, Skrtic S, Hedner T, et al. A common polymorphism in the interleukin-6 gene promoter is associated with overweight. International Journal of Obesity Related Metabolic Disorders. 2004;28(10):1272-9.
105. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? Journal of Muscle Research and Cell Motility. 2003;24(2-3):113-9.
106. Zirlik A, Abdullah SM, Gerdes N, MacFarlane L, Schonbeck U, Khera A, et al. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: results from the Dallas Heart Study. Arteriosclerosis, Thrombosis, and Vascular Biology. 2007;27(9):2043-9.
107. Zilverschoon GR, Tack CJ, Joosten LA, Kullberg BJ, van der Meer JW, Netea MG. Interleukin-18 resistance in patients with obesity and type 2 diabetes mellitus. International Journal of Obesity. 2008;32(9):1407-14.
108. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nature Medicine. 2006;12(6):650-6.
109. Inadera H. The usefulness of circulating adipokine levels for the assessment of obesity-related health problems. International Journal of Medical Sciences. 2008;5(5):248-62.
110. Parissis JT, Venetsanou KF, Kalantzi MV, Mentzikof DD, Karas SM. Serum profiles of granulocyte-macrophage colony-stimulating factor and C-C chemokines in hypertensive patients with or without significant hyperlipidemia. The American Journal of Cardiology. 2000;85(6):777-9, A9.
111. Garlichs CD, John S, Schmeisser A, Eskafi S, Stumpf C, Karl M, et al. Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia. Circulation. 2001;104(20):2395-400.
112. de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation. 2003;107(5):690-5.
113. Kralisch S, Bluher M, Paschke R, Stumvoll M, Fasshauer M. Adipokines and adipocyte targets in the future management of obesity and the metabolic syndrome. Mini Reviews in Medicinal Chemistry. 2007;7(1):39-45.
114. Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP, et al. Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. Diabetes Care. 2003;26(10):2883-9.
115. Schernthaner GH, Kopp HP, Kriwanek S, Krzyzanowska K, Satler M, Koppensteiner R, et al. Effect of massive weight loss induced by bariatric surgery on serum levels of interleukin-18 and monocyte-chemoattractant-protein-1 in morbid obesity. Obesity Surgery. 2006;16(6):709-15.
116. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of Clinical Investigation. 2006;116(6):1494-505.
117. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. The Journal of Clinical Investigation. 2006;116(1):115-24.
118. Dulak J, Jozkowicz A, Frick M, Alber HF, Dichtl W, Schwarzacher SP, et al. Vascular endothelial growth factor: angiogenesis, atherogenesis or both? Journal of the American College of Cardiology. 2001;38(7):2137-8.
119. Claffey KP, Wilkison WO, Spiegelman BM. Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. The Journal of Biological Chemistry. 1992;267(23):16317-22.
120. Mick GJ, Wang X, McCormick K. White adipocyte vascular endothelial growth factor: regulation by insulin. Endocrinology. 2002;143(3):948-53.
121. Miyazawa-Hoshimoto S, Takahashi K, Bujo H, Hashimoto N, Saito Y. Elevated serum vascular endothelial growth factor is associated with visceral fat accumulation in human obese subjects. Diabetologia. 2003;46(11):1483-8.
122. Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. The Journal of Experimental Medicine. 1999;190(12):1733-40.
123. Mortensen RF. C-reactive protein, inflammation, and innate immunity. Immunologic Research. 2001;24(2):163-76.
124. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, et al. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. Diabetes. 2002;51(5):1596-600.
125. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. The Journal of the American Medical Association. 2003;289(14):1799-804.
126. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation. 2003;107(3):391-7.
127. Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. Atherosclerosis. 2003;167(1):73-9.
128. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. Circulation. 2004;109(21 Suppl 1):II2-10.
129. Calabro P, Chang DW, Willerson JT, Yeh ET. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. Journal of the American College of Cardiology. 2005;46(6):1112-3.
130. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005;436(7049):356-62.

131. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. The Journal of Clinical Endocrinology and Metabolism. 2007;92(3):1168-71.
132. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care. 2006;29(11):2457-61.
133. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. The New England Journal of Medicine. 2006;354(24):2552-63.
134. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Schleicher E, et al. High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. Diabetes Care. 2007;30(5):1173-8.
135. Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS, et al. Retinoids and retinoid-binding protein expression in rat adipocytes. The Journal of Biological Chemistry. 1992;267(3):1805-10.
136. Qi Q, Yu Z, Ye X, Zhao F, Huang P, Hu FB, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. The Journal of Clinical Endocrinology and Metabolism. 2007;92(12):4827-34.
137. von Eynatten M, Lepper PM, Liu D, Lang K, Baumann M, Nawroth PP, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia. 2007;50(9):1930-7.
138. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. Diabetes. 1997;46(5):860-7.
139. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. International Journal of Obesity Related Metabolic Disorders. 2004;28(11):1357-64.
140. Juhan-Vague I, Roul C, Alessi MC, Ardissone JP, Heim M, Vague P. Increased plasminogen activator inhibitor activity in non insulin dependent diabetic patients--relationship with plasma insulin. Thrombosis and Haemostasis. 1989;61(3):370-3.
141. Mertens I, Van der Planken M, Corthouts B, Wauters M, Peiffer F, De Leeuw I, et al. Visceral fat is a determinant of PAI-1 activity in diabetic and non-diabetic overweight and obese women. Hormone and Metabolic Research. 2001;33(10):602-7.
142. Primrose JN, Davies JA, Prentice CR, Hughes R, Johnston D. Reduction in factor VII, fibrinogen and plasminogen activator inhibitor-1 activity after surgical treatment of morbid obesity. Thrombosis and Haemostasis. 1992;68(4):396-9.
143. Maruyoshi H, Kojima S, Funahashi T, Miyamoto S, Hokamaki J, Soejima H, et al. Adiponectin is inversely related to plasminogen activator inhibitor type 1 in patients with stable exertional angina. Thrombosis and Haemostasis. 2004;91(5):1026-30.
144. De Pergola G, Pannacciulli N. Coagulation and fibrinolysis abnormalities in obesity. Journal of Endocrinological Investigation. 2002;25(10):899-904.
145. Aso Y. Plasminogen activator inhibitor (PAI)-1 in vascular inflammation and thrombosis. Front Bioscience. 2007;12:2957-66.
146. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. Endocrine. 2006;29(1):81-90.
147. Tsao TS. Assembly of adiponectin oligomers. Reviews in Endocrine & Metabolic Disorders. 2013.
148. Pedrosa C, Oliveira BM, Albuquerque I, Simoes-Pereira C, Vaz-de-Almeida MD, Correia F. Metabolic syndrome, adipokines and ghrelin in overweight and obese schoolchildren: results of a 1-year lifestyle intervention programme. European Journal of Pediatrics. 2011;170(4):483-92.
149. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochemical and Biophysical Research Communications. 1996;221(2):286-9.
150. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocrine Reviews. 2005;26(3):439-51.
151. Simpson F, Whitehead JP. Adiponectin--it's all about the modifications. International Journal of Biochemical Cell Biology. 2010;42(6):785-8.
152. Saito K, Tobe T, Minoshima S, Asakawa S, Sumiya J, Yoda M, et al. Organization of the gene for gelatin-binding protein (GBP28). Gene. 1999;229(1-2):67-73.
153. Schober F, Neumeier M, Weigert J, Wurm S, Wanninger J, Schaffler A, et al. Low molecular weight adiponectin negatively correlates with the waist circumference and monocytic IL-6 release. Biochemical and Biophysical Research Communications. 2007;361(4):968-73.
154. Wang Y, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. The Biochemical Journal. 2008;409(3):623-33.
155. Wang ZV, Schraw TD, Kim JY, Khan T, Rajala MW, Follenzi A, et al. Secretion of the adipocyte-specific secretory protein adiponectin critically depends on thiol-mediated protein retention. Molecular and Cellular Biology. 2007;27(10):3716-31.
156. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003;423(6941):762-9.
157. Bjursell M, Ahnmark A, Bohlooly YM, William-Olsson L, Rhedin M, Peng XR, et al. Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. Diabetes. 2007;56(3):583-93.
158. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(28):10308-13.
159. Haqq AM, Muehlbauer M, Svetkey LP, Newgard CB, Purnell JQ, Grambow SC, et al. Altered distribution of adiponectin isoforms in children with Prader-Willi syndrome (PWS): association with insulin sensitivity and circulating satiety peptide hormones. Clinical Endocrinology. 2007;67(6):944-51.
