DIET, BODY FAT DISTRIBUTION AND CORONARY DISEASE - UNDERSTANDING THE EFFECT OF INFLAMMATION

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IV. **Oliveira A**, Rodríguez-Artalejo F, Lopes C. Alcohol intake and systemic markers of inflammation – shape of the association according to sex and body mass index. Alcohol Alcohol 2010; 45(2):119-25.


VI. **Oliveira A**, Lopes C, Rodriguez-Artalejo F. Adherence to the Southern-European Atlantic diet and risk of non-fatal acute myocardial infarction. [Submetido para publicação]
Esta investigação foi realizada no Serviço de Higiene e Epidemiologia da Faculdade de Medicina da Universidade do Porto e no Instituto de Saúde Pública da Universidade do Porto, sob orientação da Professora Doutora Carla Lopes (Faculdade de Medicina e Instituto de Saúde Pública da Universidade do Porto) e co-orientação do Professor Doutor Fernando Rodríguez Artalejo (Faculdade de Medicina da Universidade Autónoma de Madrid).

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List of original publications

This doctoral dissertation is based upon the following six papers, which will be referred to in the text by their Roman numerals.


IV. Oliveira A, Rodríguez-Artalejo F, Lopes C. Alcohol intake and systemic markers of inflammation – shape of the association according to sex and body mass index. Alcohol Alcohol 2010; 45(2):119-25.

V. Oliveira A, Rodríguez-Artalejo F, Gaio R, Santos A, Ramos E, Lopes C. Major dietary patterns are associated with acute myocardial infarction and cardiovascular risk markers in a Southern-European population. [Submitted]

VI. Oliveira A, Lopes C, Rodríguez-Artalejo F. Adherence to the Southern-European Atlantic diet and risk of non-fatal acute myocardial infarction. [Submitted]
Abstract
Aims

This research aims to evaluate the effect of body fat distribution and diet on coronary heart disease and whether these associations could be mediated by an inflammation pathway. To accomplish this general objective, 6 studies (corresponding to 6 papers) were performed with the following specific objectives:

1. To assess, using principal component analysis, the independent associations of general, central and peripheral subcutaneous body fat with high-sensitivity C-reactive protein (hs-CRP), in men and women from the general population.

2. To assess the effect of general, central and peripheral subcutaneous body fat on the occurrence of non-fatal acute myocardial infarction (AMI), by sex.

3. To evaluate the effect of fruit, vegetables, antioxidant vitamins and fibre intake on hs-CRP levels in individuals from the general population, and to examine the modification of these associations by sex and body mass index (BMI).

4. To assess the association of alcohol intake with hs-CRP, uric acid and leukocyte count, and whether sex and BMI modify these associations.

5. To assess the association of a posteriori dietary patterns with AMI and cardiovascular risk markers in the adult population of Porto, Portugal.

6. To examine the association between adherence to the Southern-European Atlantic diet, which is culturally rooted in Northern Portugal and Galicia, Spain, and the risk of non-fatal AMI.

Research Design and Methods

Participants are part of the EPIPorto cohort study and the EPICardis population-based case-control study, both conducted at Porto, Portugal during 1999-2003. Participants of the EPIPorto study were selected, by random digit dialing, among the 300 000 non-institutionalized inhabitants of Porto, aged 18 years or over (at the time of recruitment, 97% of households had a telephone number). The participation rate in the baseline cohort evaluation was 70% (n=2485). These participants, if had no previous clinical or silent infarction according to self-reported data and/or electrocardiographic evidence, were also included as controls in the EPICardis Study (n=2382). Cases were
patients admitted consecutively to the Cardiology Department of the four hospitals providing acute coronary care in Porto, with a first diagnosis of AMI, and who survived beyond the fourth day after the event (n=1248).

Community participants were invited to face-to-face interviews and physical examination, whereas AMI patients were concurrently interviewed during their hospital in-stay after clinical stabilization. All participants gave written informed consent to participate. Afterwards, data were collected by trained interviewers using structured questionnaires with information on social, demographic, clinical and behavioral characteristics. Cognitive impairment of participants was evaluated by a score less than 24 in the Mini-Mental State Examination test. Measurements of anthropometry and blood pressure, an electrocardiogram and blood sampling were also performed.

The main exposure variables in this research were anthropometrics and diet. Weight and height were objectively measured with subjects in light clothing and barefoot, and participants were classified as underweight/normal weight (BMI <25.0 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²). Waist circumference (WC) and waist-to-hip ratio (WHR) were also obtained. A skinfold composite index (∑triceps and biceps skinfolds/ ∑triceps, biceps, subscapular and suprailliac skinfolds) was used to estimate the proportion of peripheral subcutaneous fat (arms) (PSFA).

Dietary intake over the previous 12 months was assessed with an 82-item validated semi-quantitative food frequency questionnaire. Total alcohol intake was assessed with the same questionnaire, including information on wine, beer and spirits; moderate drinking was defined as alcohol intake ≤15 g/day in women and ≤30 g/day in men.

A posteriori dietary patterns were identified using multivariate finite mixture models; firstly among the community controls and then among the AMI cases. The probabilities predicting the cluster membership were 89% in women and 92% in men.

An a priori dietary pattern was also developed to represent the degree of adherence to the Southern-European Atlantic diet (SEAD), including nine key components of this diet: fresh fish excluding cod fish, cod fish, red meat and pork products, dairy products, legumes and vegetables, vegetable soup, potatoes, whole grain bread and wine. A score of 1 or 0 was respectively assigned to each food consumed at/above or below the sex-specific median in the controls, measured in g per 1000 kcal/day. For wine, up to 1 glass/day in women and 2 glasses/day in men were assigned with a score of 1, and a 0 score was assigned when above or below (nil consumption). The final index ranged from 0 to 9. An alternate SEAD index was calculated with a reverse scoring for
red meat and pork products and for potatoes (value 0 for consumption at or higher than the median, and 1 for a lower consumption).

The main outcome variables in this research were non-fatal AMI and cardiovascular risk markers, namely hs-CRP, uric acid, leucocytes and blood lipids, obtained from a venous blood sample drawn after a 12-hour overnight fast. hs-CRP levels (mg/l) were determined for 1538 individuals and categorized into <1 (low risk), 1-3 (average risk), >3 (high risk) to <10 (used in ordinal logistic regressions) or modeled as a continuous variable (used in generalized linear models).

Ordinal or unconditional logistic regressions were summarized by odds ratio and their respective 95% confidence intervals (OR, 95%CI), after adjustment for the potential confounders.

Results

Paper I

New independent anthropometric factors were identified, by principal component analysis, due to moderate-to-strong correlations between the single classical anthropometric measures: BMI, WC, WHR and PSFA. These new patterns of body fat distribution were found to have opposing effects on hs-CRP levels. A general pattern of fat distribution (high BMI and WC) in both sexes, and a central pattern (high WC and high WHR, but low BMI) in men, were directly associated with hs-CRP levels. In contrast, a peripheral fat pattern (high proportion of PSFA) seemed to be inversely associated with hs-CRP, at least in women (β=-0.071, p for trend=0.048 in women; β=0.044, p for trend=0.364 in men).

Paper II

When the same components of central and peripheral body fat were studied in relation to non-fatal AMI events, similar results were found: a central pattern of fat distribution was associated with a higher risk of AMI in both men and women. In contrast, the peripheral fat component predicted lower risk of AMI in women (upper vs. lower quartile: OR 0.59, 95%CI 0.36-0.96), but higher risk in men (upper vs. lower quartile: OR 2.45, 95%CI 1.69-3.55) (p-value for sex interaction<0.001).
Paper III

Inverse associations, measured by the probability of changing of hs-CRP category (low- to average risk or average- to high risk) per unit of dietary intake, were found for fresh fruit and vegetables, fresh fruit (OR 0.73, 95%CI 0.56-0.96 per 100 g/day), vegetables (OR 0.55, 95%CI 0.35-0.86 per 100 g/day), vitamin C (OR 0.34, 95%CI 0.14-0.80 per 100 mg/day) and vitamin E (OR 0.14, 95%CI 0.02-0.88 per 10 mg/day) in normal weight men. In overweight men, fiber was also inversely associated with hs-CRP levels (OR 0.53, 95%CI 0.37-0.76 per 10 g/day). Overall, associations tended to be weaker in overweight subjects.

In women, no significant associations between the dietary variables and hs-CRP were found. A significant modification effect of the evaluated associations was found by sex for fruit and vegetables, fruits, vitamin C and fiber. BMI did not modify the reported associations.

Paper IV

In women, adjusted hs-CRP levels (mg/l) by categories of alcohol intake (g/day) were 2.69 in non-drinkers, 2.25 in drinkers of >0-15 g/day, 2.32 in drinkers of >15-30 g/day, and 3.18 in drinkers of >30 g/day (p-value for the quadratic trend<0.001). In men, the association between alcohol intake and hs-CRP (mg/l) was positive and linear (2.12, 1.48, 2.17 and 2.24 mg/l, respectively for the same intake categories reported in women) (p-value for the linear trend=0.014; p-value for the quadratic trend=0.937).

Alcohol intake was also positively and linearly associated with uric acid in each sex. No significant association between alcohol intake and leukocyte count was found.

BMI modified these associations, which remained statistically significant only in normal weight women and overweight men for hs-CRP, and in normal weight individuals for uric acid.

Paper V

Four dietary patterns were identified by a posteriori methods, separately for men and women. In comparison to women with a “healthy” dietary pattern (higher consumption of fruits, vegetable soup and dairy products, and lower of red meat, fast-foods and soft drinks), those with a “low fruit and vegetables” and “red meat and alcohol” patterns (the last one also characterized by a lower intake of dairy products and vegetables) showed a higher risk of AMI (OR 1.85, 95%CI 1.01-3.39 and OR 1.91,
95%CI: 1.17-3.12, respectively). Female controls with the "red meat and alcohol" pattern also had a higher total-to-HDL cholesterol ratio (3.9 vs. 3.6, p=0.043).

In comparison to men with a "healthy" pattern, those with the "red meat and alcohol" intake pattern, similar to the counterparts found in women, were more likely to suffer an AMI (OR 1.98, 95%CI 1.35-2.92); male controls with this pattern had higher diastolic blood pressure (84 vs. 81 mmHg, p=0.010), hs-CRP (2.64 vs. 1.68 mg/l, p<0.008) and uric acid levels (57.0 vs. 48.8 mg/l, p<0.001).

Paper VI

The traditional diet in Northern Portugal and Galicia, a region in Northwest Spain, was conceptually represented by the SEAD index. A one-point increment in the SEAD score was associated with a 10% reduced risk of AMI (OR 0.90, 95%CI 0.85-0.96). As compared with individuals in the lower quartile of the SEAD index (lowest adherence), those in the upper quartile had a 33% lower likelihood of AMI (≤3 vs. ≥6 points: OR 0.67, 95%CI 0.51-0.88, p for trend=0.003).

A reverse scoring for the components which, individually, increased the AMI risk (red meat and pork products and potatoes) led to an even stronger inverse association between the SEAD index and AMI (upper vs. lower quartile: OR 0.40, 95%CI 0.30-0.52, p for trend<0.001).

Main Conclusions

Different patterns of body fat distribution, identified by principal component analysis, were found to have opposing effects on hs-CRP levels: while a central pattern of fat distribution was directly associated with hs-CRP levels in men, a high proportion of peripheral subcutaneous fat in the arms seemed to be inversely associated with hs-CRP, but only in women.

The same anthropometric factors were found to be associated with non-fatal AMI, but while the peripheral subcutaneous fat index predicted a lower risk of AMI in women, a higher risk was found in men.

The consumption of single dietary factors, such as fruit and vegetables, vitamin C, E and fiber were inversely associated with hs-CRP levels in men, whereas in women no associations were reported. The associations tended to be weaker in overweight individuals.
Alcohol intake showed a J-shaped relation with hs-CRP levels in women, and a positive linear-shaped relation in men, which probably reflects the drink patterns of our country. Uric acid levels increased with increasing alcohol intake in each sex. BMI modified these associations, so that they only held in normal weight women and in overweight men for hs-CRP, and in normal weight individuals for uric acid.

These previous dietary factors seem to cluster in the Portuguese population. Individuals with a higher consumption of red meat and alcohol, and lower intake of vegetables and dairy products, had an increased risk of AMI and a worse cardiovascular biomarker profile than those with a "healthy" dietary pattern.

A higher adherence to the South-European Atlantic diet (SEAD index), a highly palatable diet which is culturally rooted in Northern Portugal and Galicia, Spain was associated with a lower risk of non-fatal AMI events.
Resumo
Objectivos

Esta dissertação tem como objectivo avaliar o efeito da distribuição da gordura corporal e da alimentação na doença coronária e investigar se estas associações podem ser mediadas por uma via inflamatória. De forma a atingir este objectivo geral, foram desenvolvidos 6 estudos (correspondendo a 6 artigos) com os seguintes objectivos específicos:

1. Avaliar, através de análise de componentes principais, as associações independentes entre a gordura corporal generalizada, central e periférica subcutânea e os níveis séricos de proteína C-reactiva de alta sensibilidade (PCR-as), em homens e mulheres da população geral.

2. Estimar o efeito da gordura corporal generalizada, central e periférica na ocorrência de enfarte agudo do miocárdio (EAM) não fatal, por sexo.

3. Avaliar o efeito da ingestão de fruta, vegetais, vitaminas antioxidantes e fibra nos níveis séricos de PCR-as em indivíduos da população geral e quantificar se o sexo e o índice de massa corporal (IMC) podem modificar o efeito destas associações.

4. Estudar a associação entre a ingestão de álcool e os níveis de PCR-as, ácido úrico e contagem de leucócitos no sangue, e se estas associações podem ser modificadas pelo sexo e pelo IMC.

5. Estimar a associação entre padrões alimentares definidos a posteriori e a ocorrência de EAM e marcadores de risco cardiovascular na população adulta do Porto, Portugal.

6. Avaliar a associação entre a Alimentação Atlântica do Sul da Europa, culturalmente enraizada no Norte de Portugal e na região da Galiza em Espanha, e o risco de ocorrência de EAM.

Desenho do Estudo e Métodos

Os participantes integram o estudo de coorte EPIPorto e o estudo caso-controlo de base populacional EPICardis, ambos conduzidos no Porto, Portugal entre 1999 e 2003. Os participantes do estudo EPIPorto foram selecionados, por aleatorização de dígitos telefónicos, de entre os 300 000 habitantes não institucionalizados do Porto com idade igual ou superior a 18 anos (aquando do recrutamento, 97% das habitações
familiares usufruíam de serviço telefônico). A proporção de participação na avaliação inicial da coorte foi de 70% (n=2485). De entre estes participantes, aqueles que não apresentassem evidência prévia de enfarte clínico ou silencioso mediante informação auto-declarada e/ou evidência electrocardiográfica, foram também incluídos como controlos no estudo EPICardis (n=2382). Neste estudo, constituíram-se como casos os doentes admitidos consecutivamente ao Serviço de Cardiologia dos quatro hospitais que disponibilizam tratamento coronário agudo no Porto, com diagnóstico de primeiro EAM, e que tivessem sobrevivido além do quarto dia após o evento agudo (n=1248).

Os participantes comunitários foram convidados para uma entrevista pessoal e exame físico, enquanto os casos de EAM foram concomitantemente entrevistados durante a sua hospitalização após estabilização clínica. Todos os participantes forneceram consentimento informado escrito para participar, prévio à recolha de informação. Esta foi realizada por inquiridores treinados através de questionários estruturados, incluindo questões sobre características sociais, demográficas, clínicas e comportamentais. A disfunção cognitiva dos participantes foi avaliada por uma pontuação final inferior a 24 no teste Mini-Mental State Examination. Foram também conduzidas avaliações antropométricas e da pressão arterial, realizado um electrocardiograma e a colheita de uma amostra de sangue.

Definiram-se como principais variáveis de exposição nesta investigação os parâmetros antropométricos e a alimentação. O peso e a estatura foram objectivamente medidos, tendo-se estabelecido como critérios para a sua medição o uso de roupa leve e a ausência de calçado, e os participantes foram classificados nas seguintes categorias: magreza/ normoponderabilidade (IMC <25.0 kg/m²), excesso de peso (IMC 25.0-29.9 kg/m²) e obesidade (IMC ≥30.0 kg/m²). O perímetro da cintura (PC) e o perímetro da cintura-anca (PCA) foram também aferidos. Um índice baseado em pregas de adiposidade subcutânea (Σ pregas tricipital e bicapital/ Σ pregas bicapital, tricipital, subcapsular e suprailiaca) foi utilizado para estimar a proporção de gordura subcutânea periférica (braços).

O consumo alimentar foi estimado através de um questionário semi-quantitativo de frequência alimentar relativo aos 12 meses anteriores, previamente validado. A ingestão total de álcool foi avaliada pelo mesmo questionário incluindo informação sobre o consumo de vinho, cerveja, bebidas brancas e espirituosas; considerou-se como consumo moderado uma ingestão de álcool ≤15 g/dia nas mulheres e ≤30 g/dia homens.
Foram definidos padrões alimentares *a posteriori* por multivariate finite mixture models; primeiro nos controlos comunitários e depois nos casos de EAM. As probabilidades de pertencer a cada cluster foram de 89% nas mulheres e 92% nos homens.

Também se definiu um padrão alimentar *a priori*, construído de forma a representar a adesão à Alimentação Atlântica do Sul da Europa, englobando nove componentes chave desta alimentação: peixe fresco excluindo o bacalhau, bacalhau, carne vermelha e produtos cárneos de porco, produtos lácteos, leguminosas e vegetais, sopa de legumes, batatas, pão pouco refinado e vinho. Uma pontuação de 1 ou 0 foi respectivamente atribuída para consumos iguais/acima ou abaixo da mediana nos controlos, definida separadamente em cada sexo e quantificada em g por 1000 kcal/dia. Para o vinho, consumos até 1 ou 2 copos por dia, respectivamente para mulheres e homens, classificaram-se com 1; consumos acima ou abaixo (não consumo) destes níveis tiveram uma pontuação nula. O índice final apresentou um âmbito de variação de 0 a 9. Um índice alternativo foi construído atribuindo uma pontuação inversa aos componentes carne vermelha e produtos cárneos de porco e batatas (0 para consumos iguais ou superiores à mediana e 1 para consumos inferiores).

Definiram-se como principais variáveis *outcome* nesta investigação a ocorrência de EAM não fatal e marcadores de risco cardiovascular, nomeadamente PCR-as, ácido úrico, leucócitos e lipídios séricos, doseados após jejum de 12 horas numa amostra de sangue venoso. Os níveis séricos de PCR-as (mg/l) foram determinados para 1538 participantes e categorizados em <1 (baixo risco), 1-3 (risco moderado), >3 (elevado risco) a <10 (variável utilizada em regressões logísticas ordinais) ou utilizados como variável contínua (em modelos lineares generalizados).

*Odds ratio* e os respectivos intervalos de confiança a 95% (OR, IC95%) foram obtidos por regressão logística ordinal ou não condicional, após ajuste para os potenciais confundidores.

**Resultados**

**Artigo I**

Foram identificados, por análise de componentes principais, novos componentes antropométricos independentes entre si, dadas as correlações moderadas a fortes...
observadas entre os parâmetros antropométricos clássicos: IMC, PC, PCA e o índice de gordura subcutânea periférica. Estas novas distribuições de gordura corporal mostraram apresentar efeitos contrários nos níveis de PCR-as. Uma distribuição generalizada de gordura (elevados IMC e PC), em ambos os sexos, e uma distribuição central de gordura (elevados PC e PCA, mas baixo IMC), nos homens, associaram-se directamente com os níveis de PCR-as. Contrariamente, uma distribuição de gordura preferencialmente periférica (elevada proporção de gordura subcutânea nos braços) parece associar-se inversamente com a PCR-as, pelo menos nas mulheres (β=-0,071, p tendência=0,048 nas mulheres; β=0,044, p tendência=0,364 nos homens).

**Artigo II**

Observaram-se resultados similares ao avaliar o efeito dos mesmos componentes de gordura corporal em relação à ocorrência de eventos não fatais de EAM: uma distribuição central de gordura associou-se a um maior risco de EAM, em ambos os sexos. Contrariamente, o componente de gordura periférica associou-se a um menor risco de EAM nas mulheres (último vs. primeiro quartil: OR 0,59, IC95% 0,36-0,96), mas maior nos homens (último vs. primeiro quartil: OR 2,45, IC95% 1,69-3,55) (p interacção com o sexo<0,001).

**Artigo III**

A probabilidade de mudar de categoria de PCR-as (de baixo para risco moderado ou de moderado para elevado risco) mostrou uma associação inversa com o aumento da ingestão de fruta fresca e vegetais, fruta fresca (OR 0,73, IC95% 0,56-0,96 por 100 g/dia), vegetais (OR 0,55, IC95% 0,35-0,86 por 100 g/dia), vitamina C (OR 0,34, IC95% 0,14-0,80 por 100 mg/dia) e vitamina E (OR 0,14, IC95% 0,02-0,88 por 10 mg/dia), nos homens normoponderais. Nos homens com excesso de peso/ obesidade, a fibra também se associou inversamente com os níveis de PCR-as (OR 0,53, IC95% 0,37-0,76 por 10 g/dia). Globalmente, as associações apresentaram menor magnitude nos indivíduos com excesso de peso/ obesidade.

Nas mulheres, não se verificaram associações significativas entre as variáveis alimentares e a PCR-as. Foram encontradas modificações de feito das associações por sexo, no caso da medida sumária de fruta fresca e vegetais, fruta fresca, vitamina C e fibra. O IMC não modificou as associações encontradas.
Artigo IV

Nas mulheres, observaram-se as seguintes estimativas pontuais de PCR-as (mg/l) por categorias de ingestão de álcool (g/dia): 2,69 para consumos nulos, 2,25 para consumos >0-15 g/dia, 2,32 para consumos 15-30 g/dia e 3,18 para consumos >30 g/dia (p tendência quadrática<0,001). Nos homens, a associação entre a ingestão de álcool e a PCR-as foi positiva e linear (2,12, 1,48, 2,17 e 2,24 mg/l, respectivamente para os mesmos consumos reportados nas mulheres (p tendência linear=0,014; p tendência quadrática=0,937).

A ingestão de álcool associou-se diretamente e de forma linear com os níveis séricos de ácido úrico, em ambos os sexos. Não se encontram associações significativas entre a ingestão de álcool e a contagem de leucócitos.

O IMC modificou estas associações, que apenas se mantiveram significativas nas mulheres normoponderais e nos homens com excesso de peso/obesidade para a PCR-as e nos indivíduos normoponderais para o ácido úrico.

Artigo V

Foram identificados, por métodos a posteriori, quatro padrões alimentares em cada sexo. Comparativamente às mulheres com um padrão alimentar "saudável" (maior consumo de fruta, sopa de legumes e produtos lácteos e menor de carnes vermelhas, fast-foods e refrigerantes), aquelas com padrões alimentares caracterizados por "baixo consumo de fruta e hortícolas" e "carne vermelha e álcool" (este último igualmente caracterizado por consumos inferiores de produtos lácteos e vegetais) apresentaram um maior risco de EAM (OR 1,85, IC95% 1,01-3,39 e OR 1,91, IC95% 1,17-3,12, respectivamente). Os controlos do sexo feminino com o padrão "carne vermelha e álcool" apresentaram também uma maior razão colesterol total/ colesterol HDL (3,9 vs. 3,6, p=0,043).

Tomando como referência os homens com um padrão alimentar "saudável", aqueles com o padrão alimentar "carne vermelha e álcool", similares aos encontrados nas mulheres, apresentaram uma maior probabilidade de desenvolver EAM (OR 1,68, IC95% 1,35-2,92). Os controlos do sexo masculino com este padrão apresentaram níveis mais elevados de pressão arterial diastólica (84 vs. 81 mmHg, p=0,010), de PCR-as (2,64 vs. 1,68 mg/l, p<0,008) e de ácido úrico (57,0 vs. 48,8 mg/l, p<0,001).
Artigo VI

A alimentação tradicional do Norte de Portugal e da região da Galiza em Espanha foi conceptualmente representada na definição do índice “Alimentação Atlântica do Sul da Europa”. Cada unidade de aumento na pontuação final do índice associou-se a um decréscimo de 10% no risco de EAM (OR 0,90, IC95% 0,85-0,96). Comparativamente aos indivíduos no primeiro quartil do índice (menor adesão ao padrão), aqueles classificados no último quartil apresentaram 33% menos probabilidade de desenvolver EAM (≤3 vs. ≥6 pontos: OR 0,67, IC95% 0,51-0,88, p tendência=0,003).

Ao inverter a pontuação dos componentes que, individualmente, se associaram positivamente com o EAM (carne vermelha e produtos cárneos de porco e batatas) uma associação inversa ainda mais forte foi encontrada entre o índice a ocorrência de EAM (último vs. primeiro quartil: OR 0,40, IC95% 0,30-0,52, p tendência<0,001).

Principais Conclusões

Diferentes distribuições de gordura corporal, identificadas por análise de componentes principais, apresentaram efeitos antagónicos nos níveis de PCR-as: enquanto uma distribuição central da gordura corporal se associou directamente com os níveis de PCR-as nos homens, uma maior proporção de gordura subcutânea periférica nos braços mostrou-se inversamente associada à PCR-as, mas apenas nas mulheres.

Os mesmos componentes de gordura corporal associaram-se à ocorrência de eventos não fatais de EAM. Nas mulheres, o índice de gordura subcutânea periférica mostrou diminuir o risco de EAM, por outro lado, nos homens, este associou-se a um maior risco de EAM.

O consumo de alimentos/nutrientes isolados, nomeadamente de fruta e vegetais, vitaminas antioxidantes e fibra alimentar associou-se inversamente com os níveis de PCR-as nos homens. Estas associações não se observaram nas mulheres. As associações apresentaram uma menor magnitude nos indivíduos com excesso de peso/obesidade.

Observou-se uma relação em J entre a ingestão de álcool e a PCR-as, nas mulheres, mas nos homens esta relação mostrou-se positiva e linear, o que provavelmente se deve aos padrões de consumo no nosso país. Os níveis de ácido úrico aumentaram com um aumento da ingestão de álcool, em ambos os sexos. O
IMC modificou estas associações, que se mantiveram significativas apenas nas mulheres normoponderais e nos homens com excesso de peso, no caso da PCR-as, e nos indivíduos normoponderais, no caso do ácido úrico.

Os factores alimentares anteriormente descritos parecem agregar-se na população Portuguesa. Indivíduos com consumos superiores de carnes vermelhas e álcool e consumos inferiores de vegetais e produtos lácteos apresentaram um risco aumentado de desenvolver EAM e um perfil de biomarcadores cardiovasculares menos favorável, quando comparados com aqueles que apresentavam um padrão alimentar definido como “saudável”.

Uma maior adesão à Alimentação Atlântica do Sul da Europa, uma alimentação culturalmente enraizada no Norte de Portugal e na região da Galicia em Espanha, associou-se a um menor risco de ocorrência de eventos não fatais de EAM.
Resumen
Objetivos

Esta investigación tiene como objetivo evaluar el efecto de la distribución de grasa corporal y la dieta en las enfermedades coronarias y si estas asociaciones podrían ser mediadas por una vía inflamatoria. Para lograr este objetivo general, 6 estudios (correspondientes a 6 artículos) se realizaron con los siguientes objetivos específicos:

1. Evaluar, mediante análisis de componentes principales, las asociaciones independientes de la grasa corporal general, central y subcutánea periférica, con los niveles séricos de la proteína C-reactiva de alta sensibilidad (PCR-as), en hombres y mujeres de la población en general.

2. Evaluar el efecto de la grasa corporal general, central y periférica sobre el riesgo de infarto agudo de miocardio (IAM) no fatal, según el sexo.

3. Evaluar el efecto del consumo de frutas, verduras, vitaminas antioxidantes y fibra en los niveles de PCR-as en la población en general, y examinar la modificación de estas asociaciones por el sexo y el índice de masa corporal (IMC).

4. Valorar la asociación de la ingesta de alcohol con los niveles de PCR-as, ácido úrico y recuento de leucocitos, y si el sexo y el IMC modifican estas asociaciones.

5. Evaluar la asociación entre patrones dietéticos a posteriori y el IAM y marcadores de riesgo cardiovascular en la población adulta de Porto, Portugal.

6. Examinar la asociación entre la adherencia a la Dieta Atlántica del Sur-Europeo, que está culturalmente arraigada en el norte de Portugal y Galicia (España), y el riesgo de IAM no fatal.

Diseño de la Investigación y Métodos

Los participantes forman parte del estudio de cohorte EPIPorto y del estudio caso-controle de base poblacional EPICardis, ambos realizados en Porto, Portugal durante el periodo 1999-2003. Los participantes del estudio EPIPorto fueron seleccionados mediante marcación telefónica aleatoria entre los 300 000 habitantes no institucionalizados de 18 o más años de edad en Porto (al momento de lo reclutamiento, el 97% de los hogares tenían un número de teléfono). La tasa de participación en la primera evaluación de la cohorte fue de 70% (n=2485). Los
participantes sin evidencia de IAM, según datos auto-reportados o electrocardiografía, se incluyeron también como controles en el estudio EPICardis (n=2382). Los casos fueron pacientes con un primer diagnóstico de IAM ingresados consecutivamente en el Departamento de Cardiología de los cuatro hospitales que prestan cuidados coronarios agudos en Porto, y que sobrevivieron más allá del cuarto día después del evento (n=1248).

Los participantes de la comunidad fueron invitados a entrevistas personales y examen físico en el Departamento de Epidemiología de la Universidad de Porto; de forma concurrente, los pacientes con IAM fueron entrevistados y examinados durante su estancia en el hospital, una vez lograda su estabilización clínica. Todos los participantes dieron su consentimiento informado por escrito para participar. Los datos se recogieron por entrevistadores entrenados, utilizando cuestionarios estructurados sobre factores sociales, demográficos, clínicos y conductuales. El deterioro cognitivo fue definido por una puntuación inferior a 24 en el Mini-Mental State Examination. También se realizó antropometría, medición de presión arterial, electrocardiograma, y toma de muestras de sangre.

Las principales variables de exposición en esta investigación fueron la antropometría y la dieta. El peso y altura se midieron con los sujetos en ropa ligera y descalzo, y los participantes se clasificaron como bajo peso/peso normal (IMC <25,0 kg/m²), sobrepeso (IMC 25,0-29,9 kg/m²) y obesidad (IMC ≥30,0 kg/m²). La circunferencia de la cintura (CC) y la relación cintura-cadera (RCC) también se obtuvieron. Se utilizó un índice de pliegues cutáneos (Σ pliegues del tríceps y bíceps / Σ pliegues cutáneos del tríceps, bíceps, subescapular y suprailláco) para estimar la proporción de grasa subcutánea periférica (brazos).

La ingesta dietética durante los últimos 12 meses fue evaluada con un cuestionario semi-cuantitativo de frecuencia de alimentos, previamente validado. El consumo de alcohol total fue evaluado con lo mismo cuestionario y incluyó información del consumo de vino, cerveza y bebidas espirituosas; el consumo moderado de alcohol se definió como menor o igual de 15 g/día en mujeres y de 30 g/día en hombres.

Los patrones dietéticos a posteriori fueron identificados mediante multivariate finite mixture models; en primer lugar entre los controles de la comunidad y, a continuación, entre los casos de IAM. Las probabilidades de pertenecer al cluster fueron del 89% en las mujeres y 92% en los hombres.
Para representar el grado de adherencia a la Dieta Atlántica del Sur-Europeo se desarrolló un patrón dietético a priori, que incluyó nueve componentes claves de esta dieta: pescado fresco sin bacalao, bacalao, carnes rojas y productos del cerdo, productos lácteos, legumbres y verduras, sopa de verduras, patatas, pan integral y vino. Una puntuación de 1 o 0 fue respectivamente asignada a cada alimento consumido por encima o por debajo de la mediana de cada sexo entre los controles, expresada en g por cada 1000 kcal/día. Para el vino, se asignó la puntuación 1 al consumo de hasta 1 vaso al día en las mujeres y 2 vasos al día en los hombres; se asignó un cero cuando el consumo fue nulo o superior al valor antes mencionado. El índice tuvo un rango de 0 a 9. También se calculó un índice alternativo invirtiendo la puntuación para las carnes rojas y productos del cerdo y las patatas (valor 0 para el consumo igual o superior a la mediana, y 1 para un menor consumo).

Las principales variables de resultado en esta investigación fueron el IAM no fatal y los marcadores de riesgo cardiovascular, esto es PCR-as, ácido úrico, leucocitos y lípidos en la sangre, obtenidos de una muestra de sangre venosa extraída tras 12 horas de ayuno nocturno. Los niveles de PCR-as (mg/l) se determinaron en 1538 personas y se clasificaron en <1 (bajo riesgo), 1-3 (riesgo medio), > 3 (alto riesgo) a <10 (en las regresiones logísticas ordinales); también se modelaron como una variable continua (en modelos lineales generalizados).

Las asociaciones de interés se resumieron con el odds ratio y su intervalo de confianza a 95% (OR, IC95%), obtenidos en regresiones logísticas ordinales o incondicionales con ajuste por los potenciales factores de confusión.

Resultados

Artículo I

Se han identificado, mediante el análisis de componentes principales, nuevos factores antropométricos independientes que resultan de correlaciones moderadas a fuertes entre las medidas antropométricas clásicas: IMC, CC, RCC e índice de grasa subcutánea periférica. Estos factores que miden la distribución de grasa corporal tuvieron efectos opuestos en los niveles de PCR-as. Una distribución general de la grasa (altos IMC y CC), en ambos sexos, y una distribución central de grasa (altos CC y RCC, pero bajo IMC), en los hombres, estaban directamente asociadas con los niveles de PCR-as. En cambio, una distribución de la grasa preferiblemente periférica...
parece estar inversamente relacionada con la PCR-as en las mujeres ($\beta=-0.071$, p tendencia=0,048 en las mujeres; $\beta=0.044$, p tendencia=0,364 en los hombres).

**Artículo II**

Cuando los mismos componentes de grasa corporal fueron estudiados en relación con el riesgo de IAM no fatal, se obtuvieron resultados similares: una distribución central de la grasa se asoció con un mayor riesgo de IAM en ambos los sexos. En cambio, la componente de grasa periférica predijo un menor riesgo de IAM en las mujeres (cuartil superior vs. inferior: OR 0,59, IC95% 0,36-0,96), pero un mayor riesgo en los hombres (cuartil superior vs. inferior: OR 2,45, IC95% 1,69-3,55, p interacción con el sexo<0,001).

**Artículo III**

La probabilidad de cambiar de categoría de PCR-as (de bajo riesgo a riesgo medio, o de riesgo medio a alto) mostró una asociación inversa con el incremento de la ingesta alimentaria, para las frutas y hortalizas, las frutas (OR 0,73, IC95%: 0,56-0,96 por 100 g/día), verduras (OR 0,55, IC95% 0,35-0,86 por 100 g/día), vitamina C (OR 0,34, IC95% 0,14-0,80 por 100 mg/día) y vitamina E (OR 0,14, IC95% 0,02-0,88 por 10 mg/día) en hombres con peso normal. En los hombres con sobrepeso, la fibra también se asoció inversamente con los niveles de PCR-as (OR 0,53, IC95%: 0,37-0,76 por 10 g/día). En general, las asociaciones tendieron a ser de menor magnitud en los sujetos con sobrepeso.

En las mujeres, no se hallaron asociaciones significativas entre las variables de la dieta y la PCR-as. Se observó que el sexo modificaba las asociaciones para las frutas y hortalizas, frutas, vitamina C y fibra, pero no para el IMC.

**Artículo IV**

En las mujeres, los niveles de PCR-as (mg/l) ajustados por categorías de consumo de alcohol (g/día) fueron de 2,69 en los no bebedores, 2,25 en los bebedores de >0-15 g/día, 2,32 en los bebedores de >15-30 g/día y 3,18 en los bebedores de >30 g/día (p tendencia cuadrática<0,001). En los hombres, la asociación entre el consumo de alcohol y la PCR-as (mg/l) fue positiva y lineal (2,12, 1,48, 2,17 y 2,24 mg/l, respectivamente para las categorías de consumo descritas más arriba) (p tendencia lineal=0,014; p tendencia cuadrática=0,937).
El consumo de alcohol también se ha asociado de forma positiva y lineal con el ácido úrico, en cada sexo. No se encontró asociación significativa entre el consumo de alcohol y el recuento de leucocitos.