160. Modan-Moses D, Stein D, Pariente C, Yaroslavsky A, Ram A, Faigin M, et al. Modulation of adiponectin and leptin during refeeding of female anorexia nervosa patients. The Journal of Clinical Endocrinology and Metabolism. 2007;92(5):1843-7.
161. Zhou Y, Sun X, Jin L, Stringfield T, Lin L, Chen Y. Expression profiles of adiponectin receptors in mouse embryos. Gene Expression Patterns. 2005;5(5):711-5.
162. Irving AJ, Wallace L, Durakoglugil D, Harvey J. Leptin enhances NR2B-mediated N-methyl-D-aspartate responses via a mitogen-activated protein kinase-dependent process in cerebellar granule cells. Neuroscience. 2006;138(4):1137-48.
163. Zhang M, Zhao X, Li M, Cheng H, Hou D, Wen Y, et al. Abnormal adipokines associated with various types of obesity in Chinese children and adolescents.

Biomedical and Environmental Sciences. 2011;24(1):12-21.
164. Calcaterra V, De Amici M, Klersy C, Torre C, Brizzi V, Scaglia F, et al. Adiponectin, IL-10 and metabolic syndrome in obese children and adolescents. Acta bio-medica : Atenei Parmensis. 2009;80(2):117-23.
165. Vos RC, Wit JM, Pijl H, Houdijk EC. Long-term effect of lifestyle intervention on adiposity, metabolic parameters, inflammation and physical fitness in obese children: a randomized controlled trial. Nutrition & Diabetes. 2011;1:e9.
166. Weinberger B, Archer FE, Kathiravan S, Hirsch DS, Kleinfeld AM, Vetrano AM, et al. Effects of bilirubin on neutrophil responses in newborn infants. Neonatology. 2013;103(2):105-11.
167. Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochemical Pharmacology. 1996;51(6):859-62.
168. Wu Y, Li M, Xu M, Bi Y, Li X, Chen Y, et al. Low serum total bilirubin concentrations are associated with increased prevalence of metabolic syndrome in Chinese. Journal of Diabetes. 2011;3(3):217-24.
169. Novotny L, Vitek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. Experimental Biology and Medicine. 2003;228(5):568-71.
170. Lin JP, O'Donnell CJ, Schwaiger JP, Cupples LA, Lingenhel A, Hunt SC, et al. Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. Circulation. 2006;114(14):1476-81.
171. Lin LY, Kuo HK, Hwang JJ, Lai LP, Chiang FT, Tseng CD, et al. Serum bilirubin is inversely associated with insulin resistance and metabolic syndrome among children and adolescents. Atherosclerosis. 2009;203(2):563-8.
172. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics. 2001;107(1):E13.
173. Kim JA, Park HS. White blood cell count and abdominal fat distribution in female obese adolescents. Metabolism. 2008;57(10):1375-9.
174. Balagopal P, George D, Patton N, Yarandi H, Roberts WL, Bayne E, et al. Lifestyle-only intervention attenuates the inflammatory state associated with obesity: a randomized controlled study in adolescents. Journal of Pediatrics. 2005;146(3):342-8.
175. Herishanu Y, Rogowski O, Polliack A, Marilus R. Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. Eur J Haematol. 2006;76:516–20.
176. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41.
177. Stewart RA, White HD, Kirby AC, Heritier SR, Simes RJ, Nestel PJ, et al. White blood cell count predicts reduction in coronary heart disease mortality with pravastatin. Circulation. 2005;111(14):1756-62.
178. Carreira H, Pereira M, Azevedo A, Lunet N. Effect of the type of population on estimates of mean body mass index and prevalence of overweight and obesity: a systematic review of studies of Portuguese adults. Annals of Human Biology. 2012;39(3):223-38.
179. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. Circulation. 2005;111(15):1999-2012.
180. Pulgaron ER. Childhood obesity: a review of increased risk for physical and psychological comorbidities. Clinical Therapeutics. 2013;35(1):A18-32.
181. Ignarro LJ, Balestrieri ML, Napoli C. Nutrition, physical activity, and cardiovascular disease: an update. Cardiovascular research. 2007;73(2):326-40.
182. Smith JK, Dykes R, Douglas JE, Krishnaswamy G, Berk S. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. The Journal of the American Medical Association. 1999;281(18):1722-7.
183. *COSI*. European Childhood Obesity Surveillance Initiative - In: Saúde Md, editor. 2009.