El IMC ha modificado estas asociaciones, que fueron estadísticamente significativas sólo en las mujeres de peso normal y en los hombres con sobrepeso para la PCR-as, y en personas de peso normal para el ácido úrico.

Artículo V

Mediante métodos a posteriori, se identificaron cuatro patrones dietéticos en hombres y otros tantos en mujeres. En comparación con las mujeres con un patrón dietético "saludable" (mayores consumos de frutas, sopa de verduras y productos lácteos e menores de carnes rojas, fast-foods y refrescos) aquellas con patrones "bajo de frutas y hortalizas" y "carne roja y alcohol" (este último también tiene un menor consumo de productos lácteos y hortalizas), mostraron un mayor riesgo de IAM (OR 1,85, IC95% 1,01-3,39, y OR 1,91, IC95%: 1,17-3,12, respectivamente). Los controles de sexo femenino con el patrón "carne roja y alcohol" también tenían niveles superiores de relación colesterol total/colesterol HDL (3,9 vs 3,6, p=0,043).

En comparación con los hombres con un patrón dietético "saludable", los que tienen el patrón "carne roja y alcohol", similares al de los mismos nombres en las mujeres, tenían mayores riesgo de IAM (OR 1,98, IC95% 1,35-2,92); controles de sexo masculino con este patrón presentaron mayor presión arterial diastólica (84 vs. 81 mmHg, p=0,010), PCR-as (2,64 vs. 1,68 mg/l, p<0,008) y ácido úrico (57,0 vs. 48,8 mg/l, p<0,001).

Artículo VI

La dieta tradicional en el norte de Portugal y Galicia, situada en el noroeste de España, se ha representado con un índice de "Dieta Atlántica del Sur-Europeo". Cada punto de incremento en la puntuación final del índice se asoció con un riesgo reducido del 10% de IAM (OR 0,90, IC95% 0,85-0,96). En comparación con los individuos en el cuartil inferior del índice (menor adherencia a lo patrón), aquellos en el cuartil superior tenían una probabilidad 33% menor de IAM (≤3 vs. ≥6 puntos: OR 0,67, IC95% 0,51-0,88, p tendencia=0,003).

Al invertir el sistema de puntuación de los componentes que, individualmente, aumentan el riesgo de IAM (carnes rojas y productos de cerdo y patatas) se observó
una asociación aún más fuerte entre el índice de dieta atlántica y el IAM (cuartil superior vs. inferior: OR 0,40, IC95% 0,30-0,52, p<0,001).

**Principales Conclusiones**

Diferentes patrones de distribución de grasa corporal han tenido efectos distintos en los niveles de PCR-as: una distribución central de grasa corporal estaba directamente asociada con la PCR-as en los hombres, mientras una mayor proporción de grasa subcutánea periférica en los brazos parece estar inversamente asociada con la PCR-as, pero solo en las mujeres.

Los mismos patrones de grasa corporal se asociaron con el riesgo coronario, pero mientras el índice de grasa subcutánea periférica predijo un menor riesgo de IAM en las mujeres, lo mismo predijo un mayor riesgo de IAM en los hombres.

El consumo de los factores dietéticos individuales como las frutas y verduras, vitaminas antioxidantes y fibra se asoció inversamente con los niveles de PCR-as en los hombres, mientras que en las mujeres no se observó asociación. Las asociaciones fueron de menor magnitud en los sujetos con sobrepeso/obesidad.

Se encontró una relación en J entre el consumo de alcohol y los niveles de PCR-as en las mujeres, pero en los hombres esta relación fue positiva lineal, lo que probablemente refleja los patrones de consumo de nuestro país. Los niveles de ácido úrico se incrementaron al aumentar la ingesta de alcohol, en cada sexo. El IMC ha modificado estas asociaciones, de modo que sólo se mantuvieron en las mujeres de peso normal y en los hombres con sobrepeso, en el caso de la PCR-as, y en los individuos de peso normal, en el caso de lo ácido úrico.

Estos factores dietéticos parecen agregarse en la población Portuguesa. Los individuos de ambos sexos con un mayor consumo de carnes rojas y alcohol, y un menor consumo de vegetales y productos lácteos, tenían un mayor riesgo de IAM y un peor perfil de biomarcadores cardiovasculares que aquellos con un patrón dietético "saludable".

Una mayor adherencia a la Dieta Atlántica del Sur-Europeo, una dieta culturalmente arraigada en el norte de Portugal y Galicia, se asoció con un menor riesgo de eventos de IAM no fatales.
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>AMI</td>
<td>acute myocardial infarction</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>CHD</td>
<td>coronary heart disease</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
<td>cardiovascular diseases</td>
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<td>DALYs</td>
<td>disability adjusted life years lost</td>
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<td>high-sensitivity C-reactive protein</td>
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<td>IL-6</td>
<td>interleukin-6</td>
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<td>LDL</td>
<td>low-density lipoproteins</td>
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<td>SEAD</td>
<td>Southern-European Atlantic Diet</td>
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<td>U.S.</td>
<td>United States</td>
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<tr>
<td>WC</td>
<td>waist circumference</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>WHR</td>
<td>waist-to-hip ratio</td>
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Introduction
Several epidemiological changes have occurred in the last few decades; nevertheless cardiovascular diseases (CVD) still remain as the main cause of death worldwide. Globally, CVD including both coronary heart disease (CHD) (often named as ischemic heart disease) and cerebrovascular disease (often named as stroke) account for nearly 30% of deaths all over the world (1) and almost half of the total deaths among 48 European countries (54% in women and 43% in men) (2).

Within Europe, CHD remains by itself the single most common cause of death; over one in five women (22%) and one in five men (21%) die from the disease (2). Nevertheless, in Northern, Southern and Western European countries CHD mortality rates are falling steadily (2-3). In Western Europe, in particular, between 1970 and 2000 the CHD mortality declined to approximately 50% on average, corresponding to a decline of 1.8% per year (3). Therefore, CHD mortality in these countries continues to be lower than in Central and Eastern European countries; a north–east to south–west gradient is clear (2-4). Figure 1 illustrates the age-standardized death rates from CHD in women (A) and in men (B) aged 0 to 64 years (2), across Europe.

In Portugal, according to the most updated national data (5), the age-standardized death rates (/100 000 in-habitants) in the population aged less than 65 years was 4.5 in women and 11.6 in men in 2006 (global estimate of 11.5). These estimates are in agreement with the previous data shown, placing Portugal within the countries with the lowest CHD mortality rates. Following the same trend of other Western European countries, also Portugal has been facing a decline of CHD death rates (6).

Although the total CVD mortality rates in Portugal were reported to be higher than the European average [including 27 countries with very low child and adult mortality, designated Eur-A by the World Health Organization (WHO)], this was clearly due to the cerebrovascular disease contribution, since the CHD standardized death rates in Portugal were lower than the European average.

Despite these low CHD mortality rates, CVD remains as the main cause of death in Portugal, being responsible for 36% of all deaths (8.7% from CHD and 16.8% from cerebrovascular disease) (6), thus highlighting the public health interest in continuing to reduce the number of deaths and the burden caused by these diseases.
Figure I. Age-standardized death rates from CHD in women (A) and in men (B) aged 0 to 64 years, in the latest available year [source: Allender S, et al. European Heart Network; 2008 (2)].

Trying to explain the decline in CHD mortality across several countries, the WHO MONICA Project (Monitoring trends and determinants in Cardiovascular Disease), measuring trends in CHD mortality between the early 1980s and 1990s, showed that around two-thirds of the decline in CHD mortality during this period was due to a decline in CHD incidence rates and the remaining one-third was due to improvements in survival because of better treatments (7). Similarly, in the United Kingdom (8) and in the United States (U.S.) (9), a high percentage of the decline in CHD mortality (58% for England and 44% for the U.S.) over a 20-year period (1980-2000) was attributable to reductions in major risk factors (smoking, blood pressure and blood cholesterol), whereas treatment of individuals including secondary prevention explained the remaining 42% (for England) and 46% (for the U.S.) of the mortality decline.

Europe-wide updated information of CHD morbidity data is very scarce. The existence of different measures of morbidity could have hampered the comparability of data across geographical sets and temporal series. The WHO MONICA project (7) has shown that the incidence of coronary events was higher in Northern, Central and Eastern European countries than in Southern and Western Europe. These results also suggest that the incidence of coronary events is falling fast in most of the MONICA project populations from Northern and Western Europe, but is not falling as fast in the populations from Southern, Central and Eastern Europe and in some populations is to increase. The geographical pattern of CHD incidence seems to be similar to the death rates distribution.

The disability adjusted life years lost (DALYs) - an aggregate of years of life lost due to premature death and years of healthy life lost due to disability, could be seen as an
indicator of the burden of the disease. According to data from an update of the WHO Burden of Disease project (10), in Europe, 11% of all DALYs are due to CHD (17% if considering all CVD), which after neuropsychiatric disorders represents the largest single cause of DALYs. The highest DALYs lost due to CHD were observed in Eastern Europe.

Moreover, the economic costs of CHD are staggering. In 2006, the overall cost to the European Union economy (15 member states) was over €49 billion a year (one quarter of the total cost of CVD): around 48% due to direct health care costs, 34% due to productivity losses and 18% due to informal care of people with CHD (2).

Due to the high burden of CHD, primary prevention is of top importance, even for countries where current coronary mortality rates are reported to be low. Therefore, the study of the physiological mechanisms leading to CHD and its potential leading factors has long been a focus of epidemiological research.

2 | Pathophysiological mechanisms leading to Coronary Heart Disease – the emerging of an inflammatory hypothesis

Research on the possible physiological pathways leading to CHD has been ongoing for approximately a century. Initially, many have regarded lipids as central mediators of atherosclerosis; in that way, the atherosclerotic process was considered to consist mainly of the accumulation of lipids within the artery wall. This belief supported the "oxidation hypothesis" to explain the development of CHD (11-12).

According to the oxidation hypothesis, low-density lipoproteins (LDL) are retained in the intima, and undergo oxidative modification (11-12). The susceptibility of LDL to oxidation varies according to different characteristics, such as vitamin E, the major antioxidant present in lipoproteins; increasing the vitamin E content of lipoproteins in vitro or in vivo (by dietary supplementation) increases the lag phase for initiation of LDL oxidation (13). Size and density of LDL molecules also influence the extent of oxidation; small dense LDL are more susceptible to oxidation than large floating LDL (14). There is evidence of a number of pathways, both enzymatic and non-enzymatic, involved in the oxidation of LDL (11).
Over the last decades, a plausible model linking lipids and inflammation to atherogenesis has emerged. There is now increasing evidence from basic, pathological and clinical research that atherosclerosis is by itself an inflammatory disease (15-18).

The atherosclerotic process initiates when LDL-cholesterol is accumulated in the intima, undergoes oxidative modification and activates the endothelium. Therefore, proinflammatory lipids released from LDL stimulate endothelial cells to express adhesion molecules. Circulating blood monocytes adhere to this endothelium cells and differentiate into macrophages. These macrophages are activated by pathogen associated molecular patterns and, as a result of activation, they release a host of proinflammatory mediators, including proinflammatory cytokines, vasoactive molecules, several proteases, and reactive oxygen and nitrogen species (15-18). An intensified inflammatory activation by macrophages may lead to local proteolysis, plaque rupture, and thrombus formation, which cause ischemia and infarction. The accelerated inactivation of nitric oxide by reactive oxygen species – actively involved in oxidative stress, may be on the basis of endothelium dysfunction (19).

Adipose tissue can also increase the circulating levels of proinflammatory cytokines by synthesizing them in loco (20-21). In this way, obesity by itself promotes inflammation and potentiates atherogenesis independently of the above described mechanisms.

Inflammation can be objectively measured by an array of serum or plasma markers, such as proinflammatory cytokines [e.g. interleukin-1, tumor necrosis factor-alpha (TNF-α)], adhesion molecules (e.g. intercellular adhesion molecule-1, selectins), inflammatory stimuli with hepatic effects [e.g. interleukin-6 (IL-6)] or the products of the hepatic stimulation, such as serum amyloid A and C-reactive protein (CRP).

CRP which nowadays could be measured with high-sensitivity assays and appears to be a stable analyte, has been subject to numerous studies. In general, they have pointed out that slightly elevated high-sensitivity CRP (hs-CRP) levels, which would be in the normal range of conventional assays, are a marker for an increased CHD risk (22-26). Large scale prospective studies including the Physicians’ Health Study and the Women’s Health Study have found that increased hs-CRP levels at baseline in apparently healthy individuals are associated with a 2-fold increase in the risk of a future myocardial infarction. Elevated hs-CRP levels have been also associated with the severity of coronary plaques and carotid intima-media thickness (29) and with the components of the metabolic syndrome (30).
Significant positive associations between CRP concentration and age, smoking status, blood pressure, body mass index (BMI) and other anthropometric factors, physical activity (31) and hormone replacement therapy (32-33) have been described. The ability of hs-CRP to add to the predictive capacity of other established risk factors has been examined in several studies. After stratification or multivariable statistical adjustment, hs-CRP remains independently associated with incident coronary events (34-35).

Besides its effects as an inflammatory marker (a risk marker holds a statistical association with the disease, but does not need to be causally linked with it), some discussion has been put forward on the possibility of CRP be also a cardiovascular risk factor. In other words, some authors suggest that CRP, like other mediators, could be a step in the causal pathway leading to atherosclerosis or the disease itself (23). According to the current knowledge, the role of inflammatory markers in the causal pathway for CHD is still unclear; but this fact does not depreciate them usefulness as important cardiovascular risk markers.

In general, the precision and reproducibility of inflammatory marker assays have been acceptable; for example, the coefficient of variation of hs-CRP assays is generally <10% from the 0.3 to 10 mg/l range (36). There seems to be little seasonal or diurnal variation of hs-CRP (37), but considerable intra-individual variability (36, 38). An hs-CRP assay to reduce the intra-individual variability should be performed in a metabolically stable person without obvious inflammatory or infectious conditions. If a level of hs-CRP >10 mg/l is identified, it indicates an obvious source of infection or inflammation, which could obscure any prediction of coronary risk that might be attributed to the elevated level. Thus, under this context, results of hs-CRP >10 mg/l should be discard.

Cut-points of hs-CRP levels denoting low risk (<1.0 mg/l), average risk (1.0 to 3.0 mg/l) and high risk (>3.0 mg/l) are currently proposed, corresponding to approximate tertiles of hs-CRP sample distributions from more than 15 populations involving more than 40 000 persons gathered for the purpose of the Workshop on Markers of Inflammation and Cardiovascular Disease, promoted by the Centers for Disease Control and Prevention and the American Heart Association (23).

From this Workshop, some clinical and public health recommendations were taken (39): a) the screening of entire populations for elevated hs-CRP for use in primary prevention is not yet justified (Class III, Level of Evidence: C); b) the addition of
inflammatory markers to global risk prediction in patients at intermediate risk could result in a significant increase in the "posttest probability" of CVD, which would be useful clinically; c) the non-specificity of elevated CRP may be particularly problematic when applying the population level results to individual patients, since it would imply multiple determinations and potentially expensive searches for non-cardiovascular causes of elevated hs-CRP.

3 | Risk factors to Coronary Heart Disease – the role of Lifestyles

Coronary heart disease is the result of a complex interplay between genetic features and social and behavioral exposures (40-41), but differences across populations appear to be more related with lifestyles than with genetic make-up (42-43). The modifiable pattern and the considerable impact of lifestyles to the development of CHD (44) underline their importance in primary prevention efforts.

Within the INTERHEART study (44), a large multicenter population-based case-control study, lifestyles were found to be responsible for more than 50% of acute myocardial infarction (AMI) events among men and women of all ages. The INTERHEART study also highlighted that in Western European countries central obesity, measured by an increased WHR, was the risk factor with the highest impact (population attributable fraction of 63.4%), even higher than smoking (population attributable fraction of 29.3%) or abnormal lipid profile (population attributable fraction of 44.6%).

For the Portuguese population, a previous work from our research group (45) also found that high WHR was the risk factor with the highest impact on AMI (population attributable fraction of 81.2% in younger men and 88.7% in men aged more than 45 years). Additionally, smoking (63.5%) and excessive alcohol consumption (37.7%) in younger men and physical inactivity in the older ones (44.8%) had a high impact, and thus reach an extreme importance on a public health level. Lifestyles explained more than 70% of the male myocardial infarction cases of our population.

In general, case-control studies in different population sets described highest CHD population attributable fractions for smoking, hypercholesterolemia, hypertension and a
central pattern of obesity (44, 46-48). Also, in a population-based cohort study of the European Prospective Investigation into Cancer and Nutrition - Potsdam (49), 84.3% of myocardial infarctions were attributable to the presence of smoking, hypertension, diabetes, sporting inactivity and abdominal obesity, in individuals of both sexes and all ages.

For more than 50 years, prospective cohort studies on CHD risk have been performed, and major risk factors for CHD have been established, based on their high prevalence in populations, their strong impact on coronary risk and their preventability and reversibility mainly achieved by lifestyles’ improvements (50). Six major coronary risk factors have been consistently described: cigarette smoking, high blood pressure, high serum cholesterol, diabetes, overweight/ obesity and an adverse diet. The relation between these risk factors and CHD seem to be independent, strong, continuous and graded. These studies have also suggested that the lower the risk factor profile, the lower the risk for CHD and all-cause mortality (51).

Of the six established major risk factors, diet and physical activity are the only ones that, at the same time, could have a direct impact on coronary risk or could indirectly modulate the remaining risk factors, except smoking. As stated by Stamler, a population-wide adverse eating pattern is the key etiologically for five of these six major risk factors (50).

The effect of a healthy diet and lifestyle on CHD was quantified in the Nurses’ Health Study, in which 84,129 women aged 30 to 55 years were enrolled and followed up for 14 years (52). In this study, a healthy lifestyle was defined as no smoking, consuming at least half a drink of alcoholic beverage per day, practice moderate to vigorous physical activity for more than 30 minutes per day and a BMI under 25 kg/m². Individuals were considered as having a healthy diet when scored in the highest 40% of the cohort for the consumption of cereal fiber, marine n-3 polyunsaturated fatty acids and folate, and had a high ratio of polyunsaturated to saturated fatty acids and low in trans-fatty acids and glycemic load. These lifestyle and dietary factors were related to 14-year CHD incidence; in particular, 82% of coronary events in the study cohort were attributed to lack of adherence to this low-risk pattern.

Evidence from two large prospective cohort studies, the Multiple Risk Factor Intervention Trial and the Chicago Heart Association Detection Project in Industry showed that individuals with low levels of biological risk factors, such as serum cholesterol and blood pressure, and individuals who adhere to a healthy lifestyle and
diet are at very low risk for CHD (53). However, evidence from clinical trials is needed to confirm the causality role of lifestyles, and particularly dietary factors, in the etiology of CHD.

Concurrently, the five leading global risks for worldwide mortality are high blood pressure, tobacco use, high blood glucose, physical inactivity, and overweight and obesity, which seem to be responsible for more than one third of the world’s deaths. For cardiovascular deaths in particular, eight risk factors (alcohol use, tobacco use, high blood pressure, high BMI, high cholesterol, high blood glucose, low fruit and vegetable intake and physical inactivity) account for 61% of them. Combined, these same risk factors account for over three quarters of CHD mortality (1).

According to the up-to-date WHO Report (1), health risks are in transition: populations are ageing as a result of success against infectious diseases and due to a decrease of the fertility levels; at the same time, patterns of physical activity and diet, alcohol and tobacco consumption are changing. Understanding the role of these risk factors in CHD is important for developing clear and effective preventive strategies.

Although in recent years, cardiovascular epidemiology research has focused on new areas of interest, such as genomics and imaging, the bulk is still on putative risk factors, with a current emphasis on inflammatory factors (54). Under this context, obesity and diet will be approached in the two subsequent chapters.

4 | Obesity and body fat distribution as coronary risk factors and supply of proinflammatory cytokines

Compelling evidence supports that obesity is related to a higher risk of CHD, type 2 diabetes and certain cancers, and to a shorter life expectancy (55-58), which attributes to this entity a considerable public health interest. Furthermore, obesity is considered one of the leading global risks for worldwide CHD mortality (1).

The role of obesity on CHD risk is not completely understood. Different magnitude and associations’ shape between obesity and CHD have been described; some studies have found linear associations while others have reported J- or U-shaped associations
or even no significant effects (59-61), which could be related to the use of different measures of obesity.

Several studies have suggested that the amount and type of body fat distribution seem to be more important to cardiovascular risk than overall obesity per se, often assessed by BMI. Under this context, waist circumference (WC) and WHR were found to be better predictors of cardiovascular morbidity and mortality than total body weight and BMI (60, 62-65). This could be because a higher BMI may reflect increased fatness, but also higher musculoskeletal mass; moreover BMI is not a good measure of visceral fat, considered as the key determinant of metabolic abnormalities (66-67). In fact, epidemiological studies have suggested that abdominal fat distribution is a significant predictor for CHD, independently of BMI (60, 62-63, 65).

According to the model proposed by Déprès and colleagues (68), individuals who have elevated abdominal fat are really divided into two subgroups: a visceral fat group and a subcutaneous trunk fat group, both of which can independently increase the CHD risk. Research has been suggested that the adipose tissue accumulated in the visceral region (intra-abdominal fat) seems to be associated to more metabolic complications or a more deleterious risk profile than that accumulated subcutaneously (66, 68-72).

WC, reflecting both visceral and subcutaneous fat, has been extensively used to identify individuals at increased risk for obesity-associated risk factors, due to its well-documented positive association with CVD (58, 67, 73-75). At the same time, WHR is attracting growing interest, due to the favorable role recently attributed to peripheral fat (fat located in upper and lower limbs) in the modulation of cardiovascular risk (65, 76-77). Therefore, in a recent review (77) the need for capturing the separate effects of abdominal and peripheral adiposity was highlighted, and the authors suggested that WHR is a simple and inexpensive measure which could improve the assessment of the CHD risk.

Data on the specific role of peripheral fat mass on coronary risk are scant. A few studies have suggested that fat accumulated in peripheral depots, such as arms and legs, has less adverse metabolic effects on cardiovascular risk than other types of fat store (71, 78-83). Tankó and colleagues showed that, in postmenopausal women, peripheral fat measured by dual-energy x-ray absorptiometry had a favorable long-term effect on systolic blood pressure, serum triglycerides and white blood cells, and was inversely associated with aortic calcification, a direct measure of atherosclerosis (78-
79). In another study, measuring peripheral fat with the same method, the authors concluded that some degree of protection is conferred by this type of fat accumulation, which was inversely (i.e. favorably) associated with stiffness of the brachial and the carotid-femoral segment (82).

Although the mechanisms underlying the development of obesity and its comorbidities are not well established, it has been recognized that these clusters of disorders are associated with chronic mild inflammation, in which the metabolism of fat tissue plays an important role (20-21, 84-87). Adipose tissue secretes a multiplicity of factors, commonly named adipokines with different protein structures and functions, such as cytokines or related-proteins [leptin, TNF-α, IL-6], chemoattractant proteins (monocyte chemotactic protein-1), proteins of the complement system (adipsin), proteins involved in the regulation of blood pressure, vascular haemostasis or angiogenesis (angiotensinogen, plasminogen activator inhibitor-1, vascular endothelial growth factor) and molecules involved in the glucose and lipid metabolisms (adiponectin, resistin, visfatin) (87).

The role of adipose tissue in the development of local or systemic inflammation is demanded by its heterogeneity at the cellular level. The cell content of white adipose tissue – the one considered as a major endocrine organ, with an important role in the regulation of energy intake and metabolism - is extremely heterogeneous: mature adipocytes represent no more than half of the total cell content; the remaining cell components are pre-adipocytes, fibroblast, endothelial cells and macrophages (88). During adipose tissue growth, there is an increase in the size and number of mature adipocytes differentiated from progenitor cells (e.g. preadipocytes). During this fat mass expansion, macrophages from peripheral blood seem to infiltrate within adipose tissue (88-89). The molecular mechanisms responsible for it are not yet completely understood; it seems that some adipokynes such as monocyte chemotactic protein-1 and leptin can favour the diapedesis of macrophages from circulation to adipose tissue (90). Infiltrated macrophages in the adipose tissue seem to be responsible for almost the total amount of TNF-α and a significant part of IL-6 produced (88).

There is evidence that adipocytes have distinct intrinsic characteristics (e.g., fatty acid-binding proteins and enzymes of fat metabolism), which contribute to the heterogeneity in free fatty acids handling by the different fat depots (91). It has been suggested that visceral adipocytes have higher lipolytic activity, which leads to an overexposure of the liver to free fatty acids, resulting in insulin resistance and
hyperinsulinaemia. Moreover, omental adipose tissue, which is a subfraction of visceral adipose tissue, secretes more IL-6 than subcutaneous adipose tissue (92).

On the other hand, peripheral adipose tissue seems to have a high lipoprotein lipase activity and a low fatty acid turnover; it takes up, more frequently, free fatty acids from circulation and stores them, protecting the liver from high free fatty acids exposure (93). Peripheral adipose tissue seems also to secrete higher quantities of adiponectin (94). Adiponectin may affect insulin sensitivity by acting on muscle fatty acid oxidation and hormone-sensitivity lipase. Also, by stimulating nitric oxide production and the reduction in the expression of adhesion molecules in endothelial cells, adiponectin could be responsible for some anti-hypertensive and anti-atherogenic effects (95).

A higher release of free fatty acids and glycerol from adipocytes has been described in obese than in lean individuals, probably promoting insulin resistance and type 2 diabetes through the blocking of the insulin signal transduction (96). Moreover, several procoagulant proteins such as plasminogen activator inhibitor type 1 and tissue factor show higher expression in adipose tissue of obese in comparison to lean individuals (97). This over-expression could explain, at some extent, the high atherogenic risk associated with obesity.

An improvement in the circulating proinflammatory profile appears to be attained with weight loss (98). Although knowledge on the interplay of inflammation with obesity is not fully understood, the available evidence suggests that dietary interventions will become a major element of future approaches to prevent and treat obesity, its related metabolic complications, the metabolic syndrome and, at last, CVD (87).

Similarly to what happens with CHD risk, measures of central obesity seem to be stronger and more consistent predictors of inflammation than general obesity (99-101). Panagiotakos, et al. (99) have found that subjects with central fat, compared to participants with normal body fat distribution, exhibited 53% significantly higher hs-CRP levels, 30% higher TNF-α levels, 17% higher white blood cell count and 42% higher IL-6 levels. The authors also concluded that the models that included WC or WHR as independent variables had higher explanatory ability than the models that included BMI. Also, in a large triethnic population of the Insulin Resistance Atherosclerosis Study (100), WC significantly explained 14.5% of the variability of circulating hs-CRP levels and BMI only 0.4%.

Although the consistence of the results described, it has been claimed that is relatively difficult to differentiate between the effect of abdominal obesity and total body...
fat due to the high correlation between measures of obesity, which could constitute a limitation of this type of studies.

Overall, there is an increasing understanding of the metabolic effects of abdominal fat, both visceral and subcutaneous (99-102), but much less information is available on the relative contribution of peripheral fat to inflammation (78, 99). As previously described, a study in postmenopausal women showed that peripheral fat was directly associated with white blood cells (78). Panagiotakos and colleagues (99) related hip circumference with increased levels of CRP, IL-6, TNF-α and white blood cells.

Gender-related differences seem to be a major gap under this field. Some studies have described stronger associations between measures of obesity and inflammatory markers in women than in men (103-104). The observed sex differences might be related to different body fat distributions in women and men (103), and to regional differences in secretion of cytokines and adiponectin by the different fat depots (92, 94, 105). Furthermore, sex hormones and the use of oral contraceptives and hormone replacement therapy could influence the inflammatory marker levels in women (32-33, 106).

Obesity is also an important avenue by which diet can influence the CHD risk. This issue brings into discussion what is the role of obesity in the relation between diet and CHD or its role in the relation between diet and inflammation (as a possible pathway to CHD). Whereas some studies report obesity as a confounder of these associations (107-109), it seems likely that obesity could act as a mediator between diet and CHD, and for that reason not having characteristics of confounder (as understood in its epidemiological definition). In fact, in the Danish WHO MONICA survey (110), BMI modified the effect of the Prudent and the Western dietary patterns on CHD risk, suggesting an inverse association between both patterns and CHD in persons with low BMI, while the risk of CHD seemed to be positively related to the Prudent and the Western patterns in those with high BMI. Nevertheless, further investigation is needed on the possible modifying effects of measures of obesity on the relation between diet and CHD.
5 | Diet and Coronary Heart Disease

5.1 | Different theories across time

In the first part of the twenty century, a diet-heart hypothesis (also named as the lipid hypothesis) was proposed, based on the single principle that there was a direct relation between cholesterol in the diet (i.e. eggs), cholesterol in the blood, cholesterol in the atherosclerotic plaque, and its clinical complications, such as myocardial infarction (111). This relation has probably risen from the work of Anitschkow on the cholesterol-fed rabbit model (112), years latter supported by Ancel Keys in the Seven Countries Study (113), in which was shown that dietary fat and cholesterol were correlated with the increase of CHD occurred in Western and industrialized countries at that time.

In the second part of the twenty century, it became clear that dietary cholesterol played a minor role in regulating serum cholesterol levels, and that the cholesterol-rich LDL fraction, and not total cholesterol, was most strongly related to the development of atherosclerosis and its consequences (114). Different hypotheses, compatible with each other, have been proposed to explain the launch of the atherosclerotic process (e.g. response-to-injury, response-to-retention) (115), but it was the oxidation hypothesis that became more documented (11-12). The principle of this hypothesis was based upon the oxidation of native LDL molecules, that once oxidized are preferentially taken up in the arterial wall.

The oxidation hypothesis supports an important role of diet and other lifestyles in atherogenesis, since LDL can be oxidized by smoking, for example, and oxidation can be prevented by dietary antioxidants, such as vitamins and polyphenols. Therefore, it was believed that complex interactions between diet, lifestyles and lipoprotein metabolism were the major determinants of the development of atherosclerosis and its complications. In fact, until recently, major epidemiological investigations of diet and CHD have relied on the classic diet-heart hypothesis.

Nevertheless, the diet-heart hypothesis seems to be over simplistic, because the effects of diet on CHD seem to be mediated through multiple biological pathways, others than serum total cholesterol or LDL-cholesterol, including blood pressure, insulin sensitivity, oxidative stress, endothelial dysfunction and subclinical inflammation (116).
An inflammatory hypothesis for the development of CHD is currently proposed (15-18), as previously described. As so, the understanding if diet could influence CHD, through an inflammatory pathway, is still under research.

5.2 Methodological approaches to study diet

Diet has been traditionally studied through the effects of single nutrients and/or foods on several health outcomes. Although this type of analysis has been quite valuable and has conducted to several important nutritional findings, it has several conceptual and methodological limitations (117-119). The first one is related to the simple fact that people do not eat isolated nutrients or foods, but rather meals with complex combinations of nutrients that are likely to be interactive or synergistic. Therefore, the high level of inter-correlation among nutrients makes it difficult to examine their separate effects. Moreover, the effect of a single nutrient may be too small to be detect; statistically significant associations may simply be found by chance when a large number of nutrients or foods are being study together; and finally the single nutrient analysis may potentially be confounded by the effect of an overall eating behavior (117-119). For these reasons, the single nutrient/food approach may be considered as reductionist.

Diet is a complex exposure variable, which calls for multiple approaches to look at its relation with the disease risk. Dietary patterns fit into this concept; they account for cumulative and interactive effects of nutrients and foods, and may provide a comprehensive approach to disease prevention or treatment, particularly for CHD and stroke, for which multiple dietary components are established. Moreover, studying dietary patterns could have more important public health implications because the overall patterns of dietary intake might be easily understandable for the general public and easily translated into diets (117-121).

Two general approaches have been used to define dietary patterns in observational studies: a hypothesis-oriented approach and an exploratory approach. Hypothesis-oriented dietary patterns (often named as a priori patterns, indexes or scores) are defined as a composite score from various food items or nutrients (122-124). The patterns’ definition is based on available scientific evidence for specific diseases, which may be drawn from studies on single dietary components or from studies on overall dietary habits. It is also common to use the patterns’ scores as summary measures of
the degree to which an individual's diet is in agreement with specific dietary recommendations.

Such summary variables involve some subjective decisions, namely the identification of foods to be included, as well as the scoring method to be applied. Despite these constraints, a priori dietary patterns seem to represent simple methods of summarizing total diet, with easily reproducibility, comparability, interpretation and translation to the general public (117-118, 121), which could be highly useful for public health messages.

In table 1 (for Europe) and table 2 (for the U.S.) are described different diet indexes/scores used to investigate the adherence to specific dietary patterns (125-134) or to be associated with several health outcomes (109, 135-146) or mortality risk (147-155).

The “Mediterranean Diet Score” is the most extensively used index and was originally created to measure the adherence gradient to the Greek Mediterranean dietary pattern (147). Many variants have been created for the evaluation of multiple diet–health relations (141, 144, 148, 154). In general, a final score of 4 or more (from a range of 0 to 8) was associated with a satisfactory adherence to the pattern and better health implications (147).

Other indexes have evaluated the adherence to specific dietary guidelines, such as the WHO guidelines for the prevention of chronic diseases (152), using as components both foods and nutrients. Also, in the U.S. some indexes have been using dietary guidelines, such as the Dietary Recommendations from the National Academy of Sciences (127, 133) or the Food Guide Pyramid (127) to obtain a measure of the degree of adherence to specific healthy eating behaviors. In the U.S. indexes, variety and moderation scores have been included in the indexes' definition, beyond accomplishment to food and nutrient goals (126-127, 129-130, 134).
<table>
<thead>
<tr>
<th>Designation</th>
<th>First author (year) (reference)</th>
<th>Components</th>
<th>Computation of scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediterranean Diet Score (MDS)</td>
<td>Tchopoulou (1995, 2003, 2004, 2005a,b) (147, 149-150, 155) Based on the MDS: Knoops (2004) (148), Lasheras (2000) (154), Boetti (2003) (144)</td>
<td>8 components: ratio of monounsaturated to saturated fatty acids, legumes, cereals, fruit, vegetables, meat and meat products, dairy and alcohol (minor variations are found across studies).</td>
<td>For the “healthy” components, a score of 1 is assigned to an intake &lt;sex-specific median; 0 to the others. For meat and meat products and dairy, a score of 1 is given to an intake &lt;sex-specific median intake; 0 to the others. For alcohol, a value of 1 is assigned to men consuming between 10 and 30 g/day and to women consuming between 5 and 25 g/day.</td>
</tr>
<tr>
<td>Healthy Diet Indicator (HDI)</td>
<td>Huibregts (1997) (152)</td>
<td>9 components based on the dietary guidelines for the prevention of chronic diseases, defined by the WHO (1997): &lt;10% saturated fatty acids; 5-7% polyunsaturated fatty acids; 10-15% proteins; 50-70% complex carbohydrates; 27-40 g/day fiber; &gt;400 g/day fruit and vegetables; &gt;30 g/day legumes, nuts and seeds; &lt;10% mono- and disaccharides; &lt;300 mg/day cholesterol.</td>
<td>A score of 1 is assigned to an intake within the recommended intervals of the guidelines; 0 to an intake outside of that interval or the cut-off point established.</td>
</tr>
<tr>
<td>Mediterranean Adequacy Index (MAI)</td>
<td>Alberti-Fidanza (1999, 2004) (125, 132)</td>
<td>13 food groups divided in Mediterranean (bread, cereals, legumes, potatoes, vegetables, fruit, fish, vegetable oils and red wine) and non-Mediterranean food groups (milk, cheese, meat, eggs, animal fats and margarines, cakes/pies/cookies and sugar).</td>
<td>MAI is computed dividing the sum of the Mediterranean food groups by the sum of the non-Mediterranean food groups. Intakes are adjusted to daily intakes of 2500 kcal for men and 2000 kcal for women or expressed as percentage of total daily energy intake.</td>
</tr>
<tr>
<td>Healthy Food Index (HFI)</td>
<td>Ostler (2001) (151)</td>
<td>4 components based in four characteristics of the diet: 1) not consuming butter, lard or margarine daily; 2) consuming either raw or boiled vegetables at least once daily; 3) consuming either coarse white or coarse rye bread at least once daily; 4) consuming fruit at least once daily.</td>
<td>One point is given for each of the four characteristics of the diet which is accomplished.</td>
</tr>
<tr>
<td>Mediterranean Diet Score (ATTICA study)</td>
<td>Chrysohoou and Panagiotakos (2004, 2006, 2007) (135, 139-140)</td>
<td>Components divided by daily consumption of non-refined cereals and products, fruit (4-6 servings/day), vegetables (2-3 servings/day), olive oil, and non-fat or low-fat dairy products (1-2 servings/day); weekly consumption of fish, poultry, potatoes, olives, pulses and nuts (4-6 servings/week); eggs and sweets (1-3 servings/week); monthly consumption of red meat and meat products (4-5 servings/month); moderate consumption of wine (1-2 wine glasses/day); moderate consumption of fat; high monounsaturated to saturated fat ratio.</td>
<td>For the items consumed daily or &gt;4 servings/week, a score of 0 is assigned for no consumption, a score of 1 for 1-4 times/month (t/m), 2 for 5-8 t/m, 3 for 9-12 t/m, 4 for 13-18 t/m, and 5 for &gt;18 t/m. For the consumption of foods presumed to be away from this diet (e.g. meat and meat products), opposite scores are assigned. For alcohol, a score of 5 is assigned for &lt;3 wine glasses/day, 0 for consumption of &gt;7 wine glasses/day, and scores of 1 to 4 for consumptions of 3, 4, 5, 6, and 7 wine glasses/day. The total score ranges from 0 to 55.</td>
</tr>
<tr>
<td>Mediterranean dietary pattern (Case-control study in Navarra, Spain)</td>
<td>Martinez-González (2002) (138)</td>
<td>6 food items were considered protective: 1) olive oil, 2) fiber, 3) fruit, 4) vegetables, 5) fish and 6) alcohol. More two components were considered: 7) meat/meat products and 6) some items with high glycemic load (white bread, pasta and rice).</td>
<td>For each of the first 6 components, a score of 1 to 5 is assigned according to the distribution into increasing quintiles. For meat/meat products and items with high glycemic load, the score is inversely ranked. The final score ranges between 5 and 40.</td>
</tr>
<tr>
<td>Designation</td>
<td>First author (year) (reference)</td>
<td>Components</td>
<td>Computation of scores</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mediterranean dietary pattern (Spanish EPIC cohort)</td>
<td>González (2002) (128)</td>
<td>9 food groups: vegetables and garden products, fruit, pulses, cereals, red meat, fish, olive oil, milk and milk products, and wine.</td>
<td>For fruit, vegetables, fish, pulses, cereals and olive oil, 4 to 1 points are assigned to the following intakes: &gt; 75th percentile (75P), 50P-75P; 25P-50P; &lt;25P. For red meat, milk and milk products, reverse scores are assigned. For wine (men/women), 1 point is assigned to consumptions &gt; 4020 g of ethanol; 4 points to consumptions between 1 and 100/200 ml of wine; 3 points to consumptions &gt;100/200 ml wine, but &lt; 20/40 g ethanol; 0 points to nil consumption of wine or alcohol. The final score ranges between 0 points (lower adherence) and 36 points.</td>
</tr>
<tr>
<td>Mediterranean diet score (Cross-sectional survey from Girona, Spain)</td>
<td>Schröder (2004) (143)</td>
<td>9 components: cereals, vegetables, fruit, legumes, nuts, fish, high-fat dairy products, meat and red wine.</td>
<td>For cereals, fruit, vegetables, legumes, fish and nuts, a score of 1 to 3 is assigned according to the distribution into increasing tertiles. For meat and high-fat dairy products, a score of 3 to 1 is assigned according to the distribution into increasing tertiles. Red wine consumption is computed as 1 if 0 or &gt;20 g of alcohol/day and as 3 if &gt;0 to 20 g of alcohol/day. The final score ranges between 9 and 27.</td>
</tr>
<tr>
<td>Mediterranean dietary pattern (Prospective cohort study in Navarra, Spain)</td>
<td>Sánchez-Villegas (2002) (145)</td>
<td>9 components: legumes, cereals (including bread and potatoes), fruit, vegetables, meat, milk (including dairy products), alcohol, ratio of monounsaturated to saturated fatty acids (MUFA/SFA) and trans-fatty acids.</td>
<td>Energy-adjusted daily consumptions were standardized as a z-value (observed mean/standard deviation). For scoring ‘moderate’ red wine consumption, 20 g/day for men and 10 g/day for women are assigned with the highest values and progressive lower values as the consumption is lower or higher than these values. The total score adds and subtracts the standardized components (weighted favorably for legumes, cereals, fruit and vegetables, moderate alcohol consumption and the MUFA/SFA ratio; and unfavorably for the remaining). The score is converted to relative percentage of adherence.</td>
</tr>
<tr>
<td>Mediterranean dietary pattern (Spanish SUN cohort study)</td>
<td>Sánchez-Villegas (2008) (142)</td>
<td>10 components: 8 positively weighted (cereals, vegetables, fruit, legumes, fish, nuts, olive oil and moderate red wine consumption) and 2 negatively weighted (meat/meat products and whole-fat dairy products).</td>
<td>For the 8 positively weighted, a score of 1 to 3 is assigned according to the distribution into increasing tertiles. For the 2 negatively weighted, a score of 3 to 1 is assigned according to the distribution into increasing tertiles. For scoring ‘moderate’ red wine consumption, the same methodology described in Sánchez-Villegas, 2002 was followed: the continuous variable is then categorized into tertiles and included as a positively weighted component.</td>
</tr>
<tr>
<td>Designation</td>
<td>First author (year) (reference)</td>
<td>Components</td>
<td>Computation of scores</td>
</tr>
<tr>
<td>-------------------------</td>
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<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Diet Quality Index (DQI)</td>
<td>Patterson (1994) (133)</td>
<td>8 components based on the Dietary Recommendations from the 1988 National Academy of Sciences publication Diet and Health.</td>
<td>Intakes meeting a dietary goal are assigned with a score of 0; intakes not meeting a goal, but representing a fair diet, are assigned with 1 point; and those representing a poor diet are assigned with 2 points. The final score ranges between 0 (excellent diet) and 16 (poor diet).</td>
</tr>
<tr>
<td>Diet Quality Index Revised (DQI-R)</td>
<td>Haines (1999) (127)</td>
<td>10 components based on the Dietary recommendations of the National Academy of Sciences and Food Guide Pyramid; &lt;30% total fat; &lt;10% saturated fat; &lt;300 mg/day cholesterol; 2-4 servings/day of fruit; 3-5 servings/day of vegetables; 6-11 servings/day of grains; calcium and iron as % of the sex and aged-specific Adequate Intakes or Recommended Dietary Allowances; dietary diversity score; dietary moderation score.</td>
<td>Each of the 10 components contributes 10 points for the total DQI-R. Servings of fruit, vegetables and grains are expressed as % of recommended servings. Iron and calcium range 0-100%, according to the % of the recommendation value attained. For total fat, saturated fat and cholesterol, the scoring is based in 3 levels: meeting the recommendations (10 points), within 50% of the recommendations (5 points), and greater deviation of the recommendations (0 point).</td>
</tr>
<tr>
<td>Diet Quality Index International (DQI-I)</td>
<td>Kim (2003) (126)</td>
<td>4 major categories of the index components: variety (overall variety and variety within protein sources), adequacy, moderation and overall balance.</td>
<td>Scores for each component are summarized in each of the four main categories, resulting in the total DQI-I score ranging from 0 to 100.</td>
</tr>
<tr>
<td>Healthy Eating Index (HEI)</td>
<td>Kennedy (1995) (134)</td>
<td>10 component system of 5 food groups (grains, vegetables, fruit, milk, meat), 4 nutrients (total fat, saturated fat, cholesterol, sodium), and a measure of variety in food intake.</td>
<td>Each component has a range of score from 0 to 10. The minimum and maximum scores are based in the recommendations of the USDA Food Guide Pyramid, 1992. Intermate intakes are scored proportionately between 0 and 10.</td>
</tr>
<tr>
<td>Recommended Food Score (RFS)</td>
<td>Kant (2000) (153)</td>
<td>23-items of different vegetables and fruit, protein foods, grains and dairy products.</td>
<td>Foods that are consumed at least weekly are pointed with 1. Points were summed to obtain a score ranging from 0 to 23.</td>
</tr>
<tr>
<td>Alternate Healthy Eating Index (AHEI)</td>
<td>McCullough (2002, 2006) (136-137), Fargnoll (2008) (109)</td>
<td>9 components based on guidelines and scientific evidence: vegetables, fruit, nuts and soy protein, ratio of white to red meat, cereal fiber, trans fat (% energy), ratio of polyunsaturated to saturated fatty acids, duration of vitamin use, alcohol intake.</td>
<td>Eight of the 9 components contribute 0–10 points to the total score. The multivitamin component contributes either 2.5 points (for non-use) or 7.5 points (for use). The total AHEI score ranges from 2.5 (worst) to 87.5 (best).</td>
</tr>
<tr>
<td>Alternate Mediterranean Diet Score (aMDS)</td>
<td>Fung (2005) (141)</td>
<td>Represents a MDS modified: excluding potato products from the vegetable soup, separating fruit and nuts into 2 groups, eliminating the dairy group, including only red and processed meat, and assigning 1 to alcohol intake between 5-15 g/day.</td>
<td>For all food components, but red and processed meats: a score of 1 is assigned to an intake &lt;= sex-specific median; 0 to the others. For red and processed meat, the scoring is reversed. The final score ranges from 0 to 9.</td>
</tr>
<tr>
<td>Dietary Variety Score</td>
<td>Drewowski (1996, 1997) (129-130)</td>
<td>Based on the cumulative number of different foods consumed over a 15-day period.</td>
<td>For all 24-hour recall is evaluated for the consumption of dairy, meat, grain, fruit and vegetables (Food Group Score). Other scoring method (Serving Score) evaluates every recall for consumption of at least two servings each from dairy, meat, fruit and vegetables and four servings from the grain group.</td>
</tr>
<tr>
<td>Food Group Score and Serving Score</td>
<td>Kant (1991) (131)</td>
<td>Based on the consumption of dairy, meat, grain, fruit and vegetables.</td>
<td>Food groups were rated by authors as positive (anticipated to have favorably affect CVD), negative or neutral. Then, they were weighted: +1 for &quot;positive &quot; and -1 for &quot;negative&quot; food groups. Individuals were assigned a total score based on their category rank for each food group multiplied by the food group’s assigned positive or negative constant, summed across all food groups.</td>
</tr>
<tr>
<td>Comprehensive Healthy Dietary Pattern</td>
<td>Nettleton (2008) (146)</td>
<td>47 pre-defined food groups</td>
<td></td>
</tr>
</tbody>
</table>
In contrast, exploratory methods rely totally on the data at hand and derive patterns purely empirically, and for that reason are often named as *a posteriori* dietary patterns. Principal component analysis/exploratory factor analysis and cluster analysis are the main exploratory methods applied in nutritional epidemiology (156).

Principal component analysis or factor analysis identifies foods that are frequently consumed together. They aggregate food items or food groups on the basis of the degree to which they are correlated with one another. The goal is to identify linear composites of food items or food groups which account for the largest amount of variation in diet between individuals. While in principal component analysis the component score represents a mathematical transformation (a linear combination) of the observed variables, factor scores, computed in factor analysis, are considered only estimates of where individuals stand on the actual underlying and unobservable factor (156).

Cluster analysis aggregates individuals into relatively homogeneous subgroups (clusters) with similar diets (156). Therefore, it defines mutually exclusive clusters of individuals, based on distance measures between observations, which can be frequency of food consumption, average grams of food intake or percentage of energy provided by each food or food group.

Both methods involve arbitrary choices, such as the number of food groups to be included and the number of factors or clusters to be retained. Also, the validity and reproducibility of the patterns identified by these methods have been questioned (117-118, 121). Moreover, the relatively low amount of the total dietary variance explained (in the case of factor analysis) and the low probability of classification of individuals in each cluster (in the case of cluster analysis) could be overcome by other statistical methods.

Recent research suggests the use of finite mixture models for the identification of dietary patterns (157), which seems to bring some advantages over the previous approaches. In these models, data are viewed as coming from a mixture of probability densities, each one representing a different cluster and, if necessary, both the mixing proportions and the probability densities can be conditioned on covariates of interest. This method provides measures of uncertainty of the classification of individuals in each cluster; allows the adjustment of food consumption covariates simultaneously with the fitting process; and allows the dichotomization of food groups with high frequencies of zeros, and therefore makes possible the convergence of the used algorithm. It also
allows that problems of determining the number of clusters and of choosing an appropriate clustering method to be recast as statistical model choice problems.

Additionally, reduced rank regression has been described as an additional tool for dietary pattern analysis (118, 158). Reduced rank regression identifies linear functions of predictors (e.g. food groups) which explain as much variation as possible in a set of intermediate response variables, for example nutrients or biomarkers. However, with this approach, dietary patterns totally dependent of the outcome and it is unlikely that it identifies a dietary pattern that is linked to most or all potential pathways by which diet may influence the disease of interest.

5.3 | Diet and Coronary Heart Disease – the current knowledge

The knowledge on which nutrients, foods or dietary patterns are likely related to a reduced CHD risk has strongly increased. The conduction of studies on the molecular mechanisms of atherosclerosis and on the metabolic effects of nutrients and foods has contributed to this knowledge. Moreover, large and carefully conducted prospective cohort studies and dietary intervention trials have added some important insights into this field (116, 159).

Single nutrients/foods and alcohol

Different dietary strategies are believed to prevent CHD: 1) increase the consumption of omega-3 fatty acids from fish or plant sources; 2) substitute saturated and trans-fats for non-hydrogenated unsaturated fats; and 3) have a diet high in fruit, vegetables, nuts and whole grains, and low in refined grains (116, 160).

These recommendations are supported by important epidemiological findings. The results of prospective cohort studies show that the consumption of fish or fish oil containing the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with a decreased CHD risk (161-163). Omega-3 fatty acids may reduce coronary risk by preventing cardiac arrhythmia, lowering serum triglyceride levels and improving endothelial dysfunction (164).

In the Nurses’ Health Study, the cohort which probably holds the largest and most detailed dietary data over 20 years, was found that polyunsaturated fat intake was
inversely associated with CHD risk, whereas trans-fat intake increased it (165). In addition, other large prospective studies have positively associated saturate and trans-fatty acid intake with CHD (166-168). Main food sources of saturated fat are red meat and dairy products. The high content of fat but also of heme iron in red meat might account for the increased coronary risk attributed to these foods (169). Also, it has been postulated that the consumption of high-fat dairy products should be avoided in order to decrease cardiovascular risk (160), but systematic reviews of prospective cohort studies have shown no clear evidence of consistent associations between dairy products and CHD (170-171).

On the other hand, in several prospective studies, the consumption of fruit and vegetables has been consistently associated with a reduced CHD risk (172-173). The ability of fruit and vegetables to reduce LDL-cholesterol oxidation is often claimed as a possible explanation (159, 174), but research has also focused on some anti-inflammatory effects (175). These accumulating evidence contributed to the current recommendation of >400 g/day or ≥5 portions/day of fruit and vegetables (176).

Observational studies have also found that the use of supplements such as antioxidant vitamins could prevent CHD (177-179). Despite of a potential plausibility for the protective role of vitamin supplements on chronic diseases (they exert inhibition effects of LDL-cholesterol oxidation), epidemiological findings remain controversial, since randomized clinical trials do not support this beneficial effect (180-181).

In several prospective studies, the consumption of whole grains as a replacement for refined grains has been also claimed to hold an inverse association with coronary endpoints (182). Contrarily, many starchy foods such as potatoes and white bread have been associated with unfavorable effects on metabolic risk factors, due to them high glycemic index (183).

Prospective evidence suggests that moderate consumption of alcohol is associated with a decreased risk of CHD (184-186). This association could be mediated by the beneficial effects of alcohol on lipids (187), haemostatic factors (188), insulin sensitivity (189-190) and inflammation (191-192), but the precise mechanisms are not fully understood. Moreover, the specific shape of the association may vary across studies depending on the used tool to assess alcohol intake, the thresholds selected in the analyses, the inclusion of ex-drinkers, and the number and type of confounders controlled for. A recent meta-analysis (193) even suggests that a systematic error may exist when reporting that moderate alcohol is "protective" against CVD. The authors
suggest that the abstainer category may represent occasional or former drinkers, who quitted drinking because of health conditions; it might explain the higher risk of abstainers compared with moderate drinkers.

**Dietary patterns**

The need of studying diet taking into account cumulative effects of several nutrients and foods (117-119) is clearly supported by the definition and dissemination of different dietary patterns, such as the Mediterranean diet and the Prudent diet, which have long been associated with lower morbidity and mortality rates from CVD and related conditions, in different population sets (110, 121-124, 194-197).

The mainstream dietary pattern analysis has relied on *a priori* approaches; in Southern Europe, usually using Mediterranean diet indexes (121, 123, 194). Most of them have been related to overall survival and not particularly with CHD. In a case-control study in Spain, a greater adherence to a Mediterranean diet index led to a reduced risk of suffering an AMI (138).

Although Portugal is geographically close to the Mediterranean region, there is no updated information on the national individual dietary intake that would support the accomplishment of such pattern related-characteristics. Based on the Portuguese Household Budget Survey, the adherence to the Mediterranean diet was reported to be poor in all Portuguese regions (198). Moreover, some authors (199-200) have suggested that the geographical and cultural proximities of areas such as Galicia in Spain, characterized by a specific high consumption of fish and other sea foods, red meats and soup, could represent more important influences to the Portuguese eating habits than those from other Mediterranean countries.

Actually, over recent years, increasingly interest has been endorsed to the observation that within the Atlantic arch (represented by countries with straight link with the Atlantic Ocean, especially Northern Portugal and Galicia, Spain) there is a specific high consumption of protein-rich foods, but simultaneously upholding the low CHD mortality rates (24, 201-202). Thus, the concept of an Atlantic diet has been strengthening, despite no conceptual definition has been put forward until now.

The Atlantic diet seems to differ from the traditional Mediterranean diet in some important aspects: 1) unlike the Mediterranean diet, the Atlantic diet is characterized by
a high consumption of red meat, pork and fish; 2) in the Atlantic diet olive oil is not the main source of total fat, and nuts are not a relevant food component. However, like the Mediterranean diet, the Atlantic diet is characterized by a high intake of vegetables and whole foods and by wine consumption during meals.

Several studies have assessed the association between a posteriori dietary patterns and CHD (110, 195-197, 203). In the literature, two major a posteriori patterns, identified by factor analysis, have been related to CHD risk - the "Prudent" pattern, corresponding to a high intake of fruit and vegetables, and the "Western" pattern which reflects a high intake of red meat and fats (110, 195-197). Overall, the first one has been inversely associated with CHD risk, whereas the "Western" pattern increased the risk of CHD (110, 195-196).

In the ATTICA Study of Greece (203), in which 15 dietary patterns were extracted by principal component analysis, the patterns characterized by the high intake of red meat, alcohol, sweets and hard cheese were prospectively associated with CVD risk, on the other hand the dietary pattern that was mainly characterized by cereals, small fish, hardtack and olive oil intake was associated with lower cardiovascular risk, as well as the pattern characterized by fruit and vegetable intake and olive oil use in daily cooking.

5.4 | Diet and Coronary Heart Disease – the inflammatory pathway

CHD is a multifactorial disease, thus study each biological system involved in its etiology is a new and promising approach. Inflammation is believed to be one of the most important mechanisms linking healthy diets to a reduced CHD risk (Figure II), as already discussed. Under this point of view, Giugliano and colleagues (204) suggested that each dietary strategy associated with a lower risk of chronic diseases, such as obesity, insulin resistance, metabolic syndrome and CHD may be associated with a lower generation of a proinflammatory milieu (Figure II).
Figure II: Some mechanisms through which diet may lead to coronary heart disease (CHD) and a proinflammatory milieu [source: Giugliano, et al; 2006 (204)].

But what is the state of the art on the relation of diet with inflammation? Scientific research in the last several years supported a beneficial cardiovascular effect of nutrients, such as fatty acids (monounsaturated, polyunsaturated n-3), antioxidant vitamins (E, C, β-carotene), fiber and ethanol involved in inflammatory and antioxidant processes (116, 204-205). Dietary patterns have been also related with several proinflammatory cytokines (107-109, 139-141, 146, 197, 206-209).

Single nutrients/ foods

The intake of EPA and DHA n-3 fatty acids has been inversely associated with plasma levels of CRP (210-212), IL-6 (211), E-selectin (211) and TNF-α (213). Dietary supplementation with α-linolenic acid (n-3 fatty acid) seems also to decrease cytokine levels in dyslipidemic individuals (214-215), more than the linoleic acid (n-6 fatty acid) (216). N-3 fatty acids decrease the arachidonic acid content of cell membranes, resulting in the synthesis of eicosanoids, which have fewer inflammatory properties than those derived from n-6 fatty acids. Other in vitro studies provided support for an anti-inflammatory role of n-3 polyunsaturated fatty acids (217-219). A synergetic effect between n-3 and n-6 fatty acids may also be present; a study among US women and
men has found that the n-3 and n-6 fatty acids combination was associated with lower levels of inflammation than either type of fatty acid alone (213).

Saturated and trans-fatty acids have been also positively associated to inflammation (220-223). In fact, it seems that dietary fatty acids can differently modulate markers of inflammation, e.g. Baer, et al. (224) in a randomized crossover study with 50 men consuming controlled diets for 5 weeks showed that CRP levels were higher after consumption of the “trans-fatty acids diet” than after consumption of the “carbohydrate diet”, but were not significantly different after consumption of the “trans-fatty acids” and “trans-fatty acids plus stearic acid diets” than after consumption of the “saturated fatty acids diet” (8% of energy provided by lauric, myristic and palmitic fatty acids). Additionally, IL-6 concentrations were lower after consumption of the “oleic acid diet” than after consumption of the “saturated fatty acids”, “trans-fatty acids”, and “stearic acid” diets.

Antioxidant vitamins have been related to inflammatory markers, but most studies have focused on plasma levels of antioxidant vitamins (225-231), rather than dietary vitamin intake (232-233) or vitamin supplement use (234-235). Whereas observational studies held inverse and independent associations, supplementation studies found inconsistent results regarding the ability of antioxidant vitamins to reduce systemic and vascular inflammation in vivo, especially when dietary rather than pharmacological amounts are considered (235-236).

Fruit and vegetables are main dietary sources of antioxidant vitamins. Several studies have related fruit and vegetable consumption to increased inflammatory marker levels (236-243). Most studies were randomized trials with very specific exposures such as high-pressurized orange juice (236), carotenoid-rich vegetables and fruit (239), sweet cherries (240), berries and apple (241), vegetable soup "gazpacho" (242), and provide non-conclusive results, since most of them found decreased inflammatory levels with the intake of these food items, but one failed to show a reduction of CRP after several weeks of intervention (241). From the few observational studies conducted, some have reported an inverse association between fruit and vegetable intake and inflammatory markers (243), mainly in the elderly (237-238), but further research is needed on the separate effects of fruit and vegetables, by sex and in a broader age spectrum. The antioxidant components of fruit and vegetables, including vitamins and flavonoids, are believed to contribute to their anti-inflammatory effects (175).
Fruit and vegetables are also major sources of fiber. Epidemiologic evidence supports a possible metabolic effect of dietary fiber on markers of systemic inflammation (244-248). A study with individuals who took part in the U.S. National Health and Nutrition Examination Survey (NHANES 99-00) was one of the first to show a specific link between dietary fiber and CRP levels (245). Although the mechanisms underlying these associations are not fully understood, it is believed that short-term acute hyperglycemia (conducing to uncontrolled production of free radicals which may promote atherogenesis) may increase circulating levels of free radicals and proinflammatory cytokines such as IL-6, IL-18, and TNF-α (249), providing a plausible explanation for the deleterious effects of rapid glycemic waves on vasculature. On the other hand, a high quantity of fiber of a high-carbohydrate meal seems to decrease the inflammatory levels, through the inhibition of IL-18 and stimulation of adiponectin (250).

In the Women’s Health Study, a randomized double-blind placebo-controlled trial conducted in healthy middle-aged women, a significant positive association between the dietary glycemic load and plasma CRP levels was also found (251). Moreover, a dose response gradient between the dietary glycemic load and plasma hs-CRP concentrations was most apparent in overweight women. Overweight individuals have elevated concentrations of insulin and counterregulatory hormones, which are directly associated with hepatic production of CRP (252).

Alcohol

Growing evidence supports the hypothesis that the cardiovascular protective effect of moderate alcohol intake could be partly mediated through inflammation (191-192).

The association between alcohol consumption and inflammation has strong biological plausibility. Ethanol in high quantities and its metabolites may exert direct inflammatory effects on the liver, and acetaldehyde, in particular, may induce free radical production and subsequently increase lipid peroxidation and tissue inflammation (253), and lead to changes in uric acid metabolism (254). While excessive ethanol has also been associated with increased IL-6 production, lower concentrations, on the other hand, may inhibit IL-6 secretion from adipocytes (255).

Several studies have investigated the association between alcohol consumption and biomarkers of inflammation (256-269). The results are mainly dependent of the categories of alcohol intake considered and the exposure range of each specific
population. Overall, seems to be a strong inverse association between alcohol intake, regardless of the type of alcoholic beverage, and biomarkers of inflammation. A U or J-shaped associations, with different nadirs, were found in most populations. The European region shows one of the highest alcohol intakes in the world (270). In Portugal, alcohol consumption is particularly high and a considerable wide range of alcohol exposure is present, particularly in men (271).

Some authors have also suggested that ingredients of alcoholic beverages other than ethanol might explain the beneficial effects on CHD risk, especially in the case of wine (272-273). However, lower levels of inflammatory markers have been reported for moderate consumption of either wine or beer (274-276), suggesting that ethanol itself might be largely responsible for the potential anti-inflammatory effects of these beverages.

In sub-samples from two large ongoing cohort studies - the Health Professionals’ Follow-up Study and the Nurses’ Health Study (263), lower concentrations of TNF receptors 1 and 2, hs-CRP and IL-6 were found among participants who consumed moderate amounts of alcohol (1–2 drinks/day for men and half a drink per day for women), compared to non-drinkers. The results suggest a U-shaped association with increasing alcohol intake for CRP and IL-6 in both sexes. Additionally, a strong inverse linear trend was found with increasing alcohol consumption for TNF receptors.

In the Health, Aging and Body Composition study conducted with 2574 individuals aged 70-79 years, Volpato, et al. (268) reported a significant J-shaped association between alcohol intake and mean IL-6 levels with the nadir at 1–7 drinks per week, and a similar trend for hs-CRP, which was significant only among women. Also, in the Women’s Health Study, women who reported to consume alcohol at least weekly had lower concentrations of IL-6 compared to non-drinkers (258).

A cross-sectional analysis of data from the Third National Health and Nutrition Examination Survey (NHANES III) (267) showed that moderate alcohol drinkers (11 to 30 drinks per month) of the adult US population had 26% decreased probability of having an elevated level of hs-CRP, but was not found a U-shaped relation for men or for the overall population. Contrarily, in a German national health survey, a significant U-shaped association was observed between alcohol intake and hs-CRP concentrations in men with the nadir at 20–40 g/day; the association among women was not significant (261). Also, Wang, et al. described that daily alcohol intake showed
an apparent U-shaped association with hs-CRP and fibrinogen values in men, with lowest levels at an alcohol intake of 20-70 g daily (277).

Among participants in the Pravastatin Inflammation/CRP Evaluation Study (256), the hs-CRP levels were found to be lower in moderate drinkers who consumed 5–7 drinks weekly compared to light or occasional drinkers, independently of alcohol-related effects on lipids. The association was present among men, non-smokers, individuals with and without a history of CVD and women not taking hormone replacement therapy. This last finding may reflect, in part, a “first-pass” effect of hormone replacement therapy on CRP.

Alcohol consumption has been also associated with white blood cells count and fibrinogen. Nakanishi, et al. (262) examined the association between alcohol consumption and white blood cells count by smoking status, and found that white blood cells count was inversely associated with alcohol consumption in both non-smokers and smokers, however the association was less clear in smokers.

Mennen, et al. (269) showed that in French men, fibrinogen concentration was strongly associated with alcohol intake in a U-shaped fashion (nadir: 20-60 g alcohol/day), in particular for the intake of wine and spirits.

In cross-sectional samples, representatives of the general population from Germany, Scotland, and France (276), moderate consumption of either wine or beer was associated with lower levels of systemic inflammatory markers, such as hs-CRP, fibrinogen and white blood cell count.

Several intervention studies have also showed a reduction of systemic inflammatory markers after controlled consumption of moderate amounts of alcohol during a few weeks of intervention (257, 259, 264-266).

**Dietary patterns**

The study of diet indexes/scores (scoring methods previously described in table 2) in relation to markers of inflammation is quite limited. Fung, et al. (141) in the Nurses’ Health Study examined the association between several diet-quality scores and plasma concentrations of markers of inflammation. The authors found that the alternate Healthy Eating Index and the alternate Mediterranean Diet Index had the strongest inverse associations with hs-CRP and IL-6 concentrations. The other scores (Healthy
Eating Index, Diet Quality Index Revised, Recommended Food Score) had little association with these biomarkers. The authors concluded that diet indexes reflecting current intake guidelines seem to be not predictive of biomarkers of inflammation, while the alternate versions may be useful as guidelines for reducing the risk of diseases involving such biological pathways.

Fargnoli, et al. again in the Nurses' Health Study (109) have evaluated if the adherence to the alternate Healthy Eating Index was associated with lower concentrations of biomarkers of inflammation. Women with the highest adherence to the AHEI had 41% lower hs-CRP, 19% lower E-selectin and 16% lower resistin levels, than did women with the lowest adherence to the AHEI. Associations with TNF-α, IL-6, soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 did not remain significant after adjustment for BMI.

A Healthy Dietary Pattern was also a priori defined by the authors of the Multi-Ethnic Study of Atherosclerosis (MESA), reflecting a cardioprotective balance among 36 food groups: 21 food groups rated as positive and 15 food groups rated as negative. The Healthy Dietary Pattern was inversely associated with concentrations of hs-CRP, IL-6, homocysteine and fibrinogen (146).

Because it has been suggested that the Mediterranean diet protects against the development and progression of CHD, several authors have hypothesized that the benefits of adherence to the Mediterranean diet could be due to its ability to modulate low-grade systemic inflammation and coagulation mechanisms. Within the ATTICA Study (139-140), participants who were closer to the Mediterranean diet had lower hs-CRP, IL-6 and fibrinogen levels, as well as white blood cell count, as compared with those who were "away" from this dietary pattern.

A randomized trial conducted by Esposito, et al. also evaluated the Mediterranean dietary pattern in relation to markers of inflammation and endothelial dysfunction, but only among people with the metabolic syndrome (278). The study randomized 180 patients to receive either the Mediterranean diet (detailed advice about how to increase daily consumption of whole grains, fruit, vegetables, nuts and olive oil) or a “prudent diet” low in fat (50–60% carbohydrates, 15–20% protein, and <30% fat) and followed them for 2 years. The level of hs-CRP decreased from 2.8 to 1.7 mg/l (p=0.010) in the intervention group (following the Mediterranean diet), while the level did not change in the other group. Because the results were adjusted for body weight changes, these findings suggest that, largely independent of concomitant changes in body weight, a
Mediterranean-style diet might play a role in reducing the inflammatory state associated with the metabolic syndrome.

Also, *a posteriori* dietary patterns have been related to inflammation. "Prudent" dietary patterns, rich in plant-based foods, have been associated with a more favorable biomarkers' profile (107, 206) including lower hs-CRP, lower fasting insulin, lower homocysteine and higher folate concentrations. On the other hand, the "Western" pattern, characterized by higher intake of red meat, processed meat, refined grains, sweets and dessert, French fries, and high-fat dairy products, has been associated to higher hs-CRP (107, 206), IL-6 (108), C-peptide, insulin (197, 206), leptin and homocysteine concentrations (206).

Nettleton et al. (207), also looked at the associations of four dietary patterns, identified by factor analysis, with inflammatory markers in participants of the Multi-Ethnic Study of Atherosclerosis. The fats and processed meats pattern (fats, oils, processed meats, fried potatoes, salty snacks and desserts) was positively and linearly associated with hs-CRP and IL-6. In contrast, the whole grains and fruit pattern (whole grains, fruit, nuts, and green leafy vegetables) was inversely associated with hs-CRP, IL-6 and soluble intercellular adhesion molecule-1, as well as the vegetables and fish pattern (fish and dark yellow, cruciferous and other vegetables), which was inversely related to IL-6.

More recently, Hamer and Mishra have also identified four dietary patterns similar across genders, named as fast-food, health aware, traditional and sweet (208). Only the 'health aware' diet pattern (higher loadings for fruit, salad and raw vegetables, wholemeal bread and oil fish) was inversely associated with concentrations of hs-CRP and homocysteine, and positively with high-density lipoprotein cholesterol. Similarly, in a Japanese population, out of four dietary patterns derived from principal component analysis (healthy, high-fat, seafood and Westernized breakfast), only the healthy pattern, characterized by high intakes of vegetables, fruit, soy products and fish, was significantly and inversely related to hs-CRP concentrations (209).

In the Moli-sani project in Italy (279), more three dietary patterns were identified by factor analysis. The "Olive Oil and Vegetables" pattern (high intake of olive oil, vegetables, legumes, soups, fruits and fish) was associated with relatively lower values of glucose, lipids, hs-CRP, blood pressure and a cardiovascular risk score. The "Pasta and Meat" pattern (high intake of pasta, tomato sauce, red meat, animal fats and alcohol) was positively associated with glucose, lipids, hs-CRP and the cardiovascular
risk score. The "Eggs and Sweets" pattern (positive loadings of eggs, processed meat, margarines, butter, sugar and sweets) was positively associated with hs-CRP.

Together, all these findings suggest that inflammation could be a potential pathway, by which diet can modulate the coronary risk. However, research is still in progress and many scientific questions have not yet feasible answers. Most of the cohort studies providing dietary evaluations were conducted in the U.S, but the higher food diversity and wide ranges in dietary exposure, frequently observed in European populations, could provide novel and interesting insights into this filed. The use of different methodological approaches to study diet in a same population, and further providing straight comparisons by sex and obesity status would represent enormous advantages for the clear understanding of the role of diet and obesity in the modulation of coronary risk.
Aims
This research aims to evaluate the effect of body fat distribution and diet on coronary heart disease and whether these associations could be mediated by an inflammatory pathway.

The specific questions to be answered with this dissertation are:

A. Have body fat distribution different effects on hs-CRP and on CHD in men and women from the general population? [papers I and II]

B. What is the relation of dietary factors with cardiovascular risk markers and with the coronary endpoint, using different methodological approaches to measure diet, namely:

   b1. single dietary factors (fruit and vegetables, antioxidant vitamins, fiber and alcohol intake)? [papers III and IV]

   b2. a posteriori dietary patterns? [paper V]

   b3. a priori dietary patterns? [paper VI]

C. How sex and body fat can modify the associations between diet and cardiovascular outcomes? [papers I to VI]

To answer these questions, 6 studies (corresponding to 6 papers) were performed with the following specific objectives:

1. To assess, using principal component analysis, the independent associations of general, central and peripheral subcutaneous fat with hs-CRP, in men and women from the general population.

2. To assess the effect of surrogate measures for general, central and peripheral body fat on the occurrence of non-fatal AMI, by sex.

3. To evaluate the effect of fruit, vegetables, antioxidant vitamins and fiber intake on hs-CRP levels in individuals from the general population, and to examine the modification of these associations by sex and BMI.
4. To assess the association of alcohol intake with hs-CRP, uric acid and leukocyte count, and whether sex and BMI modify these associations.

5. To assess the association of *a posteriori* dietary patterns with AMI and cardiovascular risk markers in the general adult population of Porto, Portugal.

6. To examine the association between adherence to the Southern-European Atlantic diet (SEAD), which is culturally rooted in Northern Portugal and Galicia, Spain, and the risk of non-fatal AMI.
Research Design and Methods
This PhD thesis is based upon two projects: the EPI Porto study [POCTI/SAU-ESP/61160/2004] and the EPICardis study [POCTI/ESP/42361/2001], both conducted at the Department of Hygiene and Epidemiology of the University of Porto Medical School, Portugal.

1 | Setting and participants’ assembling

EPI Porto study

The EPI Porto study is a cohort study of adults, assembled to evaluate health determinants, mainly cardiovascular-related determinants, of the non-institutionalized in-habits of Porto, a large urban centre in Northwest Portugal with approximately 300 000 inhabitants.

Participants were recruited by random digit dialing (280) using households as the sample frame (at the time of recruitment, 97% of households had a telephone number, which assured the universal representativeness of eligible households at that period). Once a household was selected, all permanent residents were identified by age and sex, and one of them was selected by simple random sampling to participate. All participants were white, had Portuguese nationality, and were aged 18 years or over. Participants had no financial compensation and refusals were not replaced.