184. Kennedy MJ, Jellerson KD, Snow MZ, Zacchetti ML. Challenges in the Pharmacologic Management of Obesity and Secondary Dyslipidemia in Children and Adolescents. Paediatric drugs. 2013.
185. Cook S, Kavey RE. Dyslipidemia and pediatric obesity. Pediatric Clinics of North America. 2011;58(6):1363-73, ix.
186. Curtiss LK, Boisvert WA. Apolipoprotein E and atherosclerosis. Current Opinion in Lipidology. 2000;11(3):243-51.
187. Guerra A, Rego C, Castro EM, Seixas S, Rocha J. Influence of apolipoprotein e polymorphism on cardiovascular risk factors in obese children. Annals of Nutrition & Metabolism. 2003;47(2):49-54.
188. Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. Journal of lipid research. 1992;33(4):447-54. Epub 1992/04/01.
189. McGladdery SH, Frohlich JJ. Lipoprotein lipase and apoE polymorphisms: relationship to hypertriglyceridemia during pregnancy. Journal of Lipid Research. 2001;42(11):1905-12.
190. Goldenberg NA, Bernard TJ, Hillhouse J, Armstrong-Wells J, Galinkin J, Knapp-Clevenger R, et al. Elevated lipoprotein (a), small apolipoprotein (a), and the risk of arterial ischemic stroke in North American children. Haematologica. 2013;98(5):802-7.
191. Berglund L, Ramakrishnan R. Lipoprotein(a): an elusive cardiovascular risk factor. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;24(12):2219-26.
192. Trommsdorff M, Kochl S, Lingenhel A, Kronenberg F, Delport R, Vermaak H, et al. A pentanucleotide repeat polymorphism in the 5' control region of the apolipoprotein(a) gene is associated with lipoprotein(a) plasma concentrations in Caucasians. The Journal of Clinical Investigation. 1995;96(1):150-7.
193. Ferreira H, Costa E, Vieira E, Leao A, Magalhaes R, Gomes JL, et al. Pentanucleotide repeat (TTTTA)n polymorphism in the 5' control region of the apoliprotein (A) gene and atherothrombotic serum lipoprotein (A) concentration, in a pediatric population. Haematologica. 2003;88(3):ELT07.
194. Witztum JL. Susceptibility of low-density lipoprotein to oxidative modification. The American Journal of Medicine. 1993;94(4):347-9.
195. Byrne CD. Triglyceride-rich lipoproteins: are links with atherosclerosis mediated by a procoagulant and proinflammatory phenotype? Atherosclerosis. 1999;145(1):1-15.
196. Packard C, Caslake M, Shepherd J. The role of small, dense low density lipoprotein (LDL): a new look. International Journal of Cardiology. 2000;74 Suppl 1:S17-22.
197. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, Draznin B, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. The Journal of Clinical Endocrinology and Metabolism. 2009;94(10):3687-95.
198. Morrison JA, Glueck CJ, Daniels S, Wang P, Stroop D. Paradoxically high adiponectin in obese 16-year-old girls protects against appearance of the metabolic

syndrome and its components seven years later. The Journal of Pediatrics. 2011;158(2):208-14 e1.
199. Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, et al. Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. Circulation. 2012;126(5):598-603.
200. Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, et al. Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics. 2001;11(4):357-68.
201. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. The New England Journal of Medicine. 1995;333(18):1171-5.
202. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. Circulation Research. 2001;89(9):763-71.
203. Ernst E, Hammerschmidt DE, Bagge U, Matrai A, Dormandy JA. Leukocytes and the risk of ischemic diseases. Journal of the American Medical Association. 1987;257(17):2318-24.
204. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. Journal of the American Medical Association. 1998;279(18):1477-82.
205. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. Britsh Medical Journal. 2000;321(7255):199-204.
206. Huang L, Tao FB, Wan YH, Xing C, Hao J, Su PY, et al. Self-reported weight status rather than BMI may be closely related to psychopathological symptoms among Mainland Chinese adolescents. Journal of Tropical Pediatrics. 2011;57(4):307-11.
207. Boyd NF, Stone J, Vogt KN, Connelly BS, Martin LJ, Minkin S. Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. British Journal of Cancer. 2003;89(9):1672-85.