The baseline evaluation of participants was carried out between 1999 and 2003 and a participation proportion of 70% was achieved (281). Figure III illustrates the geographical distribution of the participants of the baseline evaluation of the EPI Porto study, by residence, in Porto. Approximately 68% of these participants were re-evaluated during 2005-2008, but information collected during this period was not used for the present investigation.

The participants cross-sectionally evaluated in the EPI Porto study (1999-2003) were also included as controls in the EPI Cardis Study.
Figure III. Geographical distribution of the EPIPorto participants in their baseline evaluation (1999-2003), by residence, in the city of Porto.

**EPICardis study**

The EPICardis study is a population-based case-control study on risk factors for AMI, conducted from 1999 to 2003.

Cases were patients who had been admitted consecutively to the Cardiology Department of the four hospitals providing acute coronary care in Porto (Hospital de São João, Hospital Geral de Santo António, Hospital Pedro Hispano e Centro Hospitalar de Vila Nova de Gaia), with a first diagnosis of AMI, and who survived beyond the fourth day after the event. The AMI diagnosis was established according to the clinical, electrocardiographic and/or enzymatic criteria defined by the European Society of Cardiology and the American College of Cardiology (282), considering AMI with and without ST segment elevation.

Community controls were individuals from the catchment area of participating hospitals (assembled as participants of the EPIPorto study), who had no previous clinical or silent infarction, according to self-reported data and/or electrocardiographic evidence.
In Figure IV is represented the time frame and the sample sizes of both studies, in which the present investigation is based upon.

![Figure IV. Time frame and sample sizes of the EPIPorto study and the EPICardis study.](image)

2 | Data collection

Selected participants from the community were invited to visit the Department of Hygiene and Epidemiology of the University of Porto Medical School to face-to-face interviews and physical examination. AMI patients were interviewed during their hospital in-stay, after clinical stabilization, usually between the fourth and the eighth day after the coronary event. The same instruments and procedures of assessing information were applied to both community participants and AMI patients.

Data were collected by trained interviewers using structured questionnaires with information on social, demographic, clinical and behavioral characteristics (including smoking, physical activity and diet). A rapid evaluation of the participants’ cognitive function was done in individuals older than 64 years, using the Mini-Mental State Examination test (283). Cognitive impairment was considered when subjects scored less than 24 points (284). A physical examination was also undergone, including measurements of anthropometry and blood pressure, an electrocardiogram and blood sampling.
A more detailed description of the study variables follows and schematic descriptions are represented by figure V (to the exposure and confounding variables) and figure VI (for the outcome variables) at the end of this chapter.

2.1 | Social and demographic characteristics

The questionnaire assessed information on completed years of aging, years of formal education, current marital status and current and past occupational activities. The occupational activities classification followed the Registrar’s General Social Classification criteria.

2.2 | Personal and family medical history

Previously diagnosed medical conditions of the participant and of his parents and siblings were assessed. Information on dislipidemia, hypertension and diabetes were self-reported, based on the question: “Has a doctor ever told you that you have…?” Additionally, objective measurements were obtained, namely for systolic and diastolic blood pressure, which were measured on a single occasion using a standard mercury sphygmomanometer after a 10-minutes rest (the mean of two blood pressure readings was considered) (285). Personal history of cardiovascular disease included previous cardiovascular disorders diagnosed by a physician, namely arterial hypertension, angina pectoris, AMI, stroke and heart failure. A family history of AMI was also registered when one or more first-degree relatives had suffered an AMI, regardless of age at occurrence.

The questionnaire also included data on medication used in the previous year, therefore coded by a trained pharmacist according to the WHO Anatomical Therapeutic Chemical (ATC) Classification System - International Classification of Diseases (ICD - version 10).

Information on menopausal status and hormone replacement therapy were recorded for all women.
2.3 | Smoking

Current and past smoking habits were recorded by questionnaire and participants were classified based on the WHO categories (286) as never-smokers, current smokers (including daily smokers - at least one cigarette a day, and occasional smokers - less than one cigarette a day) and former smokers (smokers with at least six-month abstinence). A subject who quit smoking less than six months ago was considered as a current smoker (regular or occasional smoker, according to the number of cigarettes smoked per day).

Current and former smokers were asked about the duration, age at smoking initiation and number of cigarettes smoked.

The use of other types of tobacco was also questioned and recorded, but due the almost nil contribution of other types of tobacco, only cigarette smoking was considered.

2.4 | Physical activity

Total physical activity energy expenditure was ascertained with a questionnaire exploring all professional, domestic and leisure time activities over the previous 12 months, quantified in standard metabolic equivalents (MET*hour/day) (287).

Subjects reported their daily or weekly participation in each activity, as well as the average time spent in each of them. Regular physical exercise was ascertained as the regular practice, for at least 30 minutes per week, of any leisure-time physical activity with energy expenditure higher than 2.5 metabolic equivalents during the previous year.

2.5 | Diet

Dietary intake over the previous 12 months was assessed with a validated semi-quantitative food frequency questionnaire. The questionnaire comprises a list of 82 foods or food groups and a closed section with nine categories of frequency of consumption ranging from "never or less than once a month" to "six or more times a day"; it also includes two other closed sections for the average portion consumed
(lower, equal or higher than the mean portion size) and the seasonal variation of consumption. A photographic album was used to help the decision of the average portion size consumed. Any foods that were not included in the food list but eaten regularly (once a week or more frequently) were listed in an open section. Detailed information on the development, structure, validity and reproducibility of the food frequency questionnaire is reported elsewhere (288-289).

Food and beverage consumption was converted into total energy, nutrient and alcohol intake with the software Food Processor Plus® (ESHA Research, Salem-Oregon, 1997), which has been adapted to the traditional Portuguese foods, dishes and drinks.

*Fruit and vegetables*

Fruit and vegetable intake was assessed using the food frequency questionnaire previously described. The questionnaire comprises 16 items related to vegetables, only considering fresh vegetables and vegetable soup, and 16 items related to fruit, only considering fresh fruit and natural fruit juices.

*Alcohol intake*

Alcohol intake, in grams per day (g/day), was assessed using the same food frequency questionnaire. Each subject was asked about the mean frequency of consumption of different types of alcoholic beverages, including wine, beer, and spirits - liquors, gin, rum, vodka, cocktails or other mixed drinks. The average portion consumed was asked to be lower, equal or higher than a glass of 125 ml for wine, a bottle or can of 330 ml for beer, and a cup of 40 ml for spirits. The alcoholic beverages consumption was converted into total alcohol intake with the software Food Processor Plus®, previously referred, using an algorithm that assumed the following alcohol concentrations in volume: 12% for wine, 4.7% for beer, 25% for liquors and similar beverages, and 50% for vodka and the like. The algorithm was adapted to Portuguese drinks (e.g. Porto wine).
Dietary Patterns

A posteriori dietary patterns were identified using multivariate finite mixture models (157). The mean (and standard deviation) of the individual posterior probabilities (probabilities predicting the cluster membership) were 89% (0.14) in women and 92% (0.13) in men. The dietary patterns were firstly identified among the community controls, and then from the individual component membership probabilities of the model among community controls, they were similarly identified among the AMI patients.

An a priori dietary pattern was also developed to represent the degree of adherence to the Southern-European Atlantic Diet (SEAD). The index included nine key components of this diet, established according to previous conceptual definitions of the SEAD (24, 199-202): fresh fish (lean and fatty fish, excluding cod fish and canned preparations), cod fish (either fresh or dried and salted), red meat and pork products (beef, pork and pork products, including smoked ham, bacon and sausages), dairy products (skimmed, semi-skimmed and whole milk, yogurt and cheese), legumes and vegetables (beans, peas and vegetables not eaten as soup ingredients), vegetable soup (cooked with vegetables, small amounts of potatoes and some drops of olive oil), potatoes regardless of the cooking method, whole bread (non-refined bread made of different cereals, such as wheat, corn, rye) and wine (red or white).

Each SEAD component (apart from alcohol) was measured as grams per 1000 kcal/day (to express intake as energy density). The consumption of each food, except wine, which was at or higher than the sex-specific median in controls was assigned a score of 1; a lower consumption was given a 0 score. For wine, consumptions up to 1 glass/day in women and up to 2 glasses/day in men were considered to fairly represented the meal drinking pattern characteristic of the SEAD, and thus a score of 1 was given to this level of consumption and a 0 score when above or below (nil consumption). For each participant, the scores (0 or 1) for the 9 food components of the SEAD were summed; thus the index ranged from 0 (lowest adherence to SEAD) to 9.

2.6 | Anthropometrics

Anthropometrics were obtained by trained personnel, according to standard procedures, with subjects in light clothing and barefoot (290).
Body height was measured to the nearest 0.1 kg using a digital scale (SECA®, Columbia, USA) and height was measured to the nearest cm using a wall stadiometer (SECA®, Hamburg, Germany). BMI was calculated as the value of weight (kg) over the height squared (m). Participants were classified as underweight/normal weight (BMI <25.0 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²) (291).

WC was measured midway between the lower limit of the rib cage and the iliac crest, and hip circumference on the maximum circumference over the femoral trochanter; both were measured to the nearest cm using a flexible and non-distensible tape. The WHR was calculated dividing the waist by the hip circumference. The sex-specific cut-offs used to identify increased relative risk for the development of obesity-associated risk factors were: WC >88 cm in women and >102 cm in men, and WHR >0.85 in women and >0.90 in men (291).

The thickness of triceps, biceps, subscapular and suprailiac skinfolds was measured with a Harpenden® calliper at the non-dominant side of the body. All the skinfolds were grasped with the thumb and index finger about one cm proximal to the skinfold selected site, and were measured three times at each site to the nearest 0.5 mm, registering the average value. A skinfold composite index (Σ triceps and biceps skinfolds/ Σ triceps, biceps, subscapular and suprailiac skinfold) was developed to estimate the proportion of subcutaneous fat of the arms.

2.7 | Blood sampling and biochemistry analyses

A venous blood sample was drawn after a 12-hour overnight fast. Leukocyte count was determined from the whole blood, immediately after collection. Serum samples were stored at -20°C, until analysis.

hs-CRP levels were determined by means of particle-enhanced immunonephelometry using an auto-analyser Behring, Nephelometer II, BN II® (Dade Behring™ Marburg GMBH, D-35041 Marburg, Germany) (coefficient of variation=7.6%). Firstly, they were determined for 1168 individuals of the general population; and then were determined for more 370 individuals.

For all participants, uric acid, glucose, cholesterol and triglyceride levels were determined by standard enzymatic methods (292-293). High-density lipoprotein
cholesterol was determined after precipitation of apolipoprotein B-containing lipoproteins (294).

All the samples were analyzed at the central laboratory of the São João University Hospital.

In figures V and VI are summarized the descriptions of the study variables.

3 | Quality control

All interviewers were rigorously trained, using a structured protocol addressing all the questionnaires’ queries. A periodic supervision of their work was undergone over time and comparisons between observers were performed to avoid possible interviewer bias.

A multidisciplinary team (including nutritionists for the dietary and anthropometric assessments, and physicians for the physical examinations, among others) with experience in other national and international projects was responsible for the staff training and the development of the questionnaires applied.
Social and demographic characteristics

- Age: completed years of aging
- Education: completed years of formal education
- Current marital status: married/in fact union, single, divorced, widow
- Occupational activity: white-collar, blue-collar, unemployed/housewife/students, retired

Personal and family medical history

- Dislipidemia/diabetes: self-reported
- Hypertension: self-reported; blood pressure objectively measured on a single occasion using a standard mercury sphygmomanometer
- Personal history of CVD: previous cardiovascular disorders diagnosed by a physician: arterial hypertension, angina pectoris, AMI, stroke and heart failure
- Family history of AMI: occurrence of an AMI in one or more first-degree relatives, regardless of age at occurrence
- Use of medication: as an open question, and thus coded according to the ICD-10
- Gynecological history: menopausal status and use of hormone replacement therapy in the previous year

Lifestyles

- Smoking status: defined as never smokers, current smokers, former smokers (smokers with at least six-month abstinence)
- Physical activity: assessed by a questionnaire exploring professional, domestic and leisure time activities over the previous year; regular physical exercise defined as the regular practice, for at least 30 min per week, of any leisure-time physical activity with energy expenditure > 2.5 MET
- Diet: food and nutrient intake assessed by a validated 82-item semi-quantitative food frequency questionnaire over the year before interview

Anthropometrics

- Body mass index: defined as weight (kg) over the height squared (m), both objectively measured; participants classified as underweight/normal weight (BMI <25.0 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²)
- Waist circumference: measured midway between the lower limit of the rib cage and the iliac crest; participants classified as at increased risk if >88 cm (women) and >102 cm (men)
- Hip circumference: measured on the maximum circumference over the femoral trochanter
- Waist-to-hip ratio: calculated dividing the waist by the hip circumference; participants classified as at increased risk if >0.85 (women) and >0.90 (men)
- Skinfold composite index: defined as the sum of triceps and biceps skinfolds divided by the sum of...

CVD: cardiovascular diseases, AMI: acute myocardial infarction, ICD: International Classification of Diseases, MET: metabolic equivalent of task

Figure V. Description of the main exposure and confounding variables.
6. Description of the outcome variables.

4 | Statistical analyses

Differences in proportions were tested with the Chi-square test or the Fisher-exact test, as appropriate. The Mann-Whitney test was used to compare continuous variables between two independent samples. Correlations between continuous variables were evaluated by Spearman correlation coefficients. Normality of study variables was checked using the Shapiro-Wilk criterion.
According to the type of dependent variable (continuous or categorical with \( n \) categories), different models were performed. When a continuous dependent variable was considered (e.g. hs-CRP levels), associations were summarized with generalized linear models with a log link function and Gaussian error distribution \( (295) \) [papers I and IV]. When a two-categories dependent variable was considered (AMI occurrence: yes/no), associations were summarized with unconditional logistic regressions [papers II, V and VI]. When a three-categories depend variable was considered (hs-CRP <1.00; 1.00-3.00; >3.00 mg/l), associations were summarized with ordinal logistic regression; the odds ratio indicating the probability of changing of hs-CRP category [low risk (<1.00 mg/l) to average risk (1.00-3.00 mg/l) or average to high risk (>3.00 mg/l)] for each unit increase of the independent variable [paper III]. Odds ratio and their respective 95% confidence intervals (OR, 95%CI) were obtained from the three models considered.

The dose-response relationship of variables was assessed with tests for linear trend, by modeling a categorical measure as a continuous variable. The linear and quadratic trends of the associations between alcohol intake and the inflammatory markers (paper IV) were obtained modeling the medians (\( p \) value for the linear trend) and the squared medians (\( p \) value for the quadratic trend) of each category of alcohol intake as a continuous variable.

Interactions between two variables were studied, including in the models interaction terms constructed as the product of these two variables.

Additionally, principal component analysis \( (296) \) with varimax rotation was used to identify factors representing uncorrelated components of body fat [papers I and II]. The number of factors to identify had to explain more than 90% of the total variance of anthropometry. Factor's interpretation was based on the correlations between the anthropometrics and the identified factors, obtained from the factor's loadings.

Lastly, dietary patterns were identified by multivariate finite mixture models, or equivalently, mixture of regressions with concomitant variables as independent variables \( (157) \). Due to significant differences in food group distributions among sex \( (p<0.001) \), the dietary patterns were identified by sex. Food groups with non-consumption higher than 45% were dichotomized into consumption and non-consumption. The variables that were not dichotomized were log-transformed, after a sum with 1 (g) to avoid zeros. For the model structure, variances of the continuous food group intakes were allowed to vary within and between clusters. The final number of
clusters (n=4, for both men and women) was established by the cessation of the monotonically decrease of the Bayesian Information Criterion (BIC).

In all analyses, statistical significance was set at two-sided p<0.05. Analyses were performed with the software STATA® (StataCorp, USA) and R-Gui® (R Foundation for Statistical Computing, Austria).

5 | Ethical considerations

The Ethics Committees of the four participating hospitals approved the study protocol. The EPIporto study has also ethical approval from the Portuguese Authority of Data Protection.

All participants gave written informed consent to participate, after having received an explanation of the study purposes.
Papers
Paper I

Body fat distribution and C-reactive protein – a principal component analysis

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Body fat distribution and C-reactive protein – a principal component analysis

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KEYWORDS
C-reactive protein; Body fat distribution; Peripheral fat; Sex differences

Abstract Background and Aims: To assess, using principal component analysis, the independent associations of general, central and peripheral subcutaneous fat with high-sensitivity C-reactive protein (hs-CRP), in men and women from the general population.

Methods and results: We studied 833 women and 486 men, randomly selected from the non-institutionalized population of Porto, Portugal, with information on hs-CRP (≤10 mg/l) and anthropometrics (1999–2003). Body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and a skinfold composite index to estimate the proportion of arm subcutaneous fat (PSFA), were ascertained by trained personnel. Beta regression coefficients were obtained from generalized linear models with adjustment for the main confounders.

Direct associations were found between BMI, WC, WHR and hs-CRP. PSFA was inversely associated with hs-CRP in women (β = -0.080, p-trend = 0.010). Since the anthropometric measures were strongly correlated, we used principal component analysis to identify new independent anthropometric factors. The first one, representing a generalized fat distribution (high BMI and WC), was directly associated with hs-CRP (β = 0.226, p-trend < 0.001 in women; β = 0.138, p-trend = 0.002 in men). The second factor, characterized by a high PSFA, showed an inverse association with hs-CRP in women (β = -0.071, p-trend = 0.048). The third factor, representing a central pattern of fat distribution (low BMI, but high WC and high WHR), was directly associated with hs-CRP in men (β = 0.090, p-trend = 0.005).

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Introduction

Obesity has been associated with metabolic complications, such as insulin resistance, hypertension and dyslipidemia [1–3]. These effects could be mediated, in part, by the relationship between obesity, mainly total and central obesity, and a state of low-grade chronic inflammation [4–10]. Although its patho-physiologic basis is incompletely understood, it is known that adipose tissue expresses a variety of proinflammatory cytokines, namely interleukin-6, tumor necrosis factor-α, and complement C3 [11,12], which stimulate the production of acute phase proteins, such as C-reactive protein [13].

Measures of central obesity, such as waist circumference (WC) and waist-to-hip ratio (WHR), are stronger and more consistent predictors of inflammation than general obesity which is frequently estimated from the body mass index (BMI) [4–6]. There is an increasing understanding of the metabolic effects of abdominal fat, both visceral and subcutaneous [14–16], but less information is available on the relative contribution of central and peripheral fat (fat accumulated in peripheral depots, such as upper and lower limbs), which seem to confer opposing effects — adverse for central and protective for peripheral fat — on cardiovascular risk [17–20]. In fact, the finding that the adverse effects of a high WHR could be due not only to a larger waist but also to narrower hip or thigh circumferences [21,22], suggests a cardio-protective effect of peripheral fat.

Although peripheral fat was previously associated with different cardiovascular risk markers, to our knowledge very few studies [5,19] have examined the relationship between peripheral fat and markers of systemic inflammation. Since men and women have different ranges of variation in levels of body fat, a sex-effect in this relationship could be expected. However, sex differences and the potential confounding effects of lifestyles on the associations between body fat and inflammatory markers are often disregarded. Moreover, it is relatively difficult to distinguish between the effects of abdominal, peripheral and total body fat, due to the strong correlation between the different locations of fat. As a result, traditional regression methods of adjusting the effects of one anthropometric measure for the others might not suffice. The use of principal component analysis to identify uncorrelated measures of obesity could be a useful approach to assess the independent effect of fat location on health outcomes.

This study used principal component analysis to investigate the independent associations of general, central and peripheral subcutaneous fat with high-sensitivity C-reactive protein, in men and women from the general population.

Methods

Study population

Participants were selected between 1999 and 2003, among the 300,000 non-institutionalized inhabitants of Porto, a large urban centre in the north-west of Portugal. Participants were selected by random digit dialling; in each household, permanent residents were characterized according to age and sex, and one subject aged ≥18 years was selected by simple random sampling. Eligible participants were invited to visit the Department of Hygiene and Epidemiology of the University of Porto Medical School, for interview and physical examination. Refusals were not substituted and the participation rate was 70% [23].

The study evaluated 2485 participants (1538 women and 947 men). For the present analysis, we excluded 947 individuals without C-reactive protein measurements, 101 with missing information on anthropometric measures, and 40 who lacked data on selected confounders. Seventy-eight participants (55 women and 23 men) with C-reactive protein levels >10 mg/l, which might suggest a clinically relevant inflammatory condition [24], were also excluded. Therefore, the final study sample included 1319 subjects (833 women and 486 men).

Data collection

Anthropometrics

Anthropometrics were obtained by trained personnel, according to standard procedures [25], with subjects in light clothing and barefoot. Body weight was measured to the nearest 0.1 kg using a digital scale (SECA®) and height was measured to the nearest cm using a wall stadiometer (SECA®). BMI was calculated as the value of weight (kg) over the height squared (m).

WC was measured midway between the lower limit of the rib cage and the iliac crest, and hip circumference on the maximum circumference over the femoral trochanters; both were measured to the nearest cm using a flexible and non-distensible tape. The WHR was calculated by dividing the waist by the hip circumference.

Triceps, biceps, subscapular and suprailiac skinfold thicknesses were measured with a Harpenden® caliper on the non-dominant side of the body. All the skinfolds were grasped with the thumb and index fingers about 1 cm proximal to the skinfold selected site, and were measured three times at each site to the nearest 0.5 mm; the average value was registered. We used a skinfold (SKF) composite index (∑ triceps and biceps SKF/∑ triceps, biceps, subscapular and suprailiac SKF) to estimate the proportion of subcutaneous fat of the arms (PSFA). Different skinfold
indexes have been used in the literature showing acceptable correlations with gold standard methods [26–28].

C-reactive protein assessment
Blood was sampled after a 12-h overnight fast. Serum samples were stored at −20 °C. High sensitivity C-reactive protein (hs-CRP) levels were determined through particle-enhanced immunonephelometry using an auto-analyser Behring, Nephelometer II, BN II® (Dade Behring Marburg GMBH, Germany) (coefficient of variation = 7.6%).

Social, behavioral and clinical characteristics
Data on potential confounders such as social, behavioral and clinical characteristics were collected by trained interviewers using a standard structured questionnaire.

Age and education were recorded as completed years of aging and schooling.

Total energy intake and alcohol consumption were assessed using a validated semi-quantitative food frequency questionnaire [29,30], concerning the previous 12 months. Each subject was asked about the mean frequency of consumption and the average portion consumed. The alcoholic beverage intake and other dietary intakes were converted into total alcohol intake and nutrients with the software Food Processor Plus® (ESHA Research, Salem-Oregon, 1997). Smoking was also recorded and participants were classified into current smokers (daily and occasional smokers) and non-smokers (never and former smokers with at least a 6-month abstinence). Total physical activity energy expenditure was evaluated using a questionnaire exploring all professional, domestic and leisure time activities, and was quantified in standard metabolic equivalents (MET × hour/day).

A personal history of cardiovascular disease was self-reported, registering previous cardiovascular events diagnosed by a medical doctor. The use of medications in the previous year was also asked and classified as anti-hypertensive and anti-dislipidemia drugs, according to the International Classification of Diseases system (ICD-version 10).

Information on menopause and hormone replacement therapy was recorded for all women.

Ethics
All study subjects gave written informed consent to participate. The Local Ethics Committee (São João Hospital) approved the study protocol.

Statistical analysis
Proportions were compared with the chi-square test, while the Mann–Whitney test was used to compare continuous variables between two independent samples.

The associations between the anthropometric measures (independent variables) and hs-CRP (dependent variable) were summarized with generalized linear models with a log link function and Gaussian error distribution [31]. Normality was checked using the Shapiro–Wilk criterion. Models were adjusted for age, education, alcohol consumption (as continuous variables), current smoking, total physical activity, history of cardiovascular disease, anti-hypertensive and anti-dislipidemia drugs, and menopause and hormone replacement therapy (for women) (as categorical variables).

For comparison, beta regression coefficients were standardized (z-scores), representing the change in hs-CRP levels for each standard deviation above or below the mean value of the anthropometric measure considered.

Models were stratified by sex, after studying for sex-interactions in analyses of the whole study sample, using interaction terms constructed as the product of sex by anthropometric variables.

The point estimates of hs-CRP and their respective 95% confidence intervals (95% CI) by quartiles of the anthropometric measures were also obtained from the generalized linear models described above, assuming respectively for women and men, a sample mean of 50, 52 years; 8.4, 9.1 education years; 7.3, 32.4 g/day of alcohol intake; 18.8, 32.5% of smokers; 5.9, 6.6% with a history of cardiovascular disease; 26.2, 20.6% using anti-hypertensive drugs; 8.5, 10.7% using anti-dislipidemia drugs, and 15.0, 36.9% of postmenopausal women with and without hormone replacement therapy.

Further, due to the high correlation between the anthropometric measures, as evaluated by Spearman coefficients, uncorrelated independent variables were identified through principal component analysis with varimax rotation. The scores that were entered in the models were calculated using the regression method with standardized scores. Firstly, the factors were obtained separately for each sex, but since they were very similar, the calculation of the final factors was done for the overall sample.

Since we aimed to construct uncorrelated variables explaining most of the variance of the single anthropometric measures, we identified a number of factors that explained more than 90% of the total variance. The interpretation of the factors was based on the correlations between the anthropometrics and the identified factors, obtained from the factor's loadings. The association of each factor with hs-CRP levels was summarized with generalized linear models and the models were adjusted for the confounders listed above.

Analyses were conducted using the software R-Gui® version 2.6.0.

Results
Characteristics of the study participants are shown in Table 1, by sex. Compared with men, women were less educated and reported a lower alcohol intake and smoking habits. Women reported a more frequent use of antihypertensive drugs and presented with significantly higher hip circumferences and PSFA. In contrast, men had a significantly higher WC and WHR than women.

Table 2 presents mean values of hs-CRP according to quartiles of anthropometric measures, as well as the beta- coefficients of the regression of hs-CRP on anthropometrics, by sex. In multivariate analysis, significant positive associations were found between hs-CRP and BMI, WC and WHR in each sex. Compared with subjects in the lower quartile of BMI, WC and WHR, subjects in the upper quartile had respectively 54, 53 and 41% (for women) and 39, 41 and 30% (for men) higher mean values of hs-CRP.
Table 1 Characteristics of study participants, by sex.

<table>
<thead>
<tr>
<th>Social, behavioral and clinical characteristics</th>
<th>Women n = 833</th>
<th>Men n = 486</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (25thP–75thP)</td>
<td>51 (40–62)</td>
<td>52 (40–65)</td>
<td>0.067</td>
</tr>
<tr>
<td>Education (years), median (25thP–75thP)</td>
<td>4.6 (13)</td>
<td>4 (12)</td>
<td>0.003</td>
</tr>
<tr>
<td>Alcohol intake (g/day), median (25thP–75thP)</td>
<td>1.05 (0.00–11.6)</td>
<td>26.6 (5.8–57.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total physical activity (MET × hour/day), median (25thP–75thP)</td>
<td>34.8 (33.2–38.5)</td>
<td>34.2 (32.6–39.5)</td>
<td>0.170</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>157 (18.8)</td>
<td>158 (32.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of cardiovascular disease, n (%)</td>
<td>49 (5.9)</td>
<td>32 (6.6)</td>
<td>0.608</td>
</tr>
<tr>
<td>Use of anti-hypertensive drugs, n (%)</td>
<td>218 (26.7)</td>
<td>100 (20.6)</td>
<td>0.022</td>
</tr>
<tr>
<td>Use of anti-dyslipidemia drugs, n (%)</td>
<td>71 (8.5)</td>
<td>52 (10.7)</td>
<td>0.190</td>
</tr>
<tr>
<td>Menopause with HRT, n (%)</td>
<td>125 (15.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Menopause without HRT, n (%)</td>
<td>307 (36.9)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Anthropometrics

| Body mass index, kg/m², median (25thP–75thP) | 26.2 (23.4–30.0) | 25.9 (23.7–28.7) | 0.157   |
| Waist circumference, cm, median (25thP–75thP) | 84.0 (75.6–94.0) | 92.8 (85.0–99.0) | <0.001 |
| Hip circumference, cm, median (25thP–75thP)   | 100.0 (95.0–106.5) | 99.0 (94.2–103.5) | 0.004   |
| Waist-to-hip ratio, median (25thP–75thP)      | 0.836 (0.787–0.893) | 0.936 (0.884–0.977) | <0.001 |
| Peripheral subcutaneous fat, median (25thP–75thP) | 0.466 (0.421–0.517) | 0.355 (0.315–0.412) | <0.001 |

25thP: 25th percentile; 75thP: 75th percentile; HRT: hormone replacement therapy.

* Representing a skinfold (SKF) composite index (triceps and biceps SKF).

In contrast, the PSFA was negatively associated with hs-CRP levels in women (β = −0.080, p-trend = 0.010). Compared to women in the lower quartile of PSFA, those in the upper quartile had a 22.5% lower hs-CRP.

Significant moderate-to-strong Spearman correlation coefficients (ρ) were found between BMI and WC (ρ = 0.83), BMI and WHR (ρ = 0.50), WC and WHR (ρ = 0.81), and WHR and PSFA (ρ = 0.39), which limit further adjustment for each other. Thus, principal component analysis was used to identify unrelated anthropometric factors. Three main factors, which explained 98.3% of the total variance, were found (Table 3). The first one represents individuals characterized by a generalized fat distribution, i.e. mainly represented by high BMI and high WC; the second one represents individuals with a high proportion of peripheral subcutaneous fat, and the third one is characterized by a central pattern of fat distribution, i.e. individuals predominantly with a high WC and high WHR.

Mean values of hs-CRP according to quartiles of these factors are shown in Table 4. After full adjustment for confounders, the first factor, representing a generalized fat distribution, was positively associated with hs-CRP in each sex. In men, the third factor, representing a central pattern of fat distribution, was positively associated with hs-CRP levels. Lastly, an inverse significant association was found between the second factor, representing peripheral subcutaneous fat, and hs-CRP in women.

Discussion

A general pattern of fat distribution in both sexes, and a central pattern of fat distribution in men, were directly associated with hs-CRP levels. In contrast, a high proportion of peripheral fat seemed to be inversely associated with hs-CRP levels, but only in women.

In this same population, it has previously been shown that central fat was a major determinant of increased hs-CRP levels of individuals with the metabolic syndrome [7]. Central fat appears to have more adverse effects on cardiovascular risk than the fat stored in other locations, such as the peripheral depots - arms or legs [17–21]. Although the apparent anti-atherogenic effect of peripheral fat has been previously suggested [18–21], to our knowledge, only a few studies [5,19] have examined the relation between peripheral adiposity and systemic markers of inflammation. Tókó et al. [19] showed that, in postmenopausal women, peripheral fat measured by dual-energy X-ray absorptiometry (DXA) had a favourable long-term effect on systolic blood pressure, serum triglyceride and white blood cells, and was inversely associated with aortic calcification, a direct measure of atherosclerosis.

In our study, we found an inverse association between PSFA and hs-CRP in women. Even after the construction of an independent measure (factor 2 from principal component analysis), the association maintained statistical significance. The underlying mechanisms are poorly understood, but it seems that peripheral adipose tissue has a higher lipoprotein lipase activity and low fatty acid turnover; it takes up, more frequently, free fatty acids of circulation and stores them, protecting the liver from high free fatty acids exposure, resulting in a lower production of inflammation markers [32]. Peripheral adipocytes are also believed to secrete more adiponectin, which has anti-atherogenic properties [33]. There is also evidence that adipocytes have distinct intrinsic characteristics (e.g., fatty acid-binding proteins and enzymes of fat metabolism) which further contribute to the heterogeneity in free fatty acids handling by the different fat depots [34].

Considering that regional differences in secretion of peptides, such as interleukin-6 and adiponectin, could explain different fat effects on inflammation, a sex-related effect, due to the different patterns of fat distribution between women and men, would also be expected. In the present study, men have a considerably larger proportion of fat within the abdominal area, but women have higher total
### Table 2: Crude and adjusted mean values (95% CI) of high-sensitivity C-reactive protein (hs-CRP) (mg/l) by quartiles of anthropometric measures, and beta-coefficients for the regression of hs-CRP on anthropometric measures, by sex.

#### Women

<table>
<thead>
<tr>
<th>Measures</th>
<th>Crude mean (95% CI)</th>
<th>β-sd*</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>1.50 (1.21–1.79)</td>
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<tr>
<td>Waist circumference, cm</td>
<td>1.48 (1.19–1.77)</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>1.73 (1.43–2.02)</td>
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<tr>
<td>Peripheral subcutaneous fath</td>
<td>2.86 (2.56–3.16)</td>
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<td></td>
<td><strong>Q1 (lower)</strong></td>
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<td><strong>Q2</strong></td>
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<td></td>
<td><strong>Q4 (upper)</strong></td>
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<tr>
<td>Adjusteda mean (95% CI)</td>
<td>1.53 (1.23–1.84)</td>
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<td></td>
<td>2.24 (1.94–2.53)</td>
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<td>2.90 (2.60–3.20)</td>
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<td>3.32 (3.00–3.63)</td>
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<td><strong>Q1 (lower)</strong></td>
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<td><strong>Q3</strong></td>
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<td></td>
<td><strong>Q4 (upper)</strong></td>
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</table>

#### Men

<table>
<thead>
<tr>
<th>Measures</th>
<th>Crude mean (95% CI)</th>
<th>β-sd*</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>1.47 (1.08–1.85)</td>
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<tr>
<td>Waist circumference, cm</td>
<td>1.43 (1.04–1.83)</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>1.54 (1.15–1.92)</td>
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<tr>
<td>Peripheral subcutaneous fath</td>
<td>2.05 (1.66–2.44)</td>
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<td></td>
<td><strong>Q1 (lower)</strong></td>
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<td><strong>Q2</strong></td>
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<td><strong>Q3</strong></td>
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<td></td>
<td><strong>Q4 (upper)</strong></td>
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</tr>
<tr>
<td>Adjusteda mean (95% CI)</td>
<td>1.50 (1.13–1.87)</td>
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<tr>
<td></td>
<td>2.10 (1.75–2.45)</td>
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<td></td>
<td>1.98 (1.61–2.34)</td>
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<tr>
<td></td>
<td>2.47 (2.09–2.86)</td>
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<tr>
<td></td>
<td><strong>Q1 (lower)</strong></td>
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<td><strong>Q3</strong></td>
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<td></td>
<td><strong>Q4 (upper)</strong></td>
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</table>

Q: quartiles (cut points presented in Table 1).

*β-sd: standardized beta regression coefficients, calculated from generalized linear models.

h: Representing a skinfold (SKF) composite index (Σ triceps and biceps SKF), triceps SKF, subscapular and suprailliac SKF.

*a: Adjusted for age, education, alcohol consumption, current smoking, total physical activity, history of cardiovascular disease, anti-hypertensive and anti-diabetic drugs, and menopause and hormone replacement therapy (in women).

and peripheral fat. Despite of the potential opposing effects of total and peripheral fat to CRP levels, most studies reported women presenting higher CRP levels than men [4,35–37], possibly due to the impact of hormonal factors and the much higher relative quantity of total fat, both increasing the inflammatory levels. The higher variability of hs-CRP and peripheral fat levels found in women could somewhat be responsible for the stronger association found in women than in men. Previous studies have described that the association of adiposity with low-grade systemic inflammation is considerably stronger in women [4,38]. As data for men and women were determined using the same methodology, the assessment of sex differences in a direct manner is allowed.

As often observed by other investigations [4–7], we found stronger direct associations between hs-CRP and WC than for BMI, particularly in men (beta coefficients were standardized for direct comparison). However, these results were not adjusted for other anthropometrics. After construction of independent variables, the factor characterized by high BMI, but also high WC (factor 1) had a stronger association with hs-CRP than factor 3 (high WC and WHR) — an indicator of preferential abdominal fat accumulation.

Moreover, it has been suggested that WC is a better measure of cardiovascular risk than WHR [39], due to the
Table 4: Adjusted mean values (95% CI) of high-sensitivity C-reactive protein (hs-CRP) (mg/l) by quartiles of the independent anthropometric factors identified in principal component analysis, and beta-coefficients for the regression of hs-CRP on the anthropometric factors, by sex.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude mean (95% CI)</td>
<td>β-std*</td>
<td>p-trend</td>
<td></td>
</tr>
<tr>
<td>Q1 (lower)</td>
<td>Q2 (2.02–2.59)</td>
<td>0.235</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Q2 (2.01–3.08)</td>
<td>2.49 (2.21–2.76)</td>
<td>0.069</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>Q4 (upper)</td>
<td>Q4 (2.48–3.18)</td>
<td>0.107</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>


As for hs-CRP assessment, we have collected only one sample per subject, but the problem of variability from acute infection or other short-term insult is likely to be minimal, because only sub-clinical inflammatory states are of interest in this study. Also, the measurements of body fat and hs-CRP concentrations were made at the same time, which reduces the problem of a redistribution of body fat.