208. Chavarro JE, Stampfer MJ, Campos H, Kurth T, Willett WC, Ma J. A prospective study of trans-fatty acid levels in blood and risk of prostate cancer. Cancer Epidemiol Biomarkers & Prevention. 2008;17(1):95-101.
209. MacLean CH, Newberry SJ, Mojica WA, Khanna P, Issa AM, Suttorp MJ, et al. Effects of omega-3 fatty acids on cancer risk: a systematic review. Journal of the American Medical Association. 2006;295(4):403-15.
210. Zimmet P, Alberti G, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059-61.
211. Brambilla P, Lissau I, Flodmark CE, Moreno LA, Widhalm K, Wabitsch M, et al. Metabolic risk-factor clustering estimation in children: to draw a line across pediatric metabolic syndrome. International Journal of Obesity. 2007;31(4):591-600.
212. Standards of medical care in diabetes. Diabetes Care. 2005;28 Suppl 1:S4-S36.
213. Matson KL, Fallon RM. Treatment of obesity in children and adolescents. The Journal of Pediatric Pharmacology and Therapeutics. 2012;17(1):45-57.
214. Dolinsky DH, Armstrong SC, Kinra S. The clinical treatment of childhood obesity. Indian Journal of Pediatrics. 2013;80 Suppl 1:S48-54.
215. Yeste D, Carrascosa A. Management of obesity in childhood and adolescence: from diet to surgery. Anales Pediatria. 2012;77(2):71-4.
216. Organization WH. Population-based prevention strategies for childhood obesity: report of a WHO forum and technical meeting,. Switzerland: 2010.
217. Ben Ounis O, Elloumi M, Ben Chiekh I, Zbidi A, Amri M, Lac G, et al. Effects of two-month physical-endurance and diet-restriction programmes on lipid profiles and insulin resistance in obese adolescent boys. Diabetes Metabolism. 2008;34(6 Pt 1):595-600.
218. Elloumi M, Ben Ounis O, Makni E, Van Praagh E, Tabka Z, Lac G. Effect of individualized weight-loss programmes on adiponectin, leptin and resistin levels in obese adolescent boys. Acta Paediatrica. 2009;98(9):1487-93.
219. Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. American Journal of Physiology. 2010;298(4):E824-31.
220. Lira FS, Rosa JC, Pimentel GD, Santos RV, Carnier J, Sanches PL, et al. Long-term interdisciplinary therapy reduces endotoxin level and insulin resistance in obese adolescents. Nutrition Journal. 2012;11:74.
221. Lopez-Alarcon M, Martinez-Coronado A, Velarde-Castro O, Rendon-Macias E, Fernandez J. Supplementation of n3 long-chain polyunsaturated fatty acid synergistically decreases insulin resistance with weight loss of obese prepubertal and pubertal children. Archives of Medical Research. 2011;42(6):502-8.
222. Ruiz JR, Ortega FB, Martinez-Gomez D, Labayen I, Moreno LA, De Bourdeaudhuij I, et al. Objectively measured physical activity and sedentary time in European adolescents: the HELENA study. American Journal of Epidemiology. 2011;174(2):173-84.
223. Tsai AC, Sandretto A, Chung YC. Dieting is more effective in reducing weight but exercise is more effective in reducing fat during the early phase of a weight-reducing program in healthy humans. The Journal of Nutritional Biochemistry. 2003;14(9):541-9.
224. Verdaet D, Dendale P, De Bacquer D, Delanghe J, Block P, De Backer G. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. Atherosclerosis. 2004;176(2):303-10.
225. Balagopal P, George D, Patton N, Yarandi H, Roberts WL, Bayne E, et al. Lifestyle-only intervention attenuates the inflammatory state associated with obesity: a randomized controlled study in adolescents. The Journal of Pediatrics. 2005;146(3):342-8.
226. Reinehr T, Stoffel-Wagner B, Roth CL, Andler W. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. Metabolism: Clinical and Experimental. 2005;54(9):1155-61.
227. Roth CL, Kratz M, Ralston MM, Reinehr T. Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. Metabolism: Clinical and Experimental. 2011;60(4):445-52.
228. Lee MK, Jekal Y, Im JA, Kim E, Lee SH, Park JH, et al. Reduced serum vaspin concentrations in obese children following short-term intensive lifestyle modification. Clinica chimica acta; International Journal of Clinical Chemistry. 2010;411(5-6):381-5.