The assessment of hs-CRP was not planned at the beginning of the participants’ evaluation. Thus, although hs-CRP measurements were performed consecutively, they are only available for a subset of participants. Comparing those with and without hs-CRP measurements (n = 1319 vs. 1166), subjects with hs-CRP assessment were significantly younger (51 vs. 59 median years, p < 0.001), and presented slightly lower median values of BMI (26.1 vs. 26.5 kg/m², p = 0.030), WC (87.5 vs. 90 cm, p < 0.001), and WHR (0.674 vs. 0.892, p < 0.001). No differences were found regarding sex (women: 63.2% vs. 60.5%, p = 0.168), education (8 vs. 8 median years, p = 0.147), current smoking (23.9% vs. 24.9%, p = 0.549), physical activity practice (36.4% vs. 33.4%, p = 0.120) and the PSFA measure (0.430 vs. 0.429, p = 0.580). Knowing that CRP increases with age and with increasing BMI and WC, an underestimation of
inflammatory levels is probably present in our sample. However, this underestimation is expected to be non-differential with respect to anthropometrics, suggesting that the reported associations could be even stronger. Regarding the magnitude of the differences between participants and non-participants characteristics, it is not likely to affect the results.

In most studies, the independent effects of obesity measures on health outcomes have been assessed by using adjustment procedures in multivariable models; however due to collinearity between the fat measures, this could have led to biased results, specially when adjustments for total obesity were made. To circumvent this problem of collinearity, firstly we constructed independent fat variables through principal component analysis, and afterwards evaluated their independent effects on hs-CRP levels.

An opposing effect on hs-CRP levels was found: a general and a central pattern of fat distribution were directly associated with hs-CRP, while PSFA seemed to be inversely associated with hs-CRP, at least in women. Therefore, the possible role of inflammation as a link between obesity and cardiovascular disease could arise from a balance between the adverse effects of visceral and subcutaneous fat of the trunk and the protection effects conferred by a higher proportion of peripheral subcutaneous fat.

These results call for targeted efforts to identify the mechanisms by which adipocytokines could mediate the beneficial metabolic effects of peripheral fat depots.

From clinical and public health perspectives, the identification of individuals at high cardiovascular risk, only based on WC measurements, could lead to differential misclassifications, since WC could have different impacts on cardiovascular risk according to the peripheral accumulation of fat. Moreover, sustained physical activity programs promoting the loss of fat mass within the peripheral depots (upper and lower limbs) must be under public eye discussion.

Conflict of interest

No conflict of interest to declare.

Funding source

Had no involvement in the study.

Acknowledgements


References


Paper II

Indices of central and peripheral body fat: association with non-fatal acute myocardial infarction

Andreia Oliveira, Fernando Rodríguez Artalejo, Milton Severo, Carla Lopes

Int J Obes (Lond) 2010 Jan 12. [Epub ahead of print]
ORIGINAL ARTICLE

Indices of central and peripheral body fat: association with non-fatal acute myocardial infarction

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Background: The majority of the studies have focused on the effect of general and central fat on coronary risk, neglecting the potential role of peripheral body fat.

Objective: To assess the effect of surrogate measures for general, central and peripheral body fat on the occurrence of non-fatal acute myocardial infarction (AMI).

Methods: Population-based case–control study; cases were patients aged ≥40 years consecutively hospitalized with an incident AMI (n = 653), and controls were community participants without previous AMI, selected randomly from the hospital’s catchment area population (n = 1713). Body mass index (BMI), waist circumference (WC), hip circumference and a skinfolds composite index to estimate the proportion of peripheral subcutaneous fat in the arms were ascertained. Associations were summarized with odds ratios (OR) and 95% confidence intervals (95% CI), obtained from unconditional logistic regression with adjustment for the main confounders.

Results: WC, and in particular waist-to-hip ratio (WHR), had strong direct associations with AMI risk. Peripheral subcutaneous fat was inversely associated with AMI in women, but directly in men. Using principal component analysis, three uncorrelated factors were identified representing different patterns of fat distribution: (1) generalized fat, with high BMI and high WC; (2) central fat, with high WC and WHR; and (3) peripheral subcutaneous fat. The first factor showed no significant association with AMI, but the second factor increased AMI risk in each sex (upper vs lower fourth: OR 12.2, 95% CI 5.34–27.9 in women; OR 25.0, 95% CI 14.0–44.7 in men). In contrast, the third factor was inversely associated with AMI in women (upper vs lower fourth: OR 0.59, 95% CI 0.36–0.96) and directly associated in men (OR 2.45, 95% CI 1.69–3.55; P-value for sex interaction < 0.001).

Conclusions: Central fat was associated with increased risk of AMI in women and men, while the peripheral subcutaneous fat index predicted a lower risk of AMI in women and a higher risk in men.

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Keywords: body fat distribution; peripheral fat; acute myocardial infarction; case–control study; principal component analysis

Introduction

Obesity, often assessed by body mass index (BMI), has been related to several metabolic disorders,¹ but its role on cardiovascular risk is still uncertain, because some studies have found a linear association with cardiovascular outcomes whereas others have reported J- or U-shaped associations or even no significant effects.²-⁴ Moreover, the amount and type of body fat distribution seem to be more important to cardiovascular risk than overall obesity per se, because in several studies waist circumference (WC) and waist-to-hip ratio (WHR) were found to be better predictors of cardiovascular morbidity and mortality than total body weight and BMI.³,⁵-⁹ WC has been more extensively used to identify individuals at high-risk, due to its well-documented positive association with cardiovascular disease,³,⁵-⁷ but WHR is attracting greater interest,³,⁹ because of the role recently attributed to peripheral fat (fat located in upper and lower limbs) in the modulation of cardiovascular risk.

Only a few studies have addressed the cardiovascular role of peripheral fat mass,³,⁵-⁷ which might be less atherogenic than abdominal fat, due to a low fatty acid turnover, and a differential hormone production.³,⁵-⁷ Moreover, because
women and men have different patterns of fat distribution, the association of peripheral fat with coronary outcomes might also vary with sex.

Using data from a population-based case-control study, we have examined the independent effect of surrogate measures for general, central and peripheral body fat on the occurrence of non-fatal acute myocardial infarction (AMI) in each sex.

Materials and methods

Study design and participants

A population-based case-control study was conducted among Portuguese Caucasian adults in Porto, a large urban center with almost 300,000 inhabitants in the northwest of Portugal.

Cases were patients aged >40 years admitted consecutively, from 1999 through 2003, to the Cardiology Department of the four hospitals providing acute coronary care in Porto, who survived beyond the fourth day after a first AMI. Diagnosis of AMI was carried out with standard procedures. Controls were selected from the non-institutionalized adult population of the hospital's catchment area. For each household identified by random digit dialing, permanent residents were characterized according to age and sex, and one participant was selected by simple random sampling. Refusals were not substituted and the participation rate was approximately 70%.

The ethics committees of the four participating hospitals approved the study protocol and every participant gave written informed consent.

During the study period, 1106 patients aged >40 years with an incident AMI were identified. The following cases were excluded from the analyses: 60 who were unable to collaborate; 3 who refused to participate; 34 who died before data collection; 82 who did not complete the interview; 133 with missing data on anthropometrics and 3 on selected confounders; and 138 with cognitive impairment. Out of 2000 community participants, the following were excluded: 103 (5.2%) because of a previous AMI based on a self-report or an 12-lead electrocardiogram; 121 for incomplete information on anthropometrics and 13 on selected confounders; and 50 with cognitive impairment. Therefore, analyses were conducted with 653 cases (163 women and 490 men) and 1713 controls (1065 women and 648 men).

Data collection

Data on cases and controls were collected by the same set of trained interviewers. Cases were interviewed during the hospital stay after clinical stabilization, and controls were invited to visit the Department of Hygiene and Epidemiology of the University of Porto Medical School to be evaluated.

Anthropometrics. Anthropometrics were performed with participants in light clothing and barefoot under standard procedures. Body weight was measured to the nearest 0.1 kg using a digital scale (SECA, Columbia, MO, USA) and height was measured to the nearest cm using a wall stadiometer (SECA, Hamburg, Germany). BMI was calculated dividing the weight in kg by the squared height in m. Participants were classified as underweight/normal weight (BMI < 25.0 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30.0 kg/m²), also they were classified into fourths of the BMI distribution in controls.

WC was measured midway between the lower limit of the rib cage and the iliac crest, and hip circumference on the maximum circumference over the femoral trochanters; both were measured to the nearest cm with a flexible and non-distensible tape. Abdominal obesity was defined as WC >85 cm in women and >102 cm in men, and as WHR >0.85 in women and >0.90 in men.

The thickness of triceps, biceps, subscapular and suprailiac skinfolds (SKF) was measured with a Harpenden calliper (John Bull, British Indicators Ltd, Burgess Hill, UK) at the non-dominant side of the body. SKF were grasped with the thumb and index finger approximately 1 cm proximal to the SKF site, which was measured to the nearest 0.5 mm. Three measurements were taken for each SKF, obtaining the average value. A SKF composite index was calculated to estimate the proportion of subcutaneous fat of the arms (Σ triceps and biceps SKF/Σ triceps, biceps, subscapular and suprailiac SKF). Participants were classified into fourths of the distribution of the SKF composite index in controls.

Confounders. Data on potential confounders such as social, behavioral and clinical characteristics were collected with a standard structured questionnaire. Education was recorded as completed years of schooling. Participants were also classified as current smokers (daily and occasional smokers) and non-smokers (never and former smokers quitting at least 6 months ago). Alcohol consumption was assessed with a validated semi-quantitative food frequency questionnaire, concerning the previous 12 months. The intake of alcoholic beverages was converted into total alcohol intake using the software Food Processor Plus (ESHA Research, Salem, OR, USA, 1997). Physical activity corresponded to the regular practice (at least 30 min per week) of any leisure-time physical activity with energy expenditure higher than 2.5 metabolic equivalents per hour, including walking, running and the practice of any type of sport during the previous year.

A family history of AMI was registered when one or more first-degree relatives had suffered an AMI, regardless of the age at occurrence. Information on menopause and hormone replacement therapy was also recorded for all women. Individuals aged 65 or more years underwent a Mini-Mental State Examination (MMSE) to assess cognitive function (those scoring <24 in the Mini-Mental State Examination...
were considered as cognitive impaired to provide reliable information).

Statistical analysis
Characteristics of cases and controls were compared with the Mann-Whitney test for continuous variables, and with the χ² test or the Fisher's exact test, as appropriate, for categorical variables. Correlations between the anthropometric measures were evaluated by Spearman correlation coefficients.

Principal component analysis 30 with varimax rotation was used to identify factors representing uncorrelated components of body fat. The number of factors to identify must explain more than 90% of the total variance of anthropometry. Factor's interpretation was based on the correlations between the anthropometrics and the identified factors, obtained from the factor's loadings.

The association between the anthropometric variables and the risk of AMI were summarized with odds ratios (OR) and their 95% confidence intervals (95% CI), obtained from unconditional logistic regression. ORs were adjusted for age, education and alcohol consumption, modelled as continuous variables, and for current smoking, regular physical activity, and menopause and hormone replacement therapy in women, modelled as categorical variables with dummy terms. The dose–response relationship between the anthropometric variables and AMI was assessed with tests for linear trend, by modelling the anthropometric measures as continuous variables.

Analyses were conducted separately in each sex. We tested for sex interactions in analyses of the whole study sample using interaction terms, constructed as the product of sex by anthropometric variables. Statistical significance was set at P-value < 0.05. Analyses were performed using Stata, version 9 (StataCorp. College Station, TX, USA).

Results
Table 1 shows the characteristics of AMI cases and controls, by sex. In women, cases were slightly older than controls, while the opposite was found in men. As compared with controls, cases had lower education, were less physically active, and reported more frequently a family history of AMI. Male cases had also a higher alcohol intake and reported a higher prevalence of smoking than controls.

Tables 2 and 3 show the association between the main anthropometric variables and AMI in women and men, respectively. In multivariate analysis, BMI showed no clear association with AMI, either when classified in WHO (World Health Organization) categories or in fourths, in women or men. WC was associated with an increased risk of AMI, while hip circumference showed an inverse association. As a result, WHR had a strong and graded relationship with the risk of AMI. Finally, the SKF composite index, reflecting a high proportion of peripheral subcutaneous fat, was found to be inversely associated with AMI in women (upper vs lower fourth: OR 0.44, 95% CI 0.26–0.74) and directly associated with AMI in men (OR 1.97, 95% CI 1.37–2.84; P-value for the sex interaction < 0.001).

As within the general population, significant moderate-to-strong Spearman correlation coefficients (r) were found between BMI and WC (r = 0.79), BMI and WHR (r = 0.37), BMI and hip circumference (r = 0.82), WC and WHR (r = 0.75), and WHR and SKF composite index (r = 0.40), the results of Tables 2 and 3 did not present mutual adjustments.

Using principal component analysis, we identified three uncorrelated factors, which explained 97.3% of the total variance of anthropometry (Table 4). These three factors represented different patterns of fat distribution: (1) generalized fat, particularly high BMI and high WC; (2) central fat, mainly high WC and high WHR; and (3) peripheral subcutaneous fat. After adjustment for the main confounders, the first factor showed no significant association with AMI, but the second factor increased AMI risk in each sex (upper vs lower fourth: OR 12.2, 95% CI 5.34–27.9 in women; OR 25.0, 95% CI 14.0–44.7 in men) (Table 5). In contrast, the third factor was inversely associated with AMI in women (upper vs lower fourth: OR 0.59, 95% CI 0.36–0.96) and directly associated in men (OR 2.45, 95% CI 1.69–3.55; P-value for sex interaction < 0.001).

### Table 1: Characteristics of acute myocardial infarction cases and controls, by sex

<table>
<thead>
<tr>
<th></th>
<th>Women ≥ 40 years</th>
<th>Men ≥ 40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 163)</td>
<td>Controls (n = 1065)</td>
</tr>
<tr>
<td>Age, years, median (25th–75th)</td>
<td>62 (32–69)</td>
<td>56 (48–66)</td>
</tr>
<tr>
<td>Education, years, median (25th–75th)</td>
<td>4 (3–4)</td>
<td>5 (4–11)</td>
</tr>
<tr>
<td>Alcohol intake, g/day, median (25th–75th)</td>
<td>0 (0–11.6)</td>
<td>0.8 (0–11.6)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>29 (17.8)</td>
<td>143 (13.4)</td>
</tr>
<tr>
<td>Irregular physical activity, n (%)</td>
<td>18 (11.0)</td>
<td>225 (10.5)</td>
</tr>
<tr>
<td>Family history of infarction, n (%)</td>
<td>63 (38.9)</td>
<td>303 (28.5)</td>
</tr>
<tr>
<td>Menopause</td>
<td>23 (14.1)</td>
<td>198 (18.6)</td>
</tr>
<tr>
<td>without HRT</td>
<td>103 (63.2)</td>
<td>517 (48.6)</td>
</tr>
</tbody>
</table>

Abbreviations: 25th, 25th percentile; 75th, 75th percentile; HRT, hormone replacement therapy.
<table>
<thead>
<tr>
<th>WHO categories</th>
<th>Cases n = 163 (%)</th>
<th>Controls n = 1065 (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 23.0</td>
<td>43 (23.4)</td>
<td>399 (31.8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>71 (43.6)</td>
<td>420 (39.4)</td>
<td>1.33 (0.89–2.00)</td>
<td>0.90 (0.58–1.40)</td>
</tr>
<tr>
<td>≥ 30.0</td>
<td>49 (30.1)</td>
<td>396 (28.7)</td>
<td>1.26 (0.81–1.96)</td>
<td>0.70 (0.43–1.14)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td>0.298</td>
<td></td>
<td></td>
<td>0.143</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 88</td>
<td>48 (29.4)</td>
<td>383 (35.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>88–94</td>
<td>115 (70.6)</td>
<td>482 (45.3)</td>
<td>2.90 (2.03–4.16)</td>
<td>2.10 (1.42–3.11)</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.85</td>
<td>17 (10.4)</td>
<td>488 (45.8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 0.85</td>
<td>146 (89.6)</td>
<td>567 (33.2)</td>
<td>7.54 (4.50–12.6)</td>
<td>5.84 (3.37–10.1)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 94</td>
<td>16 (9.8)</td>
<td>233 (23.9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>94–100</td>
<td>26 (16.0)</td>
<td>266 (25.0)</td>
<td>1.56 (0.82–2.97)</td>
<td>1.28 (0.65–2.45)</td>
</tr>
<tr>
<td>≥ 100</td>
<td>42 (25.8)</td>
<td>268 (23.2)</td>
<td>1.20 (0.74–1.95)</td>
<td>0.67 (0.40–1.15)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td></td>
<td>0.324</td>
</tr>
<tr>
<td>WHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.85</td>
<td>79 (48.5)</td>
<td>272 (25.5)</td>
<td>4.63 (2.63–8.13)</td>
<td>2.86 (1.33–5.35)</td>
</tr>
<tr>
<td>≥ 0.85</td>
<td>264 (34.3)</td>
<td>269 (25.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peripheral subcutaneous fat (cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (lower)</td>
<td>59 (36.2)</td>
<td>260 (24.4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>32 (19.6)</td>
<td>254 (23.8)</td>
<td>0.56 (0.35–0.88)</td>
<td>0.56 (0.34–0.91)</td>
</tr>
<tr>
<td>3</td>
<td>43 (26.4)</td>
<td>272 (25.3)</td>
<td>0.67 (0.44–1.03)</td>
<td>0.57 (0.36–0.90)</td>
</tr>
<tr>
<td>4 (upper)</td>
<td>29 (17.8)</td>
<td>269 (23.3)</td>
<td>0.47 (0.20–0.76)</td>
<td>0.35 (0.21–0.58)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td>0.005</td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**Discussion**

In our study, WC and WHR were strongly associated with AMI risk, while hip circumference showed an inverse association, in men and women alike. A high proportion of subcutaneous fat in the arms, as measured by SKF thickness, predicted a lower risk of AMI in women and a higher risk in men.

A previous investigation with a smaller sample size in this same population has reported that upper-body adiposity was associated with a higher risk of AMI, and that this association was stronger than that observed for BMI. A higher BMI may...

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**Abbreviations:** OR (95% CI), odds ratio (95% confidence intervals); WHO; World Health Organization; ^1 Odds ratio adjusted for age, education, alcohol consumption, current smoking, leisure-time physical activity, family history of infarction, and menopause and hormone replacement therapy. Quantiles of the distribution of the anthropometrics in controls. Body mass index in kg m⁻²: 1 (<24.2), 2 (24.2–27.1), 3 (27.2–30.5), 4 (>30.5); Waist circumference in cm: 1 (<79.0), 2 (79.0–86.9), 3 (87.0–93.4), 4 (>93.4); Hip circumference in cm: 1 (<96.0), 2 (96.0–100.9), 3 (101.0–107.4), 4 (>107.4); Waist-to-hip ratio: 1 (<0.808), 2 (0.808–0.855), 3 (0.856–0.907), 4 (>0.907); Skinfolds index of peripheral fat: 1 (<0.182), 2 (0.182–0.468), 3 (0.469–0.515), 4 (>0.515); Skinfolds (SF) composite index: 1 (sacro and biceps SF) 2 (sacro and triceps SF) 3 (triceps and biceps SF) 4 (triceps and biceps)
Table 3  Association of anthropometric variables with acute myocardial infarction in men

<table>
<thead>
<tr>
<th>WHO categories</th>
<th>Cases n = 490 (%)</th>
<th>Controls n = 648 (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 23.0</td>
<td>160 (32.6)</td>
<td>227 (35.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>23.0-29.9</td>
<td>279 (55.9)</td>
<td>322 (49.7)</td>
<td>1.21 (0.93-1.56)</td>
<td>1.34 (1.00-1.81)</td>
</tr>
<tr>
<td>≥ 30.0</td>
<td>56 (11.4)</td>
<td>99 (15.3)</td>
<td>0.80 (0.55-1.18)</td>
<td>0.79 (0.51-1.22)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>0.709</td>
<td>0.8±2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 102</td>
<td>405 (82.2)</td>
<td>537 (82.9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 102</td>
<td>87 (17.8)</td>
<td>111 (17.1)</td>
<td>1.04 (0.77-1.42)</td>
<td>1.17 (0.83-1.66)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.90</td>
<td>15 (3.1)</td>
<td>165 (25.5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 0.90</td>
<td>475 (96.9)</td>
<td>483 (74.5)</td>
<td>10.8 (6.28-18.6)</td>
<td>10.9 (6.10-19.4)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>0.523</td>
<td>0.49</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 75</td>
<td>101 (21.2)</td>
<td>131 (23.8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 75</td>
<td>116 (23.7)</td>
<td>176 (26.2)</td>
<td>0.99 (0.70-1.40)</td>
<td>1.15 (0.78-1.70)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>0.111</td>
<td>0.011</td>
</tr>
<tr>
<td>Hip-to-hip ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.75</td>
<td>253 (51.5)</td>
<td>354 (54.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 0.75</td>
<td>120 (24.4)</td>
<td>148 (22.8)</td>
<td>0.58 (0.41-0.81)</td>
<td>0.65 (0.46-0.92)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Perineal subcutaneous fat (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 22.6</td>
<td>111 (22.6)</td>
<td>161 (24.8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 22.6</td>
<td>91 (18.6)</td>
<td>161 (24.8)</td>
<td>0.82 (0.58-1.17)</td>
<td>0.88 (0.59-1.31)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: OR (95% CI), odds ratio (95% confidence intervals); WHO, World Health Organization; *Odds ratio adjusted for age, education, alcohol consumption, current smoking, leisure-time physical activity, and family history of infarction. #Quartiles of the distribution of the anthropometrics in controls. Body mass index in kg/m²; 1 (< 23.0), 2 (23.0-26.0), 3 (26.1-28.6), 4 (28.6); Waist circumference in cm: 1 (< 87.0), 2 (87.0-93.0), 3 (93.1-99.0), 4 (> 99.0); Hip circumference in cm: 1 (< 94.5), 2 (94.5-98.9), 3 (99.0-103.4), 4 (> 103.4). Waist-to-hip ratio: 1 (< 0.865), 2 (0.865-0.945), 3 (0.945-0.981), 4 (> 0.981). Skinfolds index of peripheral fat: 1 (< 0.315), 2 (0.315-0.355), 3 (0.356-0.404), 4 (> 0.404). Skinfolds (SK) composite index: triceps and biceps SK; triceps, biceps, subcapular and suprailiac SK.

reflect increased fatness, but also higher musculoskeletal mass; moreover BMI is not a good measure of visceral fat, the key determinant of metabolic abnormalities. Thus, the association between BMI and AMI might be expected to be weak or even null after adjustment for cardiovascular risk factors.

WC, and in particular WHR, showed a strong dose-response relationship with AMI after adjustment for the main cardiovascular risk factors. Similarly, in the INTERHEART study, a case-control study with 29,972 AMI patients across 52 countries, WHR held a stronger association with AMI than BMI or WC, regardless of sex, age, region of
Table 4  Correlations between the anthropometric variables and the three anthropometric factors identified from principal component analysis

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th></th>
<th>Factor 2</th>
<th></th>
<th>Factor 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearsen rho</td>
<td>p</td>
<td>Spearsen rho</td>
<td>p</td>
<td>Spearsen rho</td>
<td>p</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.98</td>
<td>&lt;0.001</td>
<td>0.06</td>
<td>0.006</td>
<td>0.03</td>
<td>0.022</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.80</td>
<td>&lt;0.001</td>
<td>0.52</td>
<td>&lt;0.001</td>
<td>−0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip circumference</td>
<td>0.17</td>
<td>&lt;0.001</td>
<td>0.92</td>
<td>&lt;0.001</td>
<td>−0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral subcutaneous fat</td>
<td>−0.02</td>
<td>0.290</td>
<td>−0.17</td>
<td>&lt;0.001</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Total variance explained by the three factors: 97.3%. Skinfolds (SKF) composite index (Σ triceps and biceps SKF/Σ triceps, biceps, subscapular and supraclavicular SKF).

Table 5  Association between the three anthropometric factors identified from principal component analysis and acute myocardial infarction, by sex.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Controls n (%)</th>
<th>Odds OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n (%)</td>
<td>Controls n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor 1 (generalized fat), fourths</td>
<td>1 (lower)</td>
<td>35 (21.3)</td>
<td>266 (23.0)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43 (26.4)</td>
<td>266 (23.0)</td>
<td>1.23 (0.76–1.98)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46 (28.2)</td>
<td>268 (23.2)</td>
<td>1.30 (0.81–2.09)</td>
</tr>
<tr>
<td></td>
<td>4 (upper)</td>
<td>39 (23.9)</td>
<td>265 (24.8)</td>
<td>1.12 (0.69–1.82)</td>
</tr>
<tr>
<td></td>
<td>P-value for trend</td>
<td>0.619</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Controls n (%)</th>
<th>Odds OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases n (%)</td>
<td>Controls n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor 1 (generalized fat), fourths</td>
<td>1 (lower)</td>
<td>53 (32.5)</td>
<td>266 (23.0)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>39 (23.9)</td>
<td>266 (23.0)</td>
<td>0.74 (0.47–1.15)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37 (22.7)</td>
<td>266 (23.0)</td>
<td>0.70 (0.44–1.10)</td>
</tr>
<tr>
<td></td>
<td>4 (upper)</td>
<td>109 (66.9)</td>
<td>267 (23.0)</td>
<td>15.5 (7.09–33.9)</td>
</tr>
<tr>
<td></td>
<td>P-value for trend</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                  | Factor 2 (central fat), fourths |      |                  |                     |
|                  | Cases n (%) | Controls n (%) |                  |                     |
|                  | 1 (lower) | 7 (4.29) | 266 (23.0) | 1 | 1 |
|                  | 2      | 10 (6.13) | 266 (23.0) | 1.43 (0.84–2.41) | 1.12 (0.61–1.94) |
|                  | 3      | 37 (22.7) | 266 (23.0) | 5.28 (2.13–13.1) | 4.03 (1.72–9.45) |
|                  | 4 (upper) | 109 (66.9) | 267 (23.0) | 15.5 (7.09–33.9) | 12.7 (5.34–27.9) |
|                  | P-value for trend | 0.619 |            |             | 0.057 |

|                  | Factor 3 (peripheral fat), fourths |      |                  |                     |
|                  | Cases n (%) | Controls n (%) |                  |                     |
|                  | Q1 (lower) | 53 (32.5) | 266 (23.0) | 1 | 1 |
|                  | Q2      | 39 (23.9) | 266 (23.0) | 0.74 (0.47–1.15) | 0.65 (0.41–1.05) |
|                  | Q3      | 37 (22.7) | 266 (23.0) | 0.70 (0.44–1.10) | 0.60 (0.37–0.97) |
|                  | Q4 (upper) | 109 (66.9) | 267 (23.0) | 15.5 (7.09–33.9) | 12.7 (5.34–27.9) |
|                  | P-value for trend | 0.545 |            |             | 0.030 |

|                  | Factor 2 (generalized fat), fourths |      |                  |                     |
|                  | Cases n (%) | Controls n (%) |                  |                     |
|                  | 1 (lower) | 128 (20.1) | 162 (23.0) | 1 | 1 |
|                  | 2      | 145 (29.6) | 162 (23.0) | 1.05 (0.76–1.44) | 1.30 (0.90–1.86) |
|                  | 3      | 79 (16.1) | 162 (23.0) | 0.57 (0.40–0.81) | 0.67 (0.45–1.00) |
|                  | 4 (upper) | 34 (20.9) | 267 (23.0) | 0.64 (0.40–1.01) | 0.59 (0.36–0.96) |
|                  | P-value for trend | 0.013 |            |             | 0.160 |

|                  | Factor 3 (peripheral fat), fourths |      |                  |                     |
|                  | Cases n (%) | Controls n (%) |                  |                     |
|                  | 1 (lower) | 94 (23.2) | 162 (23.0) | 1 | 1 |
|                  | 2      | 85 (17.4) | 162 (23.0) | 0.90 (0.63–1.30) | 0.99 (0.66–1.49) |
|                  | 3      | 111 (22.6) | 162 (23.0) | 1.18 (0.83–1.68) | 1.43 (0.96–2.13) |
|                  | 4 (upper) | 200 (40.8) | 162 (23.0) | 2.13 (1.33–3.35) | 2.43 (1.69–3.55) |
|                  | P-value for trend | <0.001 |            |             | <0.001 |

Abbreviation: OR (95%CI), odds ratio (95% confidence intervals). *Odds ratio adjusted for age, education, total energy intake, current smoking, leisure time physical activity, family history of infarction, and menopause and hormone replacement therapy (in women).

residence and ethnicity. In a large cohort of Swedish women, after mutual adjustment, WHR was also more closely related to coronary heart disease than BMI;* moreover, in the Dallas Heart Disease study WHR was associated with prevalent atherosclerosis and provided better discrimination than either BMI or WC. A recent meta-analysis of longitudinal studies, covering 258,114 participants with 4,355 cardiovascular events, also found that WHR was more strongly
associated with cardiovascular disease than WC, although this difference was not statistically significant. The strength of association for WHR and WC was similar in men and women.

Our results suggest that the association of WHR with AMI could reflect the separate and opposing metabolic effects of central and peripheral adiposity, as assessed by waist and hip circumferences, respectively. Thus, the adverse effects of a high WHR could be due to not only a larger waist, but also to narrower hip or thigh circumferences. Therefore, a large WC could have a different effect on cardiovascular risk according to the accumulation of fat in peripheral depots. A recent review has highlighted the need for capturing the separate effects of abdominal and peripheral adiposity, and suggests that WHR is a simple and inexpensive measure to improve the assessment of coronary heart disease risk.

Data on the specific role of peripheral fat mass on coronary risk are scarce. A few studies have suggested that fat accumulated in peripheral depots, such as arms and legs, has less adverse effects on cardiovascular risk than other types of fat stores. Peripheral adipose tissue seems to have a higher lipoprotein lipase activity and a lower fatty acid turnover, and to secrete higher quantities of adiponectin with antithromogenic properties. There is also evidence that adipocytes have distinct intrinsic characteristics (for example, fatty acid-binding proteins and enzymes of fat metabolism), which further contribute to the heterogeneity in free fatty acids handling by the different fat depots.

The separate contribution of upper and lower limbs to cardiovascular risk is also currently under discussion. Some studies have found that lower limbs fat seems to be inversely related to cardiovascular risk factors. However, the issue of whether the protective role of a larger hip or thigh circumference is due to a higher fat and/or a higher lean mass in the gluteal/femoral region remains to be fully understood. On the basis of the conflicting results concerning the selective role of appendicular body fat on cardiovascular risk factors, further investigation is needed before a plausible biological explanation can be definitively put forward.

In our study, peripheral subcutaneous fat, as measured by an arm SKI composite index, yielded opposing associations with AMI in women and men. As men and women have different ranges of variation in levels of body fat, a sex effect in the relation between fat and AMI could be expected; men have a larger proportion of fat within the visceral area, while women have higher levels and higher variability of total and subcutaneous peripheral fat. Sex hormones could also contribute to some of the differences found, although analyses were adjusted for hormone replacement therapy. In an initial analysis, we observed that men in the upper fourth of the SKI composite index had also lower hip circumferences, which led us to hypothesize that the lack of a protective effect of peripheral subcutaneous fat in men could partly be due to the lower amount of fat around the hip when compared with women. However, after further adjustment for hip circumference, the SKI composite index maintained a significant direct independent association with AMI (only a small attenuation of risk estimates was observed).

Comparing our results with the literature, we found that studies conducted in women systematically concluded that peripheral body fat was inversely related to cardiovascular risk, but some studies in men have shown that peripheral fat was inversely related to insulin sensitivity and directly related to lipoprotein concentrations, blood pressure and insulin levels. In the latter study, conducted among middle-aged Portuguese men, the researchers concluded that appendicular accumulation of fat, mainly in the arms, had a detrimental effect as also exerted by subcutaneous abdominal obesity, but not as marked.

Future research addressing the metabolic effect of different depots of peripheral fat (lower vs upper limbs), and of the same peripheral fat depot separately in men and women, are warranted to clarify the mechanisms by which adipocytes influence cardiovascular risk.

Methodological aspects. In a previous comparison between community controls (participation rate 70%) and those who refused to participate in this study, non-participation had little or no effect on crude and adjusted risk estimates for AMI, which suggests a low likelihood of non-response bias. Moreover, the recruitment of AMI cases was carried out consecutively in all hospitals providing acute coronary care in Porto. As all individuals with AMI that reach any healthcare center are referred to public hospitals, cases in our study represent all cases with non-fatal AMI in Porto.

Our study included only incident cases of AMI; it contributed to exclude the effect on the study associations of behavioral modifications occurring after the acute event, which would probably influence anthropometrics in the long term. It is nevertheless possible that AMI cases tend to lose weight immediately after hospitalization. To minimize the influence of weight loss on risk estimates, cases were interviewed as soon as possible, usually between the fourth and eighth day after hospitalization. It also allowed for registering lifestyles corresponding only to the time period preceding the AMI event.

In this study, surrogates of adiposity based on body circumferences and SKI thickness were used due to logistical limitations; however, the correlation between indirect and direct measures of body fat is reasonably high. Moreover, anthropometrics represents simple, inexpensive and useful methods to be applied in large epidemiological studies. In this study, we did not obtain an estimate of total peripheral subcutaneous fat, namely, the sum of upper arm and leg SKI, but it has been suggested that the role of appendicular fat should be independently analyzed, because of a purported specific role of arms and legs fat on cardiovascular risk. In addition, anthropometrics were performed by the same set of
Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We gratefully acknowledge the Head and Staff of the Cardiology Departments of the four hospitals collaborating in this study: Hospital São João; Hospital Pedro Hispano; Centro Hospitalar Vila Nova de Gaia and Hospital Geral de Santo António. This work was funded by grant supports from Fundação para a Ciência e a Tecnologia, Portugal. (POCTI/ESP/42361/2001, POCTI/SIU/ESP/661160/2004, SFRH/BD/31131/2006).

References


Paper III

The association of fruit, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: sex and body mass index interactions

Andreia Oliveira, Fernando Rodríguez Artalejo, Carla Lopes

ORIGINAL ARTICLE

The association of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: sex and body mass index interactions

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Objective: To study the associations of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein (hs-CRP). Existing literature on these associations is scarce and has rendered conflicting results.

Methods: Cross-sectional study of 1060 individuals (675 women, 385 men), representative of the non-institutionalized population, aged ≥18 years, in Porto, Portugal (70% participation rate). Diet over the previous year was assessed with a validated food frequency questionnaire. Associations between diet and hs-CRP (categorized into < 1, 1–3, > 3 to < 10 mg/l) were obtained from ordinal logistic regression models (odds ratio, 95% confidence intervals-OR, 95% CI) adjusted for sociodemographic and behavioural variables.

Results: In normal weight men (body mass index (BMI) < 25.0 kg/m²), for each 100 g increase in fruit and vegetable intake, there was 30% less probability of changing of hs-CRP category (no risk to moderate risk, or moderate to high risk). Protective associations were also observed between hs-CRP and fruits (OR = 0.73, 95% CI 0.56–0.96 per 100 g/day), vegetables (OR = 0.55, 95% CI 0.35–0.86 per 100 g/day), vitamin C (OR = 0.34, 95% CI 0.14–0.80 per 10 mg/day) and vitamin E (OR = 0.14, 95% CI 0.02–0.88 per 1000 retinol equivalents per day). Overall, associations tended to be weaker in overweight participants. In men (BMI ≥ 25.0 kg/m²), fibre was also negatively associated with hs-CRP. In women, no significant associations were found between dietary variables and hs-CRP. A significant modification effect of the evaluated associations was found by sex for fruits and vegetables, vitamin C and fibre, but not by BMI.

Conclusion: Intake of fruits and vegetables, vitamin C, E and fibre were negatively associated with hs-CRP in men.

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Keywords: fruits; vegetables; dietary fibre; antioxidants; C-reactive protein; effect modifier epidemiology

Introduction

Adequate intake of fruits and vegetables has been recommended for the prevention of chronic diseases, particularly cardiovascular disease (Lock and Pomerleau 2005; Lichtenstein et al., 2006). Antioxidant vitamins and dietary fibre could independently or jointly be responsible for the reduced cardiovascular risk associated with fruits and vegetables (Van Duyn and Pivonka 2000; Maron, 2004), but knowledge on these specific mechanisms is still insufficient. The ability of fruits and vegetables to reduce low-density lipoprotein-cholesterol oxidation is often claimed (Van Duyn and Pivonka 2000; Hu and Willett, 2002a), but research has also focused on some anti-inflammatory effects. High-sensitivity C-reactive protein (hs-CRP), a stable acute-phase inflammatory reactant, has been proposed as a powerful predictor of cardiovascular disease (Pearson et al., 2003), which highlights the importance of understanding its relation with fruit and vegetable consumption.
Prudent dietary patterns—rich in plant-based foods—have been associated with reduced levels of inflammatory markers (Lopez-Garcia et al., 2004, Nettleton et al., 2006, Panagiotakos et al., 2006), but evidence is still scarce on the independent association between overall fruit and vegetable intake and inflammation. Most studies on this issue were randomized trials with very specific exposures, such as high-pressureized orange juice (Sanchez-Moreno et al., 2003), carotenoid-rich vegetables and fruits (Watzl et al., 2005), sweet cherries (Kelley et al., 2006), berries and apples (Freese et al., 2004) and vegetable soup 'gazpacho' (Sanchez-Moreno et al., 2004), and provided non-conclusive results, as most of them found decreased inflammatory levels with the intake of these food items, but one failed to show a reduction of CRP after several weeks of intervention (Freese et al., 2004). All of the observational studies reported an inverse association between fruit and vegetable intake and inflammatory markers (Esmailizadeh et al., 2006), mainly in the elderly (Gao et al., 2004, Wannamethee et al., 2006), but none have examined the effect of fruits separately from vegetables, in men and women of all ages from the same population.

Fruits and vegetables are major contributors to fibre and antioxidant vitamin intake. Whereas a metabolic effect of fibre on markers of inflammation has been reported (Kring, 2005), most previous studies, which evaluated the association of inflammatory markers with vitamins, have assessed vitamin intake using plasma levels (Ford et al., 2003, van Herpen-Broekmans et al., 2004) and vitamin supplement use (Bruunsgaard et al., 2003, Sanchez-Moreno et al., 2003).

Lastly, the modification of these associations by body fat, an important source of pro-inflammatory cytokines, has never been tested.

Therefore, this study aims to evaluate the effect of fruits, vegetables, antioxidant vitamins and fibre intake on hs-CRP levels in men and women from the general population, and to examine the modification of these associations by sex and body mass index (BMI).

Methods

Participants

Participants were selected, by random digit dialling (Hartge et al., 1984), within the non-institutionalized population in Porto, a large urban centre in the north-west of Portugal with almost 300000 inhabitants. For each household selected, permanent residents were identified according to sex and age, and one participant, among those aged ≥18 years, was selected by simple random sampling. The participants' evaluation was performed between 1999 and 2003 (n = 2485). Refusals were not replaced and the participation rate was 70% (Ramos et al., 2004).

A rapid evaluation of the participant's cognitive function was done using the Mini-Mental State Examination (Folstein et al., 1975) in those aged ≥65 (participants were excluded if they scored <24 points).

Data collection

Data was collected by trained interviewers using a structured questionnaire on social, demographic, clinical, behavioural and anthropometric characteristics.

Fruits, vegetables, antioxidant vitamins and fibre intake. Dietary intake over the previous 12 months was estimated with an 82-item semi-quantitative food frequency questionnaire (Lopes, 2000). Each participant was asked about the mean frequency of consumption (nine categories ranging from 'never or less than once a month' to '≥6 times a day'), the average portion consumed (lower, equal or higher than the mean portion size) and the seasonal variation of consumption. The questionnaire has 16 items related to vegetables and 16 items related to fruits. In this study, the fruit and vegetable definitions only considered fresh fruits and natural fruit juices, and only fresh vegetables and vegetable soups.

The food frequency questionnaire had been previously validated by comparison with four 7-day food records (each one in a different season of the year), among 146 participants of the Porto population (Lopes, 2000). The Spearman correlation coefficients adjusted for sex, age, education and total energy intake were 0.66 for fibre, 0.56 for vitamin C, 0.45 for vitamin E and 0.49 for carotenoids. The reproducibility of the questionnaire was also tested, applying it to a subsample of 150 individuals, 1 year after the first evaluation. The adjusted Spearman correlation coefficients obtained were 0.42, 0.48, 0.38 and 0.45, for fibre, vitamin C, vitamin E and carotenoids, respectively.

Food consumption was converted into nutrients by the software Food Processor Plus (ESHA Research, Salem, OR, USA), which has been adapted to traditional Portuguese food and dishes.

High-sensitivity C-reactive protein

Blood was sampled after a 12-h overnight fast. Serum samples were stored at −20°C. Levels of hs-CRP were determined by means of particle-enhanced immunonephelometry using an auto-analyser Behring, Nephelometer II, BN II (Dade Behring Marburg GMBH, D-35041 Marburg, Germany).

For analysis, the hs-CRP was categorized into three groups (mg/l): <1.00; 1.00–3.00; >3.00 to ≤10, corresponding, respectively, to low, moderate and high risk of
developing an auto-analyser Behring, Nephelometer II, BN IIa (Dade Behring Marburg GmbH, D-35041 Marburg, Germany), cardiovascular disease, according to the guidelines of the American Heart Association (Class IIa, Level of Evidence B) (Pearson et al., 2003).

Socio-demographic, behavioural and anthropometric characteristics. We obtained information on age and education, recorded as completed years of aging and schooling. Smoking was also recorded and participants classified into current smokers (daily and occasional smokers) and non-smokers (never and former smokers, at least 6 months ago). Physical exercise referred to the regular practice (at least 30 min per week) of any leisure-time physical activity with energy expenditure higher than 2.5 metabolic equivalents, including walking, running and any sports activities, during the previous year.

Anthropometrics were performed by trained observers, according to standard procedures with participants in light clothing and barefoot. BMI was calculated as the weight (kg) divided by the squared height (m). Normal weight and overweight were defined, respectively, by a BMI <25.0 and ≥25.0 kg/m² (Expert Panel on the Identification Evaluation Treatment of Overweight in Adults, 1998).

Ethics
The local ethics committee approved the study protocol. All participants gave a written informed consent to participation, and the study was carried out in accordance with the Helsinki Declaration II.

Statistical analysis
Proportions were compared using the χ²-test, and the Mann-Whitney test was computed to compare medians between two independent samples.

The associations between dietary intake (independent variables, treated as continuous) and hs-CRP (dependent variable into three categories: <1.00; 1.00–3.00; >3.00 to ≤10 mg/l) were tested by ordinal logistic regression. Odds ratio and the respective 95% confidence intervals (OR, 95% CI) indicate the probability of changing hs-CRP category (no risk (<1.00 mg/l) to moderate risk (1.00–3.00 mg/l), or moderate to high risk (>3.00 to ≤10 mg/l)) for each unit increase of the independent variable. Adjustments were made for age, education and total energy intake, as continuous variables, and for current smoking and exercise, as dummy variables.

When assessing the association between dietary variables and hs-CRP, an effect modification by sex and BMI was evaluated in the regression models testing interaction terms between them.

P-values were two-tailed and statistical significance was set at P < 0.05. Analyses were performed with STATA version 9.0 (StataCorp. College Station, TX, USA).

Results
Table 1 shows the demographic, behavioural and anthropometric characteristics of participants as well as hs-CRP levels by sex. Men were significantly more educated and had significantly lower levels of hs-CRP than women. Women had lower total energy intake and reported a higher intake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women (n = 675)</th>
<th>Men (n = 385)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 (43-63)</td>
<td>55 (44-66)</td>
<td>0.113</td>
</tr>
<tr>
<td>Education (years)</td>
<td>6 (4-12)</td>
<td>6 (4-12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/l)</td>
<td>1.9 (0.9-3.8)</td>
<td>1.4 (0.7-3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1980 (1643-2302)</td>
<td>2411 (2093-2869)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All fruits and vegetables (g/day)</td>
<td>487.0 (362.0-619.3)</td>
<td>453.9 (333.2-605.4)</td>
<td>0.061</td>
</tr>
<tr>
<td>Fresh fruit</td>
<td>284.2 (196.4-383.4)</td>
<td>268.4 (187.0-383.9)</td>
<td>0.162</td>
</tr>
<tr>
<td>Vegetables</td>
<td>186.5 (127.3-260.2)</td>
<td>186.2 (118.8-252.8)</td>
<td>0.178</td>
</tr>
<tr>
<td>Vegetables (without soup)</td>
<td>113.4 (70.8-172.4)</td>
<td>105.3 (62.0-159.6)</td>
<td>0.014</td>
</tr>
<tr>
<td>Vegetable soup</td>
<td>80.0 (34.3-80.0)</td>
<td>80.0 (34.3-80.0)</td>
<td>0.809</td>
</tr>
<tr>
<td>Current smoking</td>
<td>112 (16.6)</td>
<td>118 (30.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regular exercise</td>
<td>218 (32.3)</td>
<td>166 (43.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>224 (33.2)</td>
<td>126 (32.7)</td>
<td>0.879</td>
</tr>
<tr>
<td>Normal weight (&lt;25.0 kg/m²)</td>
<td>451 (66.8)</td>
<td>259 (67.3)</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; P, percentiles.
of fruits and vegetables than men, but differences were not statistically significant. Men were more likely to be smokers and to do regular exercise. There were no significant differences between sexes regarding the distribution by BMI categories.

Table 2 presents the intake of fruits, vegetables, antioxidant vitamins and fibre according to hs-CRP by sex. In men, the higher the intake of fruits, vitamin C and dietary fibre, the lower the hs-CRP levels. In women, the median intake of the food items considered did not significantly change across hs-CRP categories.

The associations between fruits, vegetables, antioxidant vitamins and fibre and hs-CRP are presented in Tables 3 and 4, for women and men, respectively.

In women, no significant associations were found between the dietary variables and hs-CRP, either in crude analyses or after adjustment for confounders.

In normal weight men, for each increase of 100 g of fruit and vegetable intake, there was 30% less probability of changing of hs-CRP category (no risk to moderate risk, or moderate to high risk) (OR=0.73, 95% CI: 0.59-0.90). The magnitude of the association decreased when considering the overweight participants (OR=0.86, 95% CI: 0.75-0.97).

Most small weight men, protective associations were observed between hs-CRP and fruits (OR=0.73, 95% CI: 0.56-0.96, for each 100 g/day), vegetables (OR=0.55, 95% CI: 0.35-0.86, for each 100 g/day), vegetables without soup (OR=0.39, 95% CI: 0.22-0.72, for each 100 g/day), vitamin C (OR=0.34, 95% CI: 0.14-0.80 for each 10 mg/day) and vitamin E (OR=0.14, 95% CI: 0.02-0.88 for each 1000 retinol equivalents per day).

In overweight men, besides the protective effect already described for all fruits and vegetables, the isolated fruit item (OR=0.80, 95% CI: 0.68-0.95, for each 100 g/day), fibre (OR=0.53, 95% CI: 0.37-0.76, for each 10 g/day) and vitamin C (OR=0.63, 95% CI: 0.41-0.97, for each 100 mg/day) were also negatively associated with hs-CRP.

On testing interaction terms in the multivariate models between sex and independent variables, we found significant sex modification effects on hs-CRP levels for all fruit and vegetable items (P<0.001), fruits (P<0.001), vitamin C (P=0.006) and fibre (P=0.006). Further, modification effects by BMI (<25 and ≥25 kg/m²) were also tested separately by sex, but the results did not achieve statistical significance, despite the slightly higher magnitude of the associations found for non-overweight men.

Discussion

The present study suggests that higher intakes of fruits and vegetables (with the exception of vegetable soup), and higher intakes of vitamins C, E and fibre are associated with lower concentrations of hs-CRP in men, but not in women.

Despite the scientific research supporting a beneficial cardiovascular effect of fruits and vegetables, potentially involved in antioxidant and inflammatory processes (Parikh et al., 2005; Hu and Willett, 2002b), very little information...
## Table 3: Associations of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein* by body mass index in women

<table>
<thead>
<tr>
<th></th>
<th>Body mass index &lt; 25.0 kg/m²</th>
<th>n = 224</th>
<th>Body mass index ≥ 25.0 kg/m²</th>
<th>n = 451</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td></td>
<td>Adjusted OR° (95% CI)</td>
<td></td>
</tr>
<tr>
<td>All fruits and vegetables (100 g/day)</td>
<td>0.96 (0.86–1.08)</td>
<td>0.98 (0.86–1.12)</td>
<td>0.99 (0.91–1.07)</td>
<td>1.00 (0.92–1.10)</td>
</tr>
<tr>
<td>Fruits (100 g/day)</td>
<td>0.96 (0.82–1.12)</td>
<td>0.98 (0.83–1.15)</td>
<td>1.04 (0.94–1.15)</td>
<td>1.05 (0.94–1.18)</td>
</tr>
<tr>
<td>Vegetables (100 g/day)</td>
<td>0.93 (0.74–1.17)</td>
<td>0.99 (0.76–1.29)</td>
<td>0.89 (0.76–1.04)</td>
<td>0.90 (0.77–1.06)</td>
</tr>
<tr>
<td>Vegetables (without soup) (100 g/day)</td>
<td>0.88 (0.66–1.17)</td>
<td>0.95 (0.69–1.30)</td>
<td>0.84 (0.69–1.02)</td>
<td>0.86 (0.70–1.05)</td>
</tr>
<tr>
<td>Soup (100 g/day)</td>
<td>1.04 (0.65–1.64)</td>
<td>1.10 (0.67–1.82)</td>
<td>0.98 (0.79–1.22)</td>
<td>0.98 (0.70–1.37)</td>
</tr>
<tr>
<td>Vitamin C (100 mg/day)</td>
<td>0.93 (0.64–1.36)</td>
<td>1.03 (0.68–1.56)</td>
<td>0.99 (0.76–1.29)</td>
<td>1.06 (0.79–1.42)</td>
</tr>
<tr>
<td>Vitamin E (10 mg/day)</td>
<td>0.74 (0.28–1.99)</td>
<td>0.90 (0.20–4.03)</td>
<td>0.91 (0.45–1.86)</td>
<td>1.01 (0.35–2.93)</td>
</tr>
<tr>
<td>Carotenoids (1000 RE/day)</td>
<td>0.58 (0.68–1.41)</td>
<td>1.09 (0.67–1.80)</td>
<td>0.81 (0.63–1.04)</td>
<td>0.73 (0.54–1.04)</td>
</tr>
<tr>
<td>Dietary fibre (10 g/day)</td>
<td>0.99 (0.73–1.33)</td>
<td>1.07 (0.71–1.61)</td>
<td>0.99 (0.98–1.02)</td>
<td>0.93 (0.71–1.23)</td>
</tr>
</tbody>
</table>

Abbreviations: RE, retinol equivalents; OR, 95% CI; odds ratio, 95% confidence intervals.
*C-reactive protein (mg/l) categories of <1.00; 1.00–3.00; >3.00 to ≤10 considered in the ordinal logistic regression. Odds ratio indicate the probability of changing hs-CRP category for each unit increase of the dietary variable.
*Adjusted for age, education, current smoking, regular exercise and total energy intake.

## Table 4: Associations of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein* by body mass index in men

<table>
<thead>
<tr>
<th></th>
<th>Body mass index &lt; 25.0 kg/m²</th>
<th>n = 126</th>
<th>Body mass index ≥ 25.0 kg/m²</th>
<th>n = 259</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td></td>
<td>Adjusted OR° (95% CI)</td>
<td></td>
</tr>
<tr>
<td>All fruits and vegetables (100 g/day)</td>
<td>0.74 (0.61–0.89)</td>
<td>0.73 (0.59–0.90)</td>
<td>0.85 (0.78–0.93)</td>
<td>0.86 (0.75–0.97)</td>
</tr>
<tr>
<td>Fruits (100 g/day)</td>
<td>0.69 (0.54–0.88)</td>
<td>0.73 (0.56–0.96)</td>
<td>0.83 (0.72–0.97)</td>
<td>0.80 (0.68–0.95)</td>
</tr>
<tr>
<td>Vegetables (100 g/day)</td>
<td>0.66 (0.44–0.98)</td>
<td>0.55 (0.35–0.86)</td>
<td>0.94 (0.75–1.18)</td>
<td>0.86 (0.67–1.10)</td>
</tr>
<tr>
<td>Vegetables (without soup) (100 g/day)</td>
<td>0.42 (0.24–0.74)</td>
<td>0.39 (0.22–0.72)</td>
<td>0.94 (0.69–1.28)</td>
<td>0.89 (0.65–1.23)</td>
</tr>
<tr>
<td>Soup (100 g/day)</td>
<td>0.72 (0.47–1.09)</td>
<td>0.90 (0.44–1.84)</td>
<td>0.71 (0.54–0.93)</td>
<td>0.79 (0.52–1.20)</td>
</tr>
<tr>
<td>Vitamin C (100 mg/day)</td>
<td>0.31 (0.15–0.65)</td>
<td>0.34 (0.14–0.80)</td>
<td>0.72 (0.49–1.08)</td>
<td>0.63 (0.41–0.97)</td>
</tr>
<tr>
<td>Vitamin E (10 mg/day)</td>
<td>0.14 (0.04–0.52)</td>
<td>0.14 (0.02–0.88)</td>
<td>0.91 (0.40–2.07)</td>
<td>0.82 (0.26–2.57)</td>
</tr>
<tr>
<td>Carotenoids (1000 RE/day)</td>
<td>0.79 (0.46–1.35)</td>
<td>0.69 (0.39–1.23)</td>
<td>0.84 (0.55–1.29)</td>
<td>0.82 (0.51–1.30)</td>
</tr>
<tr>
<td>Dietary fibre (10 g/day)</td>
<td>0.97 (0.03–1.00)</td>
<td>0.85 (0.50–1.44)</td>
<td>0.97 (0.94–0.99)</td>
<td>0.53 (0.37–0.76)</td>
</tr>
</tbody>
</table>

Abbreviations: RE, retinol equivalents; OR, 95% CI; odds ratio, 95% confidence intervals.
*C-reactive protein (mg/l) categories of <1.00; 1.00–3.00; >3.00 to ≤10 considered in the ordinal logistic regression. Odds ratio indicate the probability of changing hs-CRP category for each unit increase of the dietary variable.
*Adjusted for age, education, current smoking, regular exercise and total energy intake.

exists regarding the effects of fruits and vegetables on inflammation.

Cross-sectional studies on this issue had been carried out, but most of them were among the elderly. Gao et al., 2004 showed that among elderly individuals, a greater frequency of fruit and vegetable intake was associated with lower CRP (not hs-CRP) concentrations. In addition, Esmaillzadeh et al., 2006 showed that higher intakes of fruits and vegetables were associated with a lower risk of the metabolic syndrome, and that may be the result of lower hs-CRP concentrations. In another cross-sectional study in older men free of cardiovascular disease (Wannamethee et al., 2006), it was found that fruit and dietary vitamin C intakes were negatively associated with mean concentrations of hs-CRP, but vegetables were only associated with tissue plasminogen activator antigen.

On the other hand, the results of clinical trials are not consistent. In a 4-week randomized controlled clinical trial in 63 healthy non-smoking men (Wattz et al., 2005), the consumption of eight daily servings of carotenoid-rich fruits and vegetables significantly reduced plasma hs-CRP concentrations compared with those who consumed two daily servings. In contrast, Freese et al., 2004, in a 6-week randomized controlled study of healthy volunteers, found no difference in inflammatory markers, including serum-sensitive CRP, between those with a rich-fruits diet and those with an isocaloric diet providing lower quantities of fruit. Another two interventional studies on 12 healthy participants were conducted, showing that drinking two glasses of orange juice (250 mg of vitamin C) (Sanchez-Moreno et al., 2003) and eating vegetable soup 'gazpacho' (containing 80% raw vegetables, 2-10% olive oil and other minor components) (Sanchez-Moreno et al., 2004) decreases inflammatory levels. However, in our study, we did not find an association between vegetable soup and hs-CRP. Portuguese soups typically include other ingredients than vegetables, particu...
larly potatoes and salt, and, therefore, could be diluting the beneficial antioxidant effect of vegetables. In fact, if we compare the magnitude of the estimates between the vegetable item with and without soup, the higher protective effect on hs-CRP excluding the vegetable soup is clear.

Antioxidant diets and their effects on inflammation are also still under discussion. Although observational studies show an inverse and independent association between total dietary antioxidant capacity and markers of inflammation (Brightenti et al., 2005), supplementation studies found inconsistent results, especially when pharmacological rather than dietary amounts are considered (Bruunsgaard et al., 2003, Devaraj and Jialal 2000). In the present study, only vitamins from dietary sources were considered, as the use of vitamin supplements in our population is limited to short periods of time, and participants were not able to accurately classify the supplement type used. Moreover, there were no significant differences between the use of supplements and those not using them regarding both hs-CRP levels and fruit and vegetable consumption, hence we did not take into account the use of vitamin supplements in the final models.

A gender modification effect was observed for several of the associations studied, which might be explained in several ways. Higher levels of hs-CRP have been systematically reported among women (Festa et al., 2001, McConnell et al., 2002), which is corroborated by our study (Table 1 of participants’ profile). Women also have slightly higher intakes of fruits and vegetables than men. Together, these arguments would hypothetically suggest stronger associations among women. However, women also have a higher accumulation of fat, an important source of pro-inflammatory cytokines, which could also be responsible for the increased circulating levels of hs-CRP. Although we did not find significant differences between sex according to stratification of BMI in absolute amounts, women have significantly higher BMI than men (median 27.4 vs 26.6 kg/m², P<0.001). Therefore, we could hypothesize that in women the adipose tissue is the most important source of inflammation, and that the effect of nutrients and foods are not enough to be detected. The impact of sex hormones on circulating levels of inflammatory proteins (Barnes-Mitchell et al., 2001, Rexrode et al., 2003) could also be confounding the overall effect of diet on these levels.

Although we did not find a BMI modification effect, the same explanation could be used to explain the higher magnitude of the associations between dietary variables and hs-CRP within non-overweight participants. Increasingly, evidence has consistently related obesity with a state of low-grade chronic inflammation (Chudek and Wiecek 2006; Ferrante, 2007), hence it is possible that, in individuals with a high accumulation of fat, the latter is the most important source of inflammation, and therefore the dietary effect on hs-CRP levels is overcome.

Finally, an association between fibre intake and hs-CRP was also found among men in our study. Cross-sectional analysis with diabetics from the Health Professionals’ Follow-Up Study (Qi et al., 2005) and Nurses’ Health Study (Qi et al., 2006) pointed out that diets low in glycemic load and high in fibre may increase plasma adiponectin concentrations and, therefore, reduce inflammatory marker concentrations. An interventional study showed an inverse association between the quantity of fibre of a high-carbohydrate meal and inflammation, through the inhibition of IL-18 and stimulation of adiponectin (Esposito et al., 2003). In our study, after adjustment for potential confounders, this inverse association was only significant among overweight participants, unlike our previous findings concerning the other nutrients. The strict relation between carbohydrates and insulin metabolism could help to explain this difference. Overweight participants are more likely to have higher concentrations of insulin and counterregulatory hormones, which are directly associated with hepatic production of CRP (O’Riodain et al., 1995). In addition, the high accumulation of fat could be responsible for higher circulating levels of hs-CRP, but in this case, the effect of fat does not overcome the effect of fibre, probably because fibre, among several other nutrients, has the strongest association with hs-CRP among overweight men (regression coefficients standardized for comparison between nutrients).

Strengths and limitations

The inverse association of fruit and vegetable consumption with hs-CRP might be attributed, in part, to healthier lifestyles, frequently associated with higher intakes of these foods. Furthermore, part of the beneficial effects of fruits and vegetables could be because of their low content of energy and saturated fat. Yet, because the results from crude analyses and from models with adjustment for energy intake and major lifestyles are fairly similar, residual confounding is likely to be small.

The assessment of hs-CRP was not planned at the beginning of the participants’ evaluation. Thus, although hs-CRP measurements were performed consecutively, they are only available for a subset of participants. Comparing those with and without hs-CRP measurements, no significant differences were found regarding sex distribution, and consumption of fruits and vegetables, vitamin A and fibre. However, participants without hs-CRP assessment were slightly younger (median age 52 vs 53 years, P=0.039), more educated (median 9 vs 6 years, P<0.001) and presented lower median values of BMI (25.8 vs 27.0 kg/m², P<0.001). Given the fact that CRP increases with age and BMI, and decreases with education, higher inflammatory levels were probably observed in our sample. However, it is probable that the study associations are valid, because the hs-CRP overestimation is believed to be non-differential with respect to dietary exposures.

In the present study, only one sample per participant was collected for hs-CRP assessment. However, we are only interested in subclinical inflammation, hence the problem of variability, within participant, from acute infection or other short-term insult is likely to be minimal.
In conclusion, the consumption of fruits and vegetables (with exception of vegetable soup), and vitamins C, E and fibre were negatively associated with hs-CRP levels in men. These associations tended to be weaker in overweight individuals. Therefore, the beneficial health effects of fruits and vegetables could be partly mediated through an inflammatory pathway.

Acknowledgements

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References


Paper IV

Alcohol intake and systemic markers of inflammation – shape of the association according to sex and body mass index

Andreia Oliveira, Fernando Rodríguez Artalejo, Carla Lopes

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PHARMACOLOGY AND CELL METABOLISM

Alcohol Intake and Systemic Markers of Inflammation—Shape of the Association According to Sex and Body Mass Index

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Abstract — Aims: To assess the association of alcohol intake with high-sensitivity C-reactive protein (hs-CRP), uric acid and leucocyte count in blood, and whether sex and body mass index (BMI) modify these associations.

Methods: Individuals aged ≥18 years were randomly selected from the population of Porto, Portugal (78% of participation). A total of 640 women and 400 men with reliable information on inflammatory markers and alcohol intake, obtained from a validated food frequency questionnaire, were studied. Associations and their respective trends were estimated from generalised linear models, with adjustment for potential confounders. Analyses were stratified by sex and BMI. Results: In women, adjusted hs-CRP levels (mg/l) were 2.69 in non-drinkers, 2.25 in drinkers of 0–14 g alcohol/day, 2.32 in drinkers of 15–29 g alcohol/day and 2.83 in drinkers of ≥30 g alcohol/day (P-value for quadratic trend = 0.001). In men, the association between alcohol intake and hs-CRP was positive and linear (P-value for the linear trend = 0.014). Alcohol intake was also positively and linearly associated with uric acid in each sex. Body weight modified these associations, which remained statistically significant only in normal-weight (BMI = 18.5–26 kg/m2) women and overweight (BMI = 26.5–32 kg/m2) men for hs-CRP, and in normal-weight individuals for uric acid. No significant association between alcohol intake and leucocyte count was found.

Conclusions: The association of alcohol intake with hs-CRP was U-shaped in women but positive and linear-shaped in men. Alcohol intake was directly associated with uric acid in men and women. BMI modifies the effect of alcohol on hs-CRP and uric acid levels in each sex.

INTRODUCTION

Light to moderate alcohol consumption has been associated with lower cardiovascular risk (Di Castelnuovo et al., 2002; Mukamal et al., 2005; O’Keefe et al., 2007) and all-cause mortality (Di Castelnuovo et al., 2006; Kloner and Rezai, 2007). This association is mediated by the beneficial effects of alcohol on lipids (Rimm et al., 1999; Mukamal et al., 2007), haemostatic factors (Rimm et al., 1999; Mukamal et al., 2001) and insulin sensitivity (Sierksma et al., 2004; Greenfield et al., 2005), but the precise mechanisms are only in part understood. In fact, alcohol consumption holds a significant association with cardiovascular risk even after controlling for the above mediators, suggesting alternative pathways for this association. Growing evidence supports the hypothesis that the cardiovascular protective effect of moderate alcohol intake could also be partly mediated through an inflammatory process (Imhof and Koening, 2003; Zuuris et al., 2004). High amounts of alcohol and its metabolites may exert direct inflammatory effects on the liver and induce free-radical production, therefore increasing lipid peroxidation and tissue inflammation (Jayatilaka and Shaw, 1998), and leading to changes in uric acid metabolism (Zakhari and Li, 2007). On the other hand, low alcohol concentrations may inhibit interleukin-6 secretion from adipocytes (McCarty, 1999).

Although several studies have assessed the association between alcohol consumption and systemic markers of inflammation (Imhof et al., 2001; Mezzano et al., 2004; Sierksma et al., 2002; Stewart et al., 2002; Albert et al., 2003; Nakanshi et al., 2003; Estreich et al., 2004; Volpato et al., 2004; Reiterstol et al., 2005; Avellone et al., 2006; Pai et al., 2006), the results are highly dependent on the exposure range of each specific population. The European region shows one of the highest alcohol intakes in the world (Rehm et al., 2001). In Portugal, alcohol consumption is particularly high, which, together with the considerable wide range of alcohol exposure in men (Marques-Vidal and Dias, 2005), allows for the study of the association’s shape, taking into account sex-related differences. Moreover, body fat, as a privileged supply of pro-inflammatory cytokines (Basard et al., 2006), could modify these associations, but this effect has not been commonly approached.

This study aims to assess the association of alcohol intake with blood levels of high-sensitivity C-reactive protein (hs-CRP), uric acid and leucocyte count, and whether sex and body mass index (BMI) modify these associations.

METHODS

Participants selection

Participants were selected by random digit dialling from the non-institutionalized population of Porto, a large urban centre in the north-west of Portugal with almost 300,000 inhabitants. Once a household was selected, all permanent residents were identified by age and sex; one of them (aged ≥18 years) was randomly selected and invited to visit the Department of Hygiene and Epidemiology of the University of Porto Medical School for interview and physical examination performed between 1999 and 2003 (n = 2485). All participants were white and with Portuguese nationality. Results were not substituted and the participation proportion was 70% (Ramos et al., 2004).

In individuals aged >64 years, a rapid evaluation of cognitive function was done using the Mini-Mental State Examination test (MMSE; Folstein et al., 1975), and subjects were excluded.
when scoring ≤24 points due to cognitive impairment (Anthony et al., 1982).

For the purpose of this investigation, some individuals were excluded from analysis due to the following sort of reasons: (i) 55 unable to participate due to physical or cognitive impairment (49 formally evaluated by the MMSE and six with physical/mental apparent incapacity to answer questions); (ii) 19 with lacking data on alcohol intake; (iii) 977 without biochemistry measurement of any of the inflammatory markers under study; (iv) 81 with hs-CRP levels > 10 mg/l, which suggest a clinically relevant inflammatory condition (Yeh and Willerson, 2003), and not sub-clinical inflammation, as required; (v) 17 with missing data on potential confounders of the study; (vi) six who reported to have changed their alcohol intake in the year before interview (i.e., quit or started drinking), to ensure that the reported alcohol intake was long term. Thus, analyses were conducted with 1330 subjects (840 women and 490 men).

Data collection

Data were collected by a set of trained interviewers with a standard structured questionnaire including socio-demographic, behavioural and clinical characteristics as well as physical examination.

Alcohol intake

Alcohol intake, in gram per day (g/day), was assessed using a food frequency questionnaire covering the previous 12 months. Each subject was asked about the mean frequency of consumption of different types of alcoholic beverages, including wine, beer and spirits—liquors, gin, rum, vodka, cocktails or other mixed drinks. Frequency categories ranged from ‘never or less than once a month’ to ‘6 or more times a day’; and the average portion consumed was lower, equal or higher than a glass of 125 ml (41 oz) of wine, a bottle or can of 330 ml for beer and a cup of 40 ml for spirits. The alcoholic beverage consumption was converted into total alcohol intake with the software Food Processor Plus (ESHAM Research, Salem, OR, USA 1997) using an algorithm that assumed the following alcohol concentrations in volume: 12% for wine, 4.7% for beer, 25% for liqueurs and similar beverages, and 50% for vodka and the like. The algorithm was adapted to Portuguese drinks (e.g., Porto wine). Moderate drinking was defined as alcohol intake ≤15 g/day in women and ≤30 g/day in men (Lichtenstein et al., 2006), while excessive drinking was deemed to be above those thresholds.

The food frequency questionnaire has been validated by comparison with four 7-day food records (each one in a different season of the year) among 146 subjects of Porto (Lopes, 2000). High correlation coefficients were found for alcohol intake (unadjusted Spearman correlation $r = 0.88$; sex-, age-, education- and energy-intake-adjusted Spearman correlation $r = 0.69$).

Systemic markers of inflammation

A blood sample was drawn after a 12-h overnight fast. Leukocyte count was determined from the whole blood immediately after collection. Serum samples for hs-CRP and uric acid assessment were stored at −20°C until analysis. hs-CRP levels were determined by means of particle-enhanced immuno-
nephelometry using a Behring Nephelometer II, BN II™ autoanalyzer (Dade Behring™ Marburg GmbH, D-35041 Marburg, Germany). Uric acid levels were determined by standard enzymatic methods.

Potential confounders and effect modifiers

Age and education were recorded as completed years of schooling. Total energy intake was obtained with the same 82-item food frequency questionnaire (Lopes, 2000) used to collect alcohol intake. Smoking status was also asked and participants were classified into never-smokers, current smokers (daily and occasional smokers) and ex-smokers (smokers with at least 6-month abstinence). Total physical activity energy expenditure was ascertained with a questionnaire exploring all professional, domestic and leisure time activities, and was quantified in standard metabolic equivalents (METs/hour/day).

Anthropometries were obtained by trained observers according to standard procedures with subjects in light clothing and barefoot. Body height was measured to the nearest 0.1 kg using a digital scale (SECA®), and height was measured to the nearest centimetre with a wall stadiometer (SECA®). BMI was calculated dividing the weight (kg) by the squared height (m).

Personal history of cardiovascular disease was self-reported and included previous cardiovascular disorders diagnosed by a physician, namely arterial hypertension, angina pectoris, acute myocardial infarction, stroke and heart failure.

Information on menopausal status and hormone replacement therapy was also recorded for all women.

Ethics

The local ethics committee (São João Hospital) approved the study protocol. All participants gave written informed consent to participate; the study was carried out in accordance with the Helsinki Declaration II.

Statistical analysis

Differences between two independent samples were assessed with the chi-squared test for proportions and with the Mann–Whitney test for continuous variables.

As inflammatory marker distributions were highly skewed (normality was checked using the Shapiro–Wilk criterion), generalized linear models with a log link function and Gaussian error distribution were used to estimate the magnitude of the associations (β coefficient and the respective 95% confidence intervals (95% CI)) and the point estimates and their respective 95% CI for the inflammatory markers across categories of daily alcohol intake. Models were adjusted for age, education, total energy intake, total physical activity, BMI (as continuous variables), smoking status, personal history of cardiovascular disease, and menopause and hormone replacement therapy (as dummy variables). Analyses were also conducted with stratification by sex and BMI categories (<25 vs ≥25 kg/m²).