229. Reinehr T, Woelfle J, Roth CL. Lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children: a longitudinal analysis. Metabolism: Clinical and Experimental. 2011;60(9):1349-54.
230. Araki S, Dobashi K, Yamamoto Y, Asayama K, Kusuhara K. Increased plasma isoprostane is associated with visceral fat, high molecular weight

adiponectin, and metabolic complications in obese children. European Journal of Pediatrics. 2010;169(8):965-70.
231. Araki S, Dobashi K, Kubo K, Asayama K, Shirahata A. High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. The Journal of Clinical Endocrinology and Metabolism. 2006;91(12):5113-6.
232. Reinehr T, Stoffel-Wagner B, Roth CL. Adipocyte fatty acid-binding protein in obese children before and after weight loss. Metabolism: Clinical and Experimental. 2007;56(12):1735-41.
233. Metcalf BS, Jeffery AN, Hosking J, Voss LD, Sattar N, Wilkin TJ. Objectively measured physical activity and its association with adiponectin and other novel metabolic markers: a longitudinal study in children (EarlyBird 38). Diabetes Care. 2009;32(3):468-73.
234. de Mello MT, de Piano A, Carnier J, Sanches Pde L, Correa FA, Tock L, et al. Long-term effects of aerobic plus resistance training on the metabolic syndrome and adiponectinemia in obese adolescents. Journal of Clinical Hypertension. 2011;13(5):343-50.
235. Solbraa AKM, Asgeir; Resaland, Geir Kåre; Steene-Johannessen, Jostein ; Ylvisåker, Einar; Holme, Ingar Mortenand Anderssen, Sigmund Alfred. Level of physical activity, cardiorespiratory fitness and cardiovascular disease risk factors in a rural adult population in Sogn og Fjordane. Norsk Epidemiologi. 2011;20 ((2)):179-88.
236. McGavock JM, Torrance BD, McGuire KA, Wozny PD, Lewanczuk RZ. Cardiorespiratory fitness and the risk of overweight in youth: the Healthy Hearts Longitudinal Study of Cardiometabolic Health. Obesity (Silver Spring), Md. 2009;17(9):1802-7.
237. Reinehr T, Andler W. Changes in the atherogenic risk factor profile according to degree of weight loss. Archives of Disease in Childhood. 2004;89(5):419-22.
238. Sherafat-Kazemzadeh R, Yanovski SZ, Yanovski JA. Pharmacotherapy for childhood obesity: present and future prospects. International Journal of Obesity (2005). 2013;37(1):1-15.
239. INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde IMdS. Prontuário Terapêutico - 11. Lisboa: Ministério da Saúde; 2012.
240. INFARMED – Autoridade Nacional do Medicamento e Produtos de Saúde IMdS. 2013 [updated 2013-09-03; cited 2013 2013-09-03]; Available from: http://www.infarmed.pt/portal/page/portal/INFARMED.
241. Portugal UM. 2013 [cited 2013 2013-09-03]; Available from: http://www.simposium.pt/.
242. Pharma O. XL-S Medical. 2013 [cited 2013 2013-09-04]; Available from: http://xlsmedical.com/site/pt.
243. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
244. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
245. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. Journal of Lipid Research. 1990;31(3):545-8.
246. Gajewska J, Weker H, Ambroszkiewicz J, Chelchowska M, Wiech M, Laskowska-Klita T. Changes in concentration of serum adiponectin multimeric forms following weight reduction programme in prepubertal obese children. Medycyna Wieku Rozwojowego. 2011;15(3):298-305.
247. Schwartz J, Weiss ST. Host and environmental factors influencing the peripheral blood leukocyte count. American Journal of Epidemiology. 1991;134(12):1402-9.
248. Suwa T, Hogg JC, English D, Van-Eeden SF. Interleukin-6 induces demargination of intravascular neutrophils and shortens their transit in marrow. American Journal Physiology Heart Circulation Physiology. 2000;279(6):H2954-60.
249. Veltri S, Smith-2nd JW. Interleukin 1 trials in cancer patients: a review of the toxicity, antitumor and hematopoietic effects. Stem Cells. 1996;14(2):164-76.
250. Kushner I, Rzewnicki D. Acute phase response. In: Gallin JI, Snyderman R, Fearon DT, Haynes BF, Nathan C, editors. Inflammation: basic principles and clinical correlates. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 317-29.