The linear and quadratic trends of the associations between alcohol intake and the inflammatory markers were tested with the same generalized linear models. For the linear trend, the medians of each category of alcohol intake were modelled as a continuous variable; for the quadratic trend, the squared medians were used.
Table 1. Characteristics of the study participants, by sex

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 840)</th>
<th>Men (n = 490)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>51 (41-62)</td>
<td>52 (39-65)</td>
<td>0.285</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>6 (4-13)</td>
<td>9 (4-12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total energy intake (kcal/day)</strong></td>
<td>2010 (1661-2575)</td>
<td>2034 (2093-2882)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total physical activity (METs/hour/day)</strong></td>
<td>24.6 (23.2-28.5)</td>
<td>34.2 (22.5-39.2)</td>
<td>0.099</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.4 (23.5-30.4)</td>
<td>26.1 (23.7-28.9)</td>
<td>0.157</td>
</tr>
<tr>
<td><strong>hs-CRP (mg/l)</strong></td>
<td>1.7 (0.8-3.7)</td>
<td>1.3 (0.6-2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Uric acid (mg/l)</strong></td>
<td>30 (23-46)</td>
<td>52 (45-62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Leukocyte count (mm⁻³)</strong></td>
<td>6200 (5200-7300)</td>
<td>6400 (5600-7700)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/life union</td>
<td>530 (63.1)</td>
<td>384 (78.4)</td>
</tr>
<tr>
<td>Single</td>
<td>142 (16.9)</td>
<td>72 (14.7)</td>
</tr>
<tr>
<td>Widowed</td>
<td>111 (13.2)</td>
<td>16 (3.2)</td>
</tr>
<tr>
<td>Divorced</td>
<td>57 (6.8)</td>
<td>18 (3.7)</td>
</tr>
<tr>
<td><strong>Current occupational activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White-collar job</td>
<td>282 (33.6)</td>
<td>179 (36.5)</td>
</tr>
<tr>
<td>Blue-collar job</td>
<td>138 (16.4)</td>
<td>94 (19.2)</td>
</tr>
<tr>
<td>Employed/housewife/student</td>
<td>204 (24.5)</td>
<td>48 (9.8)</td>
</tr>
<tr>
<td>Retired</td>
<td>216 (25.7)</td>
<td>109 (21.5)</td>
</tr>
<tr>
<td><strong>Personal history of cardiovascular disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>566 (67.4)</td>
<td>353 (72.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>274 (32.6)</td>
<td>137 (28.0)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smokers</td>
<td>607 (72.3)</td>
<td>146 (29.8)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>151 (18.0)</td>
<td>139 (28.4)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>82 (9.7)</td>
<td>185 (37.8)</td>
</tr>
<tr>
<td><strong>Alcohol drinking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-drinkers</td>
<td>225 (26.8)</td>
<td>33 (6.7)</td>
</tr>
<tr>
<td>Regular drinkers (≤1 glass/week)</td>
<td>388 (46.2)</td>
<td>486 (96.4)</td>
</tr>
<tr>
<td>Occasional drinkers</td>
<td>158 (18.8)</td>
<td>30 (6.1)</td>
</tr>
<tr>
<td>Ex-drinkers</td>
<td>69 (8.2)</td>
<td>25 (4.7)</td>
</tr>
<tr>
<td><strong>Alcohol intake (g/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>367 (41.7)</td>
<td>62 (12.6)</td>
</tr>
<tr>
<td>&gt;0-15</td>
<td>345 (41.1)</td>
<td>152 (31.0)</td>
</tr>
<tr>
<td>&gt;15-30</td>
<td>97 (11.6)</td>
<td>73 (14.9)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>31 (3.7)</td>
<td>26 (5.3)</td>
</tr>
</tbody>
</table>

hs-CRP, high-sensitivity C-reactive protein.

Analyses were performed with the free software R, version 2.8.1 (R Foundation for Statistical Computing, Austria, 2008).

**RESULTS**

Characteristics of participants, by sex, are described in Table 1. Men completed more years of education and reported significantly higher total energy intake than women. Men also had a higher prevalence of current smoking and reported higher alcohol intake. Both women and men were frequently married and white-collar workers. Women presented higher hs-CRP concentration, while men had higher uric acid levels and leukocyte count.

Table 2 shows the estimates of hs-CRP, uric acid and leukocytes according to the daily alcohol intake in each sex. In women, a significant association between alcohol intake and hs-CRP levels was found; moderate drinkers (>0-15 g/day) had significantly lower hs-CRP levels than non-drinkers (2.25 vs 2.69 mg/l, P = 0.004) and excessive drinkers (2.25 vs. 3.18 mg/l, P = 0.004) (P-value for the quadratic trend <0.001). In men, the association of alcohol intake with hs-CRP was positive and linear (P-value for linear trend = 0.014). Alcohol intake was positively associated with uric acid levels in each sex (P-value for linear trend = 0.021 in women and P = 0.022 in men). No significant association was found between alcohol intake and leukocyte count.

In analyses stratified by BMI, the associations of alcohol intake with hs-CRP only held in normal-weight (BMI <25 kg/m²) women and in overweight (BMI ≥25 kg/m²) men (Table 3). For uric acid, the linear associations sustained only in normal-weight individuals of both sexes.

**DISCUSSION**

In the present study, alcohol intake showed a J-shaped relation with hs-CRP levels in women and a positive linear-shaped relation in men. Uric acid levels increased with increasing alcohol intake in each sex. Lastly, BMI modified these associations so that they only held in normal-weight women and in overweight men for hs-CRP, and in normal-weight individuals for uric acid.

Previous studies have reported a beneficial effect of moderate alcohol intake on hs-CRP levels (Inoh et al., 2001; Stewart et al., 2002; Alberi et al., 2003; Volpato et al.,...
Table 2. Estimates of high-sensitivity C-reactive protein (hs-CRP), uric acid and leukocyte count according to alcohol intake, by sex

<table>
<thead>
<tr>
<th>Alcohol intake (g/day)</th>
<th>Adjusted estimates(^a) (95% CI)</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
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<td><strong>hs-CRP (mg/L)</strong></td>
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<td>0.26</td>
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<tr>
<td><strong>Uric acid (mg/dL)</strong></td>
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<tr>
<td>0.25</td>
<td>(0.25, 0.27)</td>
<td>2.01</td>
<td>1.36</td>
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<tr>
<td><strong>Leukocyte count (mm(^3)</strong></td>
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<tr>
<td>0.90</td>
<td>(0.89, 0.91)</td>
<td>2.05</td>
<td>1.39</td>
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\(^a\) Adjusted for age, education, total energy intake, smoking status, total physical activity, BMI, personal history of cardiovascular disease and menopausal and hormone replacement therapy (in women).

\(^b\) Trends obtained from generalized linear models with a log link function and Gaussian error distribution; linear trend calculated using the median of each category of alcohol intake modelled as a continuous variable; for quadratic trends, the squared medians were used.

\(^c\) 95% CI, 95% confidence intervals.

2004; Pui et al., 2006). Most of them showed J-shaped associations between alcohol and hs-CRP in both men and women (Imhof et al., 2001; Alberi et al., 2003; Volpato et al., 2004; Pui et al., 2006). In our study, only among women, moderate alcohol intake (defined as >0-15 g/day of alcohol—approximately less than one to two drinks of alcohol per day) was significantly associated with lower hs-CRP than either lower or higher alcohol intakes; a positive linear association was found in men, which could be to some extent explained by the alcohol drinking patterns in Portugal. According to data from the World Health Organization (Rehn et al., 2001), Portugal presents with a 'high level of consumption' of alcohol, particularly among men. In our study, 42% of men reported a daily alcohol intake above 30 g/day, which is the current American Heart Association recommended threshold for those who drink (Lichtenstein et al., 2006). Although our data could somewhat suggest that male moderate drinkers have the lowest hs-CRP levels (Table 2), excessive alcohol intake was responsible for a right-skewed effect, producing an overall significant linear association. The cut points to define moderate intake could also contribute to the different results found.

In the present study, we followed the American Heart Association guidelines (Lichtenstein et al., 2006), which recommend up to one drink per day in women and two drinks in men, but across studies the cut points varied with the range of alcohol intake in each specific population.

Table 3. Estimates of high-sensitivity C-reactive protein (hs-CRP), uric acid and leukocyte count according to alcohol intake, by sex and BMI

<table>
<thead>
<tr>
<th>Alcohol intake (g/day)</th>
<th>Adjusted estimates(^a) (95% CI)</th>
<th>Women</th>
<th>Men</th>
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<tbody>
<tr>
<td></td>
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\(^a\) Adjusted for age, education, total energy intake, smoking status, total physical activity, BMI, personal history of cardiovascular disease and menopausal and hormone replacement therapy (in women).

\(^b\) Trends obtained from generalized linear models with a log link function and Gaussian error distribution; linear trend calculated using the median of each category of alcohol intake modelled as a continuous variable; for quadratic trends, the squared medians were used.

\(^c\) 95% CI, 95% confidence intervals.
Additionally, a recent meta-analysis (Fillmore et al., 2007) suggested that a systematic error may exist when reporting that moderate alcohol is ‘protective’ against cardiovascular disease. The authors suggest that the abstainer category may represent occasional or former drinkers who quit drinking because of health conditions that increase inflammation levels; it might explain the higher risk of abstainers compared with moderate drinkers. In the present study, we did not find significant differences in hs-CRP between never and former drinkers (P = 0.096 in women and P = 0.710 in men).

Women in every alcohol drinking category presented higher hs-CRP levels than men, which is in agreement with previous studies (Festa et al., 2001; McConnell et al., 2002; Connolly et al., 2003). This is often explained by the impact of sex hormones on circulating levels of inflammatory proteins (BarJustin-Mitchell et al., 2001; Rexord et al., 2003) and by a higher accumulation of fat, an important source of pro-inflammatory cytokines (Bastard et al., 2006), in women than in men.

hs-CRP fasting levels is not widely described as an inflammatory marker. However, the significant associations between uric acid and leukocyte count, CRP, and interleukins suggest that uric acid is not only a marker of the catabolic rate but may also be actively involved in the inflammatory process (Cugliandol et al., 2009) as well as CRP and leukocytes (Bastard et al., 2006). In our study, a positive dose–response association between alcohol intake and uric acid levels was found. Most studies have related alcohol intake to heightened risk of gout and alcoholic cirrhosis (Cho, 2005; Zahahri and Li, 2007), but the relation of alcohol with low-normal levels of uric acid in healthy populations seems to be quite ignored. A study among apparent healthy volunteers (Alatalo et al., 2009) suggested a gender-dependent impact of alcohol consumption on uric acid levels, but in our study the effect was similar in both sexes.

For the relation of alcohol intake with leukocyte count, the literature is scarce. Nakahishi et al. (2003) suggested an inverse association between alcohol consumption and leukocytes; however, we observed no association. The lack of specificity of this biomarker to detect sub-clinical inflammation could be responsible for the nil association evinced with alcohol intake.

In most studies, body fat is seen as a confounder of the relation between alcohol intake and cardiovascular risk markers; in our study, however, we found different results according to BMI categories. A previous study (Dom et al., 2003) reported that light to moderate drinkers seemed to have lower BMI and less frequent abdominal obesity than non-drinkers, but those consuming over two drinks showed a dose–response relation with abdominal obesity. As adipose tissue is an important source of pro-inflammatory cytokines (Bastard et al., 2006), the beneficial effect of moderate alcohol drinking reducing hs-CRP levels could result from lower fat accumulation.

In fact, in the present study, after BMI stratifications (and simultaneous adjustment for BMI to minimize residual confounding), the J-shaped association between alcohol and hs-CRP was found only in normal-weight women (BMI <25 kg/m²); among overweight women, excess body weight might counteract the effect of alcohol intake on hs-CRP levels. In contrast, in men, a positive linear association between alcohol and hs-CRP was found only among overweight individuals. This finding could be explained by two main reasons: the lower variability of hs-CRP among non-overweight men, which could be insufficient to find an association, and the higher alcohol intake of overweight men compared with normal-weight men and women, which offset the effect of body fat on inflammatory markers.

There is no evidence of a causal association between body fat and uric acid (e.g. body fat does not produce uric acid) as there is for CRP, but its documented association (Zahahri and Li, 2007) is also consistent with the above explanations.

Strengths and limitations

Apparently, the cross-sectional design of the present study could be seen as a potential limitation; however, it seems unlikely that hs-CRP and uric acid levels in healthy individuals would affect self-reported alcohol intake (i.e. reverse causality). A major problem could be the reliance on self-reporting to determine frequency and quantity of alcohol intake. Therefore, non-differential misclassification may have diluted the true association between the studied biomarkers and alcohol consumption since participants may underestimate the true consumption. Nonetheless, the food frequency questionnaire used for alcohol assessment was previously validated and found to be strongly correlated with the 7-day records (Lopes, 2000).

Due to the large contribution of wine to total alcohol intake, beverage-specific analyses were not possible. However, there is evidence that ethanol itself is largely responsible for the potential anti-inflammatory effects of alcoholic beverages (Rinn and Stampafer, 2002; Imhof et al., 2004; Levitan et al., 2005).

The relatively small sample size for men could also be seen as a limitation, leading to less precise estimates than in women. Therefore, future research in high-alcohol-intake populations with larger sample sizes should confirm the reported associations, particularly in men.

We have only one measurement of the inflammatory markers available per subject; however, we do not face the problem of variability from acute infection or other short-term insults since we are only analysing sub-clinical inflammation levels (<10 mg/L).

The assessment of hs-CRP was not planned at the beginning of the participants' evaluation. Thus, although hs-CRP measurements were consecutively performed, they were only available for a subset of participants. Comparing those with and without hs-CRP measurements (n = 1330 vs 1155), subjects with hs-CRP assessment were slightly younger (52 vs 55 median years; P < 0.001), but no significant differences were found regarding sex (63.2% vs 61.4% P = 0.163) and education (8 vs 8 median years; P = 0.197). As CRP increases with age, an underestimation of inflammatory levels is probably present in our sample, but this underestimation is expected to be non-differential with respect to alcohol intake; and it is not likely that the relatively small difference in age between participants and non-participants might have affected the results.

CONCLUSIONS

While alcohol intake showed a J-shaped association with hs-CRP levels in women, the association was positive and linear-shaped in men. It might be partly due to high alcohol
consumption observed in men. Alcohol intake was directly associated with uric acid in men and women. Lastly, the effect of alcohol on hs-CRP and uric acid varied across categories of BMI. Our results support the hypothesis that the effect of alcohol intake on cardiovascular risk could be partly explained through an inflammatory pathway.

**REFERENCES**


Paper V

Major dietary patterns are associated with acute myocardial infarction and with cardiovascular risk markers in a Southern-European population

Andreia Oliveira, Fernando Rodríguez Artalejo, Rita Galo, Ana Cristina Santos, Elisabete Ramos, Carla Lopes,

[Submitted]
Major dietary patterns are associated with acute myocardial infarction and cardiovascular risk markers in a Southern-European population

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ABSTRACT

Background: Most dietary pattern analyses in Southern Europe have relied on a priori approaches using Mediterranean Diet indexes. These methods, however, may not reflect the current population’s food consumption.

Objective: To assess the association of a posteriori dietary patterns with acute myocardial infarction (AMI) and cardiovascular risk markers in the general adult population of Porto, Portugal.

Design: A population-based case-control study was conducted. Information was collected by trained interviewers. Diet was assessed with a validated 82-item food frequency questionnaire.

Participants/setting: Cases were patients consecutively hospitalized for an incident non-fatal AMI (n=820), and controls were individuals free of previous AMI selected from the hospitals’ catchment area (n=2196) (1999-2003).

Statistical analyses: Dietary patterns were identified by multivariate finite mixture models among controls. Odds ratios (OR) and their 95% confidence intervals (CI) were obtained from unconditional logistic regression, with adjustment for main confounders.

Main outcome measure: Incident non-fatal AMI.

Results: Four dietary patterns were identified in each sex. In comparison to women with a “healthy” dietary pattern, those with a “low fruit and vegetables” and “red meat and alcohol” patterns, the last one also characterized by a lower intake of dairy products and vegetables, showed a higher risk of AMI (OR=1.85, 95% CI: 1.01-3.39 and OR=1.91, 95% CI: 1.17-3.12). Female controls with the “red meat and alcohol” pattern also had a higher total-to-HDL cholesterol ratio. In comparison to men with a “healthy” pattern, those with the “red meat and alcohol” intake pattern, similar to the counterpart found in women, were more likely to suffer an AMI (OR=1.98, 95% CI: 1.35-2.92); male controls with this pattern had higher diastolic blood pressure, C-reactive protein and uric acid levels. No associations were observed between the remaining dietary patterns and AMI.

Conclusions: The dietary patterns with higher consumption of red meat and alcohol and lower intake of dairy products and vegetables were associated with an increased risk of AMI and with a worse cardiovascular risk profile, in both men and women. These findings strengthen the importance of dietary pattern analysis, which beyond the effect of single foods, could potentiate or reduce the disease risk.

Key-words: dietary patterns; finite mixture models; myocardial infarction; cardiovascular risk markers
INTRODUCTION

The relation of diet with coronary heart disease (CHD) has traditionally been approached through the effect of single foods and nutrients but, due to the conceptual and methodological limitations of this approach, dietary patterns have attracted great interest (1-2).

The mainstream dietary pattern analysis has relied on a priori approaches; in Southern Europe, usually using Mediterranean Diet indexes (3-4). A main criticism of this approach is that it may not reflect the current population’s food consumption. Moreover, a priori methods are based on the assumption of protective or deleterious health effects for every food component, but they do not acknowledge that clustering of food components could vary across populations, reflecting their own cultural traditions. For instance, moderate alcohol consumption is usually considered a healthy behaviour (5-7), but while some studies have reported associations with healthier foods (8-9), others have found the opposite (10-11). It suggests that the health effect of a single food can be confounded by population-specific dietary behaviours. These caveats make the case for using dietary patterns defined a posteriori.

Though several studies have assessed the association between a posteriori dietary patterns and cardiovascular disease (12-16), only sparse information is available for the Southern European populations when using an exploratory approach that relies totally in data at hand (16). Portugal has a wide range of dietary exposures as well as a particularly high consumption of wine and fish (17-18), which could lead to unique food clusterings.

This study assessed the association of a posteriori dietary patterns with non-fatal acute myocardial infarction (AMI) and some cardiovascular risk markers in the adult population of Porto, Portugal.

METHODS

Study design and participants

A population based-case control study was carried out between 1999 and 2003 in Porto - an urban centre located in northwest Portugal with almost 300 000 inhabitants. Cases were selected after consecutive admission to the Cardiology Department of the four hospitals which provide acute coronary care in Porto. Cases were included in the present study if they had a first episode of AMI (19) and survived beyond the fourth day post AMI. During the study period, 1248 patients with a first AMI
were identified. Out of these, we excluded 37 who died before the interview, 65 who were unable to collaborate, three who refused to participate, 138 with cognitive impairment as indicated by a score <24 in the Mini Mental State Examination (20-21), and 162 with incomplete information. Further exclusions were made for 21 who changed diet in the year before interview, and for two whose energy intake was below or above three standard deviations of the sex-specific median intake.

Controls were selected by random digit dialing within the non-institutionalized adult population of Porto, using the households as sample units. Of note is that during the recruitment period, the proportion of households with telephone was 97%. Simple random sampling was used to select only one subject among all aged ≥18 years in each household. Refusals were not substituted within the same household and the participation rate was 70% (22). Out of 2485 participants initially selected, 103 (4.1%) were excluded due to previous clinical or silent myocardial infarction, according to self-reported data and/or electrocardiographic evidence. We also excluded 51 persons with cognitive impairment, 60 persons with incomplete information, and 75 who changed their food habits during the year before interview. For the case-control analysis, the final sample included 820 cases (194 women and 626 men) and 2196 controls (1362 women and 834 men).

To examine the association between dietary patterns and cardiovascular risk markers, a cross-sectional analysis was conducted among the controls only. Out of the 2196 controls, we further excluded 328 individuals without information on at least one of the following variables: systolic and diastolic blood pressures, serum glucose, serum total-to-HDL cholesterol ratio and triglycerides. As a result, analyses with these variables were conducted with 1868 persons. Lastly, for analyses concerning high-sensitivity C-reactive protein (hs-CRP), uric acid and leukocyte count, a sub-sample of 925 participants was obtained, after exclusion of 1209 individuals without measurement of one of the markers under study, and 62 with hs-CRP above 10 mg/l which suggests the presence of a clinically relevant inflammatory condition (23).

The Ethics Committees of the four participating hospitals approved the study protocol, and every participant gave written informed consent.

Data collection

Controls were invited to a face-to-face interview at the Department of Hygiene and Epidemiology of the University of Porto Medical School; AMI patients were interviewed during their in-hospital stay after clinical stabilization, usually between the fourth and eighth day after admission. Data on cases and controls were collected
concurrently by the same set of trained interviewers, using the same questionnaire on socio-demographic, behavioural and clinical characteristics.

Diet was collected with an 82-item semi-quantitative food frequency questionnaire, previously validated against four seven-days food records (24-25). Each subject had to report the average frequency of consumption for each food-item among nine categories, ranging from "never or less than once a month" to "6 or more times a day"; subjects also had to choose the average portion consumed (lower, equal or higher than the average portion size) and the seasonal variation of consumption. Food consumption was converted into total energy intake and nutrients with the software Food Processor Plus® (ESHA Research, Salem-Oregon, 1997), adapted to Portuguese foods and dishes.

Age and education were recorded as completed years of aging and schooling. Tobacco consumption was registered as daily (at least one cigarette/day), occasional (less than one cigarette/day), former (quit for at least six months) and never smoking. For analyses only two categories were considered: current smoking (daily and occasional smokers) and non-smoking (never and former smokers). Physical activity referred to the regular practice (at least 30 min per week) of any leisure-time physical activity with energy expenditure higher than 2.5 metabolic equivalents per hour, including walking, running and any sports activities, during the previous year. A family history of AMI was considered when at least one first-degree relative had suffered an AMI or a sudden death, regardless of age at occurrence.

Anthropometrics were performed with subjects in light clothing and barefoot under standard procedures. Body weight was measured to the nearest 0.1 kg using a digital scale (SECA®, Columbia, USA), and height to the nearest cm with a wall stadiometer (SECA®, Hamburg, Germany). Body mass index (BMI) was calculated as weight in kg divided by the squared height in m. Waist circumference (WC) was ascertained midway between the lower limit of the rib cage and the iliac crest, and hip circumference on the maximum circumference over the femoral trochanters; both were measured to the nearest cm with a flexible and non-distensible tape.

For the cross-sectional analysis, controls underwent some measurements. Systolic and diastolic blood pressures were measured on a single occasion using a standard mercury sphygmomanometer after a 10-minute rest; the mean of two blood pressure readings was used in the analyses. Also, a venous blood sample was drawn after a 12-hour overnight fast, to measure glucose, total cholesterol, HDL-cholesterol, triglycerides, hs-CRP, uric acid and blood leukocyte count. Serum glucose, cholesterol, triglycerides and uric acid levels were determined using automatic standard routine enzymatic methods. HDL-cholesterol was measured after precipitation of
apolipoprotein B-containing lipoproteins. hs-CRP was determined with particle-enhanced immunonephelometry using an auto-analyser Behring, Nephelometer II, BN II® (Dade Behring™ Marburg GMBH, D-35041 Marburg, Germany). Leukocyte count was determined from the whole blood, immediately after collection.

Statistical analysis

The food items of the food frequency questionnaire were aggregated into 14 food-groups according to nutritional similarities. Given that there were sex differences in the food-groups distribution (p-value <0.001), dietary patterns were identified separately for men and women. Patterns were firstly identified among the controls using multivariate finite mixture models, or equivalently, mixture of regressions with concomitant variables as independent variables (26). The multivariate dependent variable represented the 14 food-groups consumption.

As high frequencies of zero consumption can originate problems in model fitting, food-groups with a percentage of non-consumption higher than 45% were dichotomized into consumption and non-consumption. This was the case for alcohol in women and soft drinks in both men and women; the remaining food-groups did not present non-consumptions higher than 15%. The variables that were not dichotomized were log-transformed, after adding one (g) to avoid zero values. For the model structure, variances of the continuous food group intakes were allowed to vary within and between clusters.

Screening of outliers was done by the sigma gap method for right skewed distributions. Twenty-four men and 22 women were identified as outliers and removed for the definition of patterns. Differences in food consumption with age as well as correlations between individual food-group consumption and total energy intake were detected. Therefore, both age group and total energy intake were taken as independent and concomitant variables.

The final number of clusters (n=4, for both men and women) was established by the cessation of the monotonically decrease of the Bayesian Information Criterion (BIC). The mean (and standard deviation) of the individual posterior probabilities (probabilities predicting the cluster membership) were 89% (0.14) in women and 92% (0.13) in men. Dietary characterization of each cluster was based on results from tests of multiple comparisons of food-group means. Dietary pattern identification for AMI cases was done from the individual component membership probabilities of the model among the controls.

The association between the identified dietary patterns and the risk of AMI was summarized with odds ratios (OR) and their respective 95% confidence intervals (CI).
calculated with unconditional logistic regression. Both univariate (model 1) and multivariate models were presented; multivariate models were adjusted for age, education, total energy intake (as continuous variables), smoking (daily, occasional, never, former smokers), regular leisure-time physical activity (no, yes) and family history of AMI (no, yes) (model 2). Further adjustment for BMI (model 3) and for self-reported hypertension, dyslipidemia and diabetes mellitus (model 4) was also done, though we acknowledge that the latter variables may partly act as intermediate variables in the relationship between diet and AMI. Lastly, we examined a possible interaction of BMI in the association between dietary patterns and AMI, by using interaction terms constructed as the product of dietary pattern categories with BMI.

Statistical analyses for dietary pattern identification were conducted with the package flexmix from the software R, version 2.7.1, (R Foundation for Statistical Computing, Austria, 2008). Other analyses were performed with Stata SE, version 10 (Statacorp, Texas, 2007).

RESULTS

Table 1 shows the four different dietary patterns identified in each sex. In women, the pattern with the highest consumption of vegetables, including vegetable soup, fruits and dairy products, and with the lowest consumption of red meat, fast-foods and soft drinks was named “healthy”; the pattern characterized by the lowest intake of all food-groups, in particular fruit and vegetables, was called “low fruit and vegetables”; the pattern with the lowest consumption of dairy products and vegetable soup, and the highest of red meat and alcohol, was designated as “red meat and alcohol”; lastly, the pattern with the highest intake of white meat, sweets and fast-foods and the second highest intakes of red meat, vegetables, including vegetable soup, and dairy products was named “in transition to fast-food”.

In men, the pattern with the highest consumption of vegetable soup, fruits, dairy products and cereals, and the lowest of red meat, fast-foods and alcoholic beverages, was designated as “healthy”; the highest intake of fish and vegetables characterized the “fish” pattern; also the pattern with the highest consumption of red meat, alcohol and fast-foods, and the lowest of fruits, vegetable soup, dairy products and cereals, was named “red meat and alcohol”; lastly, a pattern with an intermediate consumption of most food-groups but of white meat, was designated as “intermediate intake” (Table 1).
Table 2 shows the characteristics of cases and controls according to their dietary patterns, in each sex. Characteristics among the controls will be compared across the different dietary patterns. Women with the “healthy” pattern were older, with lower frequency of smoking and higher of physical activity. Women with a “low fruit and vegetables” pattern presented the lowest energy intake and the highest percentage of smokers. The “red meat and alcohol” pattern corresponded to women with lower education and higher BMI and WC. Female controls in this pattern were also the least physical active. The “in transition to fast-food” pattern included younger and highly educated women, with lower BMI and WC. In men, the “healthy” pattern represented individuals less frequently smokers and more physical active. The “fish” pattern included older men with larger WC. The “red meat and alcohol” corresponded to younger men with higher energy intake and BMI. This pattern also held a stronger association with smoking and physical inactivity. Lastly, men with an “intermediate intake” pattern had both lower energy intake and BMI (Table 2).

Table 3 presents the association between the dietary patterns and the risk of AMI, in each sex. Using the “healthy” pattern as reference, women with patterns characterized by “low fruit and vegetables” consumption and high “red meat and alcohol” intake showed a higher risk of AMI (OR=1.72, 95%CI: 1.01-2.92; OR=1.86, 95%CI: 1.22-2.84, respectively). After adjustment for the main confounders (model 2), women with the “red meat and alcohol” pattern had a 76% higher risk of an AMI (OR=1.76, 95%CI: 1.11-2.78). After further adjustment for hypertension, dyslipidemia and diabetes (model 4), also the “low fruit and vegetables” pattern held a significant association with AMI (OR=1.85, 95%CI: 1.01-3.39). In men, the “red meat and alcohol” and the “intermediate intake” patterns showed a significant and positive association with AMI occurrence in unadjusted analyses; but after further adjustment for confounders only the “red meat and alcohol” pattern yielded a significant association with AMI (OR=1.83, 95%CI: 1.26-2.67) (Table 3).

Lastly, results did not vary with BMI (p for BMI-interaction=0.423 in women and 0.215 in men).

Table 4 shows the association between the dietary patterns and the cardiovascular risk markers in each sex. Again, the “healthy” pattern was used as reference. Women with the “red meat and alcohol” pattern presented a higher adjusted total-to-HDL cholesterol ratio (3.9 vs. 3.6, p=0.043). Men with the “fish” pattern showed higher uric acid levels (55.1 vs. 48.8 mg/l, p=0.002); also, those with the “intermediate intake” had a higher diastolic blood pressure (84 vs. 81 mmHg, p=0.036). Lastly, men included with the “red meat and alcohol” pattern had higher diastolic blood pressure (84 vs. 81 mmHg, p=0.010), hs-CRP (2.64 vs. 1.68 mg/l, p<0.008) and uric acid levels
(57.0 vs. 48.8 mg/l, p<0.001). For the remaining dietary patterns, no statistically significant differences were found in cardiovascular risk markers.

DISCUSSION

Individuals with a higher consumption of red meat and alcohol, and lower intake of vegetables and dairy products, had an increased risk of AMI and higher levels of cardiovascular risk markers than those with a “healthy” dietary pattern. Other dietary patterns showed no association with AMI risk.

We used an exploratory approach to identify mutually exclusive groups of individuals sharing food intake profiles, often designated as a posteriori dietary patterns. In the literature, two major a posteriori patterns, identified by factor analysis, have been related to CHD risk - the “Prudent” pattern, corresponding to a high intake of fruit and vegetables, and the “Western” pattern which reflects a high intake of red meat and fats (12-15). Overall, the first one has been inversely associated with CHD risk, whereas the “Western” pattern increased the risk of CHD (12-13, 15). Positive associations were also found between the “prudent” or “healthy” pattern and a more favourable cardiovascular biomarkers profile (27-28), and between the “Western” pattern and higher CRP (27-28), interleukin-6 (IL-6) (29), C-peptide, insulin (14, 27), leptin and homocysteine concentrations (27). Nettleton et al., also looked at the associations of four dietary patterns, identified by factor analysis, with cardiovascular risk markers in participants in the Multi-Ethnic Study of Atherosclerosis, and the fats and processed meats pattern was again positively associated with CRP, IL-6 and homocysteine (30).

These findings concur with the results of the present study where individuals of the general population, particularly men, with higher consumptions of red meat, fast-food and alcoholic beverages presented higher inflammatory biomarker levels than those with a healthy dietary pattern. As in previous studies (13, 27), other well-known cardiovascular risk factors, such as systolic blood pressure or serum lipid concentrations did not significantly differ between the dietary patterns. Overall our results highlight the potential role of chronic low-grade inflammation in the relationship between diet and coronary disease.

The dietary pattern with higher intake of red meat and alcohol was associated with an increased risk of AMI. Of note is that alcohol consumption has seldom came out as a main food component of most a posteriori dietary patterns in the literature. One reason might be the insufficient variation in alcohol intake in Northern American
populations, where most studies were conducted. On the other hand, the European region has one of the highest alcohol intakes in the world and, in particular, Portugal presents a high level and a large variation in consumption of alcohol from wine sources in men (31-32). In line with our results, in the ATTICA Study of Greece (16), in which 15 dietary patterns were extracted by principal component analysis, the pattern characterized by the high intake of red meat, alcohol, sweets and hard cheese was prospectively associated with cardiovascular disease.

Therefore, in our particular population and probably also in others with similar drinking and eating patterns, recommendations on alcohol intake must be extremely prudent, since alcohol clusters with red meat intake and is inversely associated with other healthier foods, such as fruit and vegetables (table 1), resulting in an overall increase of AMI risk in both sexes. A previous study in Porto failed to show a J-shaped association between alcohol and AMI (33), in contrast to what has been found in other populations (34).

The association between dietary patterns and socio-demographic, lifestyle and anthropometric characteristics suggest that food choices are a part of a larger pattern of health behaviours. Individuals with the "healthy" dietary pattern were more often non-smokers and physically active; in contrast, men following the "red meat and alcohol" intake pattern had higher BMI, and were less physical active and most frequently smokers. Therefore, although analyses have adjusted for these factors, it does not rule out an interaction between dietary patterns and other cardiovascular risk factors. For instance, BMI could modify the relation between dietary patterns and CHD risk (15), but we found no significant interaction between BMI and diet on AMI risk. Including BMI or WC as covariates in multivariate models resulted in a small attenuating effect, suggesting that the relation between diet and AMI could be independent of body fat, as reported elsewhere (12, 13).

It is also noteworthy the similarities between some of the identified patterns and the recent concept of an Atlantic Diet, suggested by some authors (35-36), who believe that regions with a straight link with the Atlantic Ocean (particularly Northern Portugal and Galicia, Spain) share a specific high consumption of fish and other sea foods, red meats and soups, as it is observed in the “in transition to fast-food” and “fish” patterns, identified in women and men, respectively. In women, it seems that a transition from the traditional Atlantic Diet concept to a more Westernized-type pattern is ongoing, since women in this pattern has already adopted foods, such as fast-foods, sweets and white meats as main food consumptions. In men, the “fish” pattern, characterized by the highest intake of fish, but also higher intakes of red meat, vegetables, particularly vegetable soup holds important features of the Atlantic Diet.
Methodological aspects

A previous comparison between community participants and refusals in our study (22) showed that non-participation had little or no impact on risk estimates of AMI, which suggests a low likelihood of non-response bias. Recruitment of AMI cases was done consecutively, which minimizes selection bias at this level. Moreover, participating hospitals are the ones providing acute coronary care in Porto, so that a representative sample of all non-fatal AMI patients was likely obtained.

To minimize survival and recall bias, only incident AMI cases were included. Also, data collection was done immediately after diagnosis, to avoid bias resulting from behaviour change after the coronary event. Previous research has shown that dietary patterns are fairly stable over time (27, 37), which supports a single assessment of dietary intake. Moreover, we also excluded individuals (21 cases and 75 controls) who reported dietary changes during the preceding year.

Most studies have used factor, principal component analysis or combinatorial algorithms of cluster analysis to derive \textit{a posteriori} dietary patterns (38). However, recent research suggests that the use of finite mixture models for the identification of dietary patterns might entail some advantages over the previous approaches (26). It allows that problems of determining the number of clusters and of choosing an appropriate clustering method to be recast as statistical model choice problems. It also produces posterior cluster belonging probabilities for each subject, given individual food intakes and any other relevant variables, therefore providing measures of uncertainty of the associated classification (in this study we found high individual posterior probabilities: 89% in women and 94% in men). Additionally, it allows the adjustment of food consumption covariates simultaneously with the fitting process, and allows the size of pattern to depend on a set of (concomitant) variables. Moreover, in the present study food non-consumption was not treated as a continuous variable; our solution included dichotomizing food groups whose non-consumption was, roughly, higher than 50%.

CONCLUSIONS

This study identified four dietary patterns, separately for men and women, in the adult general population of Porto, Portugal. As compared to a healthy pattern, the patterns with higher consumptions of red meat and alcohol and lower dairy products and vegetable intakes, were associated with an increased risk of AMI and with a worse
cardiovascular biomarker profile, in both men and women. These findings strengthen the importance of dietary pattern analysis, which beyond the effect of single foods, could potentiate or reduce the risk of disease.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Head and Staff of the Cardiology Departments of the four hospitals collaborating in this study: Hospital São João; Hospital Pedro Hispano; Centro Hospitalar Vila Nova de Gaia and Hospital Geral Santo António.
REFERENCES


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Table 1. Description of the mean consumptions of each food-group according to of the identified dietary patterns (DP).

<table>
<thead>
<tr>
<th>Food groups (g/day)</th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DP 1</td>
<td>DP 2</td>
<td>DP 3</td>
<td>DP 4</td>
</tr>
<tr>
<td></td>
<td>“healthy”</td>
<td>“low fruit and vegetables”</td>
<td>“red meat and alcohol”</td>
<td>“in transition to fast-food”</td>
</tr>
<tr>
<td></td>
<td>mean (standard deviation)</td>
<td>mean (standard deviation)</td>
<td>mean (standard deviation)</td>
<td>mean (standard deviation)</td>
</tr>
<tr>
<td>Red meat</td>
<td>40 (33.7)</td>
<td>61 (71.1)</td>
<td>71 (40.0)</td>
<td>68 (39.1)</td>
</tr>
<tr>
<td>White meat</td>
<td>38 (31.2)</td>
<td>31 (31.8)</td>
<td>41 (25.8)</td>
<td>44 (30.9)</td>
</tr>
<tr>
<td>Fish</td>
<td>72 (37.4)</td>
<td>67 (46.8)</td>
<td>72 (34.7)</td>
<td>72 (37.1)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>205 (128.5)</td>
<td>124 (102.6)</td>
<td>143 (103.0)</td>
<td>159 (91.8)</td>
</tr>
<tr>
<td>Vegetable soup</td>
<td>370 (161.7)</td>
<td>203 (215.8)</td>
<td>149 (193.6)</td>
<td>314 (170.0)</td>
</tr>
<tr>
<td>Fruits</td>
<td>339 (164.3)</td>
<td>226 (219.4)</td>
<td>282 (148.4)</td>
<td>299 (161.2)</td>
</tr>
<tr>
<td>Pasta/potatoes/rice</td>
<td>133 (66.9)</td>
<td>133 (87.2)</td>
<td>167 (89.4)</td>
<td>162 (72.3)</td>
</tr>
<tr>
<td>Dairy products</td>
<td>501 (259.4)</td>
<td>304 (294.7)</td>
<td>232 (261.6)</td>
<td>440 (237.8)</td>
</tr>
<tr>
<td>Cereals</td>
<td>137 (65.2)</td>
<td>117 (90.2)</td>
<td>143 (69.6)</td>
<td>131 (66.0)</td>
</tr>
<tr>
<td>Added fats</td>
<td>12 (9.5)</td>
<td>11 (9.6)</td>
<td>11 (8.6)</td>
<td>12 (8.3)</td>
</tr>
<tr>
<td>Sweets</td>
<td>21 (30.9)</td>
<td>35 (61.2)</td>
<td>31 (38.0)</td>
<td>44 (45.8)</td>
</tr>
<tr>
<td>Fast-foods</td>
<td>11 (16.2)</td>
<td>40 (56.6)</td>
<td>35 (38.7)</td>
<td>42 (35.5)</td>
</tr>
<tr>
<td>Soft drinks</td>
<td>53 (117.9)</td>
<td>107 (248.2)</td>
<td>92 (186.6)</td>
<td>89 (197.7)</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>64 (113.9)</td>
<td>91 (167.5)</td>
<td>100 (159.6)</td>
<td>74 (141.3)</td>
</tr>
</tbody>
</table>

Boldface and underline represent, respectively, the highest and lowest mean consumptions, across clusters and by sex, which have shown to be significantly different from the remaining mean consumptions, by performing multiple comparison tests.
Table 2: Socio-demographic, lifestyle and anthropometric characteristics of cases with acute myocardial infarction and controls, according to dietary patterns (DP).

<table>
<thead>
<tr>
<th>Dietary patterns</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;healthy&quot;</td>
<td>&quot;low fruit and vegetables&quot;</td>
</tr>
<tr>
<td>n cases/controls</td>
<td>41/316</td>
<td>27/121</td>
</tr>
<tr>
<td><strong>mean (SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>62.7 (11.5)</td>
<td>55.6 (12.1)</td>
</tr>
<tr>
<td>controls</td>
<td>57.3 (15.1)</td>
<td>50.8 (15.1)</td>
</tr>
<tr>
<td>Education, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>3.9 (3.1)</td>
<td>4.3 (2.2)</td>
</tr>
<tr>
<td>controls</td>
<td>7.9 (5.2)</td>
<td>8.8 (5.6)</td>
</tr>
<tr>
<td>Total energy intake, kcal/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>2096 (454)</td>
<td>1849 (554)</td>
</tr>
<tr>
<td>controls</td>
<td>1920 (480)</td>
<td>1848 (675)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>28.7 (4.5)</td>
<td>29.2 (5.4)</td>
</tr>
<tr>
<td>controls</td>
<td>27.2 (5.0)</td>
<td>27.4 (5.4)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>95.6 (11.4)</td>
<td>95.0 (12.2)</td>
</tr>
<tr>
<td>controls</td>
<td>87.0 (12.6)</td>
<td>87.7 (14.6)</td>
</tr>
<tr>
<td><strong>n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>5 (12.2)</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td>controls</td>
<td>37 (11.7)</td>
<td>36 (29.8)</td>
</tr>
<tr>
<td>Regular physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>6 (14.6)</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>controls</td>
<td>129 (40.8)</td>
<td>35 (28.9)</td>
</tr>
<tr>
<td>Family history of AMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>16 (30.0)</td>
<td>12 (44.4)</td>
</tr>
<tr>
<td>controls</td>
<td>93 (29.4)</td>
<td>21 (17.4)</td>
</tr>
</tbody>
</table>

* p < 0.05 for differences between DP categories in cases and controls, assessed by non-parametric tests.

SD: standard deviation; AMI: acute myocardial infarction.
Table 3. Association between dietary patterns and the risk of acute myocardial infarction, by sex.

<table>
<thead>
<tr>
<th></th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Model 4&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>OR (95%CI)</td>
<td>OR (95%CI)</td>
<td>OR (95%CI)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;healthy&quot;</td>
<td>41/316</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&quot;low fruit and vegetables&quot;</td>
<td>27/121</td>
<td>1.72 (1.01-2.92)</td>
<td>1.71 (0.97-3.04)</td>
<td>1.74 (0.98-3.08)</td>
</tr>
<tr>
<td>&quot;red meat and alcohol&quot;</td>
<td>66/273</td>
<td>1.86 (1.22-2.84)</td>
<td>1.76 (1.11-2.78)</td>
<td>1.78 (1.12-2.82)</td>
</tr>
<tr>
<td>&quot;in transition to fast-food&quot;</td>
<td>60/652</td>
<td>0.71 (0.47-1.08)</td>
<td>0.78 (0.50-1.24)</td>
<td>0.78 (0.50-1.23)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;healthy&quot;</td>
<td>73/171</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&quot;fish&quot;</td>
<td>207/366</td>
<td>1.32 (0.96-1.83)</td>
<td>1.05 (0.73-1.50)</td>
<td>1.03 (0.72-1.47)</td>
</tr>
<tr>
<td>&quot;red meat and alcohol&quot;</td>
<td>257/188</td>
<td>3.20 (2.30-4.46)</td>
<td>1.83 (1.26-2.67)</td>
<td>1.78 (1.22-2.60)</td>
</tr>
<tr>
<td>&quot;intermediate intake&quot;</td>
<td>89/109</td>
<td>1.91 (1.29-2.83)</td>
<td>1.36 (0.88-2.10)</td>
<td>1.34 (0.87-2.07)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Crude odds ratio.
<sup>b</sup> Odds ratio adjusted for age, education, smoking status, regular physical activity, total energy intake and family history of acute myocardial infarction.
<sup>c</sup> Model 2 plus adjustment for BMI.
<sup>d</sup> Model 2 plus adjustment for self-reported hypertension, dyslipidemia and diabetes.

OR (95%CI): Odds ratio (95% confidence interval).
Table 4. Distribution of cardiovascular risk markers, by dietary patterns (DP) and sex in a subsample of the general population.

<table>
<thead>
<tr>
<th></th>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>133 (130-135)</td>
<td>136 (132-140)</td>
<td>132 (130-135)</td>
<td>132 (131-134)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>82 (76-88)</td>
<td>93 (84-103)</td>
<td>86 (80-93)</td>
<td>83 (79-87)</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>93 (91-97)</td>
<td>96 (90-100)</td>
<td>92 (88-95)</td>
<td>92 (90-94)</td>
</tr>
<tr>
<td>Total-to-HDL cholesterol ratio</td>
<td>3.6 (3.5-3.8)</td>
<td>3.8 (3.6-4.1)</td>
<td>3.9 (3.7-4.0)*</td>
<td>3.8 (3.7-4.0)</td>
</tr>
<tr>
<td>Triglycerides, g/l</td>
<td>1.04 (0.96-1.11)</td>
<td>1.14 (1.02-1.26)</td>
<td>1.11 (1.03-1.19)</td>
<td>1.03 (0.98-1.08)</td>
</tr>
<tr>
<td>hs-CRP, mg/l</td>
<td>2.27 (1.92-2.63)</td>
<td>2.66 (1.98-3.33)</td>
<td>2.72 (2.35-3.09)</td>
<td>2.67 (2.39-2.94)</td>
</tr>
<tr>
<td>Uric acid, mg/l</td>
<td>39.8 (38.1-41.5)</td>
<td>42.6 (39.4-45.7)</td>
<td>41.4 (39.7-43.2)</td>
<td>40.2 (38.9-41.4)</td>
</tr>
<tr>
<td>Leukocytes, /mm³</td>
<td>6319 (6027-6610)</td>
<td>5936 (5422-6450)</td>
<td>6340 (6042-6637)</td>
<td>6434 (6223-6646)</td>
</tr>
</tbody>
</table>

| **Men**              |                      |                      |                      |                      |
| Systolic blood pressure, mmHg | 133 (130-136)        | 134 (132-136)        | 137 (134-140)        | 136 (133-140)        |
| Diastolic blood pressure, mmHg | 81 (79-83)           | 83 (82-84)           | 84 (82-86)*          | 84 (82-86)*          |
| Glucose, mg/dl       | 100 (95-106)         | 97 (94-100)          | 99 (94-104)          | 96 (90-102)          |
| Total-to-HDL cholesterol ratio | 4.3 (4.1-4.5)        | 4.5 (4.4-4.7)        | 4.5 (4.3-4.8)        | 4.4 (4.1-4.7)        |
| Triglycerides, g/l   | 1.18 (1.03-1.33)     | 1.35 (1.25-1.45)     | 1.37 (1.23-1.51)     | 1.17 (1.00-1.35)     |
| hs-CRP, mg/l         | 1.68 (1.20-2.17)     | 1.96 (1.60-2.32)     | 2.64 (2.18-3.10)*    | 1.95 (1.33-2.56)     |
| Uric acid, mg/l      | 48.8 (45.6-52.1)     | 55.1 (52.9-57.4)*    | 57.0 (54.2-59.9)**   | 50.5 (46.2-54.8)     |
| Leukocytes, /mm³     | 6662 (6239-7084)     | 6898 (6606-7189)     | 6378 (6021-6735)     | 6121 (5589-6654)     |

Women: DP1=“healthy”; DP2=“low fruit and vegetables”; DP3=“red meat and alcohol”; DP4=“in transition to fast-food”.
Men: DP1=“healthy”; DP2=“fish”; DP3=“red meat and alcohol”; DP4=“intermediate intake”.

95%CI: 95% confidence interval; CRP: C-reactive protein; HDL: high density lipoprotein; LDL: low density lipoprotein.
Differences between dietary patterns obtained by generalized linear models: *p<0.05; **p<0.001 comparing with the reference class: DP1.

* Means adjusted for age, education, smoking status, regular physical activity, total energy intake and family history of acute myocardial infarction.
Paper VI

Adherence to the Southern-European Atlantic diet and risk of non-fatal acute myocardial infarction

Andreia Oliveira, Carla Lopes, Fernando Rodríguez Artalejo

[Submitted]
Adherence to the Southern-European Atlantic diet and risk of non-fatal acute myocardial infarction

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ABSTRACT

Background: The Southern-European Atlantic diet (SEAD) is the traditional diet in Northern Portugal and Galicia, a region in Northwest Spain.

Objective: To examine the association between adherence to the SEAD and the risk of non-fatal acute myocardial infarction (AMI).

Design: Population-based case-control study in Porto, Portugal. Cases were patients aged ≥18 years hospitalized with an incident AMI (n=820), and controls were individuals without AMI selected randomly from the resident population of the hospitals' catchment area (n=2196). A validated food frequency questionnaire was used in face-to-face interviews to assess dietary intake in the previous year. We developed a SEAD adherence index with nine key components: fresh fish excluding cod fish, cod fish, red meat and pork products, dairy products, legumes and vegetables, vegetable soup, potatoes, whole grain bread, and wine. A score of 1 or 0 was respectively assigned to each food consumed above or below the sex-specific median in the controls.

Results: After adjustment for the main confounders, a one-point increment in the SEAD score was associated with a 10% reduced risk of AMI (odds ratio [OR] 0.90, 95% confidence interval [95% CI] 0.85,0.96). As compared with individuals in the lower quartile of the SEAD index (lowest adherence), those in the upper quartile had a 33% lower likelihood of AMI (OR 0.67, 95% CI 0.51,0.88; p for trend=0.003).

Conclusions: Adherence to the SEAD was associated with lower risk of non-fatal AMI. SEAD might contribute to the very low coronary mortality in Northern Portugal and Galicia.

Key-words: Dietary patterns; Atlantic diet; Myocardial Infarction; Case-control study
INTRODUCTION

The Southern-European Atlantic diet (SEAD) is the traditional diet in the Northwest of the Iberian Peninsula, in particular, Northern Portugal and Galicia, a region in Northwest Spain (1-5) (Supplemental Figure 1). These regions have geographical, climatic and cultural characteristics that have led to a diet of their own. Because they are located on the Atlantic coast and fishery has been a main local industry, fresh fish and, in particular, cod fish, either fresh or dried and salted, are key components of the SEAD. Also, thanks to the high rainfall in the region, good pastures abound, which favors extensive cattle and pig breeding; as a result, red meat, pork and dairy products are staple foods. Farm land has traditionally been organized in small family properties, ensuring a supply of seasonal legumes, vegetables and potatoes. Whole grain bread, either from corn or wheat, is served as a side dish or as the base for meat and fish pies. Also, a vegetable soup ("caldo") is the first course in many meals throughout the year. Lastly, despite being rainy, the weather is also fairly sunny with mild temperatures, allowing for grape cultivation. Consequently, wine is a component of the SEAD (1-5).

The SEAD differs from the traditional Mediterranean diet (6) in at least two important aspects. First, unlike the Mediterranean diet, the SEAD is characterized by a high consumption of red meat, pork and fish. Second, olive oil is not the principal source of total fat in the SEAD, and nuts and fruits are not prominent components of the diet. However, like the Mediterranean diet, SEAD is characterized by a high intake of vegetables and whole foods and by wine consumption during meals.

Also of note is that these Southern-European Atlantic regions show a very low mortality from coronary heart disease (CHD), with age-adjusted rates of 30.6/100 000 in 2006 for Northern Portugal (7) and 69/100 000 in 2007 for Galicia (8). These rates are comparable to or slightly lower than those in Mediterranean countries such as France, Italy or Greece, but much lower than in Northern and Eastern European countries (9).

Although the health effects of the Mediterranean diet have been widely studied (10-11), this is the first study to examine the association between adherence to the SEAD and risk of acute myocardial infarction (AMI). These results might help to support dietary recommendations for the populations of these regions, and to understand the contribution of diet to their low coronary risk.
SUBJECTS AND METHODS

Study design and participants

From 1999 to 2003 we conducted the EPICardis study, a population-based case-control study among Caucasian adults in Porto, an urban centre with approximately 300,000 inhabitants in Northwest Portugal. Cases were patients consecutively admitted with a first AMI (12) to the Cardiology Department of the four hospitals providing acute coronary care in Porto, and who survived beyond the fourth day after the coronary event. Controls were individuals from the catchment area of participating hospitals, with no previous clinical or silent infarction, according to self-reported data or and electrocardiographic evidence. Controls were selected from random digit dial of households (13), and then from simple random sampling of permanent residents aged ≥18 years. Refusals were not replaced, and the participation rate was 70% (14). The Ethics Committees of the four participating hospitals approved the study protocol, and all participants gave written informed consent.

Of the 1248 patients with a first AMI consecutively identified, we excluded 65 who were unable to collaborate, 37 who died before the interview, 3 who refused to participate, 138 with cognitive impairment as indicated by a score of <24 in the Mini Mental State Examination (15-16), and 162 who did not finish the interview or provided incomplete information. We further excluded 21 patients who changed diet the year before and 2 with possible unreliable data because energy intake exceeded three standard deviations of the sex-specific population median intake. Of the 2485 community participants, we excluded 103 due to previous clinical or silent infarction, 60 because of physical disability or incomplete information, 51 with cognitive impairment, and 75 who changed diet the year before. Thus, the analyses were conducted with 820 cases (194 women, 626 men) and 2196 controls (1362 women, 834 men).

Data collection and study variables

Data were collected concurrently in cases and controls, by the same set of trained interviewers using the same structured questionnaire. Patients with AMI were interviewed during their hospital stay, after clinical stabilization, usually between the 4th and the 8th day after the coronary event. Controls were invited to face-to-face interviews and physical examination at the Department of Hygiene and Epidemiology of the University of Porto Medical School.
Diet

Dietary intake over the previous 12 months was assessed with a validated semi-quantitative food frequency questionnaire. The questionnaire comprises a list of 82 foods or food groups and a closed section with nine categories of frequency of consumption ranging from “never or less than once a month” to “six or more times a day”; it also includes two other closed sections for the average portion consumed (lower, equal or higher than the mean portion size) and the seasonal variation of consumption. A photographic album was used to help the decision of the average portion size consumed. Detailed information on the development, structure, validity and reproducibility of the food frequency questionnaire is reported elsewhere (17-18). Food consumption was converted into total energy intake with the software Food Processor Plus® (ESHA Research, Salem-Oregon, 1997), which has been adapted to the traditional Portuguese foods.

Operational definition of the Southern-European Atlantic Diet

Diet indexes are often used to describe total diet (19). To represent the degree of adherence to the SEAD, we developed an index based upon the conceptual definition of SEAD proposed in several international meetings over the last years (1-5). The two main operational decisions involved were the selection of the types of food and the methods of scoring. From the conceptual definition of SEAD, it followed that the index should contained at least these foods or food groups: fresh fish (lean and fatty fish, excluding cod fish and canned preparations), cod fish (either fresh or dried and salted), red meat and pork products (beef, pork and pork products, including smoked ham, bacon, and sausages), dairy products (skimmed, semi-skimmed and whole milk, yogurt and cheese), legumes and vegetables (beans, peas and vegetables not eaten as soup ingredients), vegetable soup (cooked with vegetables, small amounts of potatoes and some drops of olive oil), potatoes regardless of the cooking method, whole grain bread (non-refined grain bread made of different cereals, such as wheat, corn, rye) and wine (red or white).

Although shellfish is commonly considered a characteristic component of SEAD, it is typically consumed in small amounts; hence, it was not included as a component of the SEAD index.

Each SEAD component (apart from alcohol) was measured as grams per 1000 kcal/day (to express intake as energy density). As in other well-known diet indexes, such as the Mediterranean diet developed by Trichopoulou et al. (20), consumption of each food, except wine, which was at or higher than the sex-specific median in controls was assigned a score of 1; a lower consumption was given a 0 score. For wine, we
considered that consumption up to 1 glass/day in women, and up to 2 glasses/day in men, fairly represented the meal drinking pattern characteristic of the SEAD. Thus, a score of 1 was given to this level of consumption, and a 0 score when above or below (nil consumption). For each participant, the scores (0 or 1) for the 9 food components of the SEAD were summed; thus the index ranged from 0 (lowest adherence to SEAD) to 9.

Socio-demographic, lifestyle, clinical and anthropometric characteristics

For both AMI cases and controls, we obtained information on age and education (completed years of schooling), tobacco smoking (current smoking, including regular and occasional smokers; and non-current smoking, including never and former smokers with at least six months without smoking), and physical exercise (regular practice for at least 30 min per week of any leisure-time physical activity with energy expenditure higher than 2.5 metabolic equivalents during the previous year). A family history of AMI was also registered when one or more first-degree relatives had suffered an AMI, regardless of age at occurrence. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m), measured with participants in light clothing and barefoot. Information on menopausal status and hormone replacement therapy was also recorded for all women.

Statistics

Differences in proportions were tested with the Chi-square test or the Fisher-exact test, as appropriate. The Kruskal-Wallis test was used to compare continuous variables between two independent samples.

The association between adherence to the SEAD and risk of non-fatal AMI was summarized with odds ratios (OR) and their 95% confidence intervals (95% CI), obtained from unconditional logistic regression models. The SEAD score was modeled both as a continuous variable and in quartiles, using the lowest quartile as reference. Models were adjusted for sex, age, education, total energy intake, and BMI, as continuous variables, and for current smoking (no, yes), regular physical activity (no, yes), and menopause and hormone replacement therapy in women (no, yes). Models were further adjusted for consumption of fruits, refined cereals and white meat, as continuous variables, because these types of food were not part of the SEAD index. The dose-response relationship between the SEAD index and AMI was assessed with tests for linear trend, by modeling the quartiles of the SEAD index as a continuous variable (values 1 to 4). Also we examined whether the association between the SEAD index and AMI varied with sex and BMI (<30.0 vs. ≥30.0 kg/m²); to this end, the models
included interaction terms constructed as the product of SEAD quartiles by sex and BMI categories. Likelihood ratio tests were used to compare models with and without interaction terms.

To assess the robustness of results, we conducted sensitivity analyses using two different procedures to obtain the SEAD index. First, the score used sex-specific quartiles of consumption of each type of food (measured in g per 1000 kcal/day); values assigned ranged from 1 for the lowest quartile to 4 for the highest. Second, the score assigned values to the frequency of food consumption: 1 for less than weekly, 2 for one-to-six times per week, and 3 for daily consumption. In both cases, the scoring for alcohol consumption remained the same as for the main analyses.

Lastly, to place the association between the SEAD and AMI into the context of what can be expected from a healthy diet, we calculated the Mediterranean diet index proposed by Trichopoulou et al. (20) and examined its association with AMI in our study. To this end, we replicated the analyses described above replacing the SEAD score by the Mediterranean diet score.

Statistical significance was set at two-sided p<0.05. Analyses were performed with STATA/SE 10.0 (USA, StataCorp; 2007).

RESULTS

Table 1 shows the personal characteristics of AMI cases and controls by quartiles of the SEAD index. Regardless of adherence to the SEAD, AMI cases were more frequently men and had lower education than controls. They also reported higher total energy intake and higher prevalence of smoking and physical inactivity. Moreover, in the lower quartile of the SEAD score, BMI was higher in cases than in controls. Among controls, age increased from the lowest to the highest quartile of the SEAD score, whereas total energy intake decreased. Also, controls in the lower quartile of the SEAD score were more frequently smokers and inactive.

Table 2 shows the association between adherence to the SEAD and risk of non-fatal AMI. After adjustment for the main confounders, a 1 point increase in SEAD score was associated with a 10% reduced risk of AMI (odds ratio [OR] 0.90, 95% confidence interval [95% CI] 0.85, 0.96). As compared with individuals in the lower quartile of the SEAD score (lower adherence), those in the upper quartile had a 33% lower likelihood of AMI (OR 0.67, 95% CI 0.51, 0.88; p for trend=0.003). These results did not vary with sex or BMI (p for sex-interaction=0.661, p for BMI-interaction=0.218).
Results were also similar in the sensitivity analyses where, rather than median food consumption, the SEAD scoring used sex-specific quartiles (OR 0.61, 95% CI 0.46, 0.80; p for trend <0.001) or frequency with which each food was consumed (OR 0.58, 95% CI 0.43, 0.79; p for trend<0.001).

The Mediterranean diet index showed an inverse association with the risk of AMI. This association was slightly stronger than that found between the SEAD and AMI. As compared with the first quartile of the Mediterranean index score (lower adherence), the adjusted OR (95% CI) for AMI was 0.85 (0.66, 1.10) for the second quartile, 0.78 (0.60, 1.02) for the third quartile, and 0.61 (0.47, 0.81) for the fourth quartile.

To understand the health effects underlying the association between adherence to the SEAD and risk of AMI, we ran models where SEAD score was replaced by each of the nine individual food components (table 3). As compared to those with a lower than the median consumption, those with a higher consumption of cod fish, dairy products, legumes and vegetables, whole grain bread, and wine showed a reduced risk of AMI. In contrast, a higher consumption of red meat and pork products and potatoes was associated with an increased risk. Consumption of fresh fish or vegetable soup did not show an association with AMI.

Of note is that a SEAD index calculated with reverse scoring for red meat and pork products and for potatoes (value 0 for consumption at or higher than the median, and 1 for a lower consumption) led to an even stronger inverse association between the SEAD and AMI. As compared with individuals in the lower quartile of the SEAD score, those in the upper quartile had a 60% lower risk of AMI (OR 0.40, 95% CI 0.30, 0.52; p for trend<0.001).

DISCUSSION

Our results show that a higher adherence to the SEAD, a highly palatable diet which is culturally rooted in Northern Portugal and Galicia, is associated with a lower risk of non-fatal AMI.

Although the SEAD index has been derived from a conceptual definition of SEAD based on a consensus of international experts, it is also supported by food consumption data. Portugal has a high availability of fish, particularly of cod fish (21-22). Also, evidence exists that the national availability of meat and dairy products is relatively high and has been increasing, almost reaching the present-day Northern-European levels (23). The availability of vegetables throughout the year is also
relatively high, when compared with other European countries (21-23). As a natural wine producer, Portugal also has very frequent alcohol consumption, mainly from wine sources (24).

Individually, most food components of the SEAD have been previously associated with CHD (25-26). In several prospective studies, consumption of fish (27), fruits and vegetables (28), and whole grains as a replacement for refined grains (29), have been consistently associated with a reduced risk of CHD. For dairy products, a review of 12 prospective cohort studies has shown no clear evidence of consistent associations with a higher risk of CHD (30). In our study, an inverse association with AMI was found. Though our results should be replicated in prospective studies, several authors have reported an inverse association between dairy consumption and the occurrence of one or several facets of the metabolic syndrome, including high blood pressure (31-32), which might account for our findings. Prospective evidence also suggests an inverse relationship between light-to-moderate wine consumption and cardiovascular risk (33-34). The exact form of the association may, nevertheless, vary across studies depending on the instrument used to assess alcohol, the thresholds selected in the analyses, the inclusion of ex-drinkers, and the number and type of confounders controlled for. In our study we assigned a value of 1 only to moderate consumption of wine; this decision aimed to reflect the meals-associated drinking pattern characteristic of SEAD. Also, the use of a lower threshold for women than for men is due to the traditionally lower consumption in women, which continues to be observed today (24). Of note is that our findings on the inverse association between the SEAD and CHD risk did not change materially when we replicated the analyses with a SEAD score which assigned a value of 1 to a consumption of ≥1 glass/day in women and ≥2 glasses/day in men (data not shown).

Although fish was considered a main component of the SEAD, the intake of red meat and pork products was even higher, and almost double the consumption of white meat (mainly poultry). The high content of heme iron and fat in red meat, particularly saturated fat and cholesterol, together with the high salt content of pork products, might account for an increased coronary risk (35). Also, potatoes have a high glycemic index, which has been associated with unfavorable effects on metabolic risk factors (36). Although in our study both variables were associated with a higher frequency of CHD, this does not preclude an inverse association between adherence to the SEAD and CHD risk. In fact, the use of a diet index allows for the measurement of the combined effect (including interactions) of all food components of SEAD, some of them with a clear protective influence on CHD (e.g. vegetables, whole grains, wine). Lastly, despite the widely-accepted beneficial effects of fish consumption on CHD (27), we found no
association of fresh fish (excluding cod fish) with CHD. Other studies using a Mediterranean diet index have also reported no associations between many food components of the diet, including non-cod fish, and CHD risk (20, 37).

For a correct interpretation of our findings, some methodological comments are needed. Case-control studies are relatively prone to several types of error, particularly in sample selection and information recall. In the present study, cases of AMI were approached during their in-hospital stay and only incident cases were selected, which should reduce potential bias from behavior change after the coronary event. Furthermore, given that all diagnosed AMI cases in Portugal are admitted to public hospitals, that all AMI cases invited consented to participate, that the response rate among controls was fairly high (70%), and that previous research has shown that non-participation had little or no impact on risk estimates of AMI (14), it is not likely that selection bias exerted a substantial influence on our results. Even so, to confirm our results future research with a prospective design is needed.

Moreover, our food frequency questionnaire has shown good reproducibility and validity in this specific population (17-18). Additionally, data collection was conducted by trained personnel in face-to-face interviews, using a photograph album to help with the selection of portion sizes; this might have minimized the expected underestimation of intake, which is frequent in food consumption studies. Also, the exclusion of individuals who changed their diet during the previous year undoubtedly improved the assessment of long-term intake.

Lastly, as with any a priori dietary patterns, several subjective decisions were made to build the SEAD index. However, results were similar when using different methods of scoring, which indicates that our results are fairly robust.

Our findings are of practical importance. First, they indicate that the traditional diet of Northern Portugal and Galicia is healthy from a CHD standpoint, and support the development of dietary guidelines for these populations based upon their own cultural roots. Also, our results suggest that a reduction in consumption of red meat, pork products and potatoes might increase the benefit of SEAD on CHD, becoming closer to what might be achieved by a Mediterranean diet. However, more research is needed on this issue because intervention on a few food components might lead to compensatory diet changes, whose overall health effects are as yet unknown. Third, they suggest that SEAD might contribute to the low coronary mortality traditionally registered in Northern Portugal and Galicia, helping to shed light on a long-standing scientific issue. Finally, the effect of adherence to the SEAD on other important disorders, such as stroke or gastric cancer, which represent a large disease burden in Portugal and Spain, should also be investigated.
ACKNOWLEDGEMENTS

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REFERENCES


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Table 1. Characteristics of cases with non-fatal acute myocardial infarction and controls, by adherence to the Southern-European Atlantic Diet (SEAD).

<table>
<thead>
<tr>
<th></th>
<th>Quartiles of the SEAD score&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (lower adherence)</td>
</tr>
<tr>
<td>n cases/controls</td>
<td>333/663</td>
</tr>
<tr>
<td><strong>mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>48.7 (12.2)</td>
</tr>
<tr>
<td>controls</td>
<td>48.9 (15.9)</td>
</tr>
<tr>
<td>Education, years</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>6.3 (3.7)</td>
</tr>
<tr>
<td>controls</td>
<td>8.6 (5.0)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total energy intake, kcal/day</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>2780 (799)</td>
</tr>
<tr>
<td>controls</td>
<td>2451 (737)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>27.1 (3.9)</td>
</tr>
<tr>
<td>controls</td>
<td>26.5 (4.8)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Sex (women)</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>64 (19.2)</td>
</tr>
<tr>
<td>controls</td>
<td>391 (59.0)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>261 (78.4)</td>
</tr>
<tr>
<td>controls</td>
<td>336 (60.7)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>Regular physical activity</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>57 (17.1)</td>
</tr>
<tr>
<td>controls</td>
<td>199 (30.0)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
<tr>
<td>Family history of infarction</td>
<td></td>
</tr>
<tr>
<td>cases</td>
<td>113 (33.9)</td>
</tr>
<tr>
<td>controls</td>
<td>136 (20.5)&lt;sup&gt;‘&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Quartile (Q) values of the SEAD score in controls: Q1 (lower adherence): ≤3; Q2: 4; Q3: 5; Q4: ≥6 in women; Q1: ≤ 3; Q2: 4; Q3: 6; Q4: ≥6 in men.

<sup>‘</sup>p<0.05: differences in personal characteristics between cases and controls in each quartile of the SEAD score, tested by the Kruskal-Wallis test (for continuous variables) and the Chi-square test (for dummy variables).

<sup>‘‘</sup>p<0.05: differences in personal characteristics between quartiles of the SEAD score in cases or controls, tested by the Kruskal-Wallis test (for continuous variables) and the Chi-square test (for dummy variables).
Table 2. Association between adherence to the Southern-European Atlantic Diet (SEAD) and risk of non-fatal acute myocardial infarction.

<table>
<thead>
<tr>
<th></th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR 1 (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SEAD scoring above/below sex-specific median food consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEAD score</td>
<td>820 (100)</td>
<td>2196 (100)</td>
<td>0.83 (0.78,0.87)</td>
<td>0.90 (0.85,0.96)</td>
</tr>
<tr>
<td>Quartiles of the SEAD score 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (lower adherence)</td>
<td>333 (40.6)</td>
<td>663 (30.2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>205 (25.0)</td>
<td>506 (23.0)</td>
<td>0.81 (0.66,0.99)</td>
<td>1.06 (0.83,1.36)</td>
</tr>
<tr>
<td>3</td>
<td>195 (19.0)</td>
<td>523 (23.8)</td>
<td>0.62 (0.49,0.77)</td>
<td>0.83 (0.64,1.08)</td>
</tr>
<tr>
<td>4 (higher adherence)</td>
<td>126 (15.4)</td>
<td>522 (23.8)</td>
<td>0.48 (0.38,0.61)</td>
<td>0.67 (0.51,0.88)</td>
</tr>
<tr>
<td><strong>p-trend</strong></td>
<td>&lt;0.001</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **SEAD scoring based on sex-specific quartiles of food consumption** |             |                 |                   |                     |
| SEAD score           | 820 (100)   | 2196 (100)     | 0.91 (0.86,0.93)  | 0.95 (0.92,0.97)    |
| Quartiles of the SEAD score 3 |             |                 |                   |                     |
| 1 (lower adherence)  | 318 (38.8)  | 557 (25.4)     | 1                 | 1                   |
| 2                    | 167 (20.4)  | 431 (19.6)     | 0.68 (0.54,0.85)  | 0.90 (0.69,1.18)    |
| 3                    | 199 (24.3)  | 649 (29.6)     | 0.54 (0.44,0.66)  | 0.69 (0.54,0.89)    |
| 4 (higher adherence) | 136 (16.6)  | 559 (25.5)     | 0.43 (0.34,0.54)  | 0.61 (0.46,0.80)    |
| **p-trend**          | <0.001      | <0.001          |                   |                     |

| **SEAD scoring based on frequency of food consumption** |             |                 |                   |                     |
| SEAD score           | 820 (100)   | 2196 (100)     | 0.93 (0.89,0.96)  | 0.91 (0.87,0.95)    |
| Quartiles of the SEAD score 4 |             |                 |                   |                     |
| 1 (lower adherence)  | 213 (26.0)  | 480 (21.9)     | 1                 | 1                   |
| 2                    | 274 (33.4)  | 616 (28.0)     | 1.00 (0.81,1.24)  | 0.92 (0.71,1.19)    |
| 3                    | 205 (29.0)  | 637 (29.0)     | 0.72 (0.58,0.91)  | 0.64 (0.49,0.84)    |
| 4 (higher adherence) | 128 (15.6)  | 463 (21.1)     | 0.62 (0.48,0.80)  | 0.58 (0.43,0.79)    |
| **p-trend**          | <0.001      | <0.001          |                   |                     |

1 Unconditional logistic regression models adjusted for sex, age, education, total energy intake, fruits, refined cereals, white meat, smoking status, regular physical activity, family history of infarction, body mass index, and menopause and hormone replacement therapy in women.

2 Quartile (Q) values of the SEAD score in controls: Q1 (lower adherence): ≤3; Q2: 4; Q3: 5; Q4: ≥6 in women; Q1: ≤3, Q2: 4, Q3: 6, Q4: ≥6 in men.


4 Quartile (Q) values of the SEAD score in controls: Q1 (lower adherence): ≤15; Q2: 16-17; Q3: 18-19; Q4: ≥20.

OR: odds ratio; 95% CI: 95% confidence interval.
Table 3. Association between each of the nine food components of the Southern-European Atlantic Diet and risk of non-fatal AMI.

<table>
<thead>
<tr>
<th>Food components ¹ (g per 1000 kcal/day)</th>
<th>Median intake in controls ² (25th-75th P)</th>
<th>Women Cases (n, %)</th>
<th>Men Cases (n, %)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR ³ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fish (excluding cod fish)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>19.1 (10.9, 29.5)</td>
<td>417 (50.8)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>16.4 (9.1, 26.1)</td>
<td>403 (49.2)</td>
<td>1098 (50.0)</td>
<td>0.97 (0.82, 1.13)</td>
<td>1.16 (0.96, 1.41)</td>
</tr>
<tr>
<td>Cod fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>6.3 (3.8, 10.6)</td>
<td>445 (54.3)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>7.2 (4.3, 12.7)</td>
<td>375 (45.7)</td>
<td>1098 (50.0)</td>
<td>0.64 (0.72, 0.99)</td>
<td>0.62 (0.68, 0.99)</td>
</tr>
<tr>
<td>Red meat and pork products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>28.7 (17.1-41.3)</td>
<td>289 (35.2)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>32.4 (23.2, 46.8)</td>
<td>531 (64.8)</td>
<td>1098 (50.0)</td>
<td>1.84 (1.56, 2.17)</td>
<td>1.74 (1.43, 2.12)</td>
</tr>
<tr>
<td>Dairy products</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>176.9 (101.7-284.2)</td>
<td>552 (67.3)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>113.3 (64.6, 185.3)</td>
<td>268 (32.7)</td>
<td>1098 (50.0)</td>
<td>0.48 (0.41, 0.57)</td>
<td>0.65 (0.53, 0.79)</td>
</tr>
<tr>
<td>Legumes and vegetables</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>70.8 (45.5-105.0)</td>
<td>550 (67.1)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>54.0 (35.2, 80.2)</td>
<td>270 (32.9)</td>
<td>1098 (50.0)</td>
<td>0.49 (0.42, 0.58)</td>
<td>0.64 (0.53, 0.78)</td>
</tr>
<tr>
<td>Vegetable soup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>132.7 (63.4-200.9)</td>
<td>462 (56.3)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>103.9 (46.4, 176.6)</td>
<td>358 (43.7)</td>
<td>1098 (50.0)</td>
<td>0.77 (0.66, 0.91)</td>
<td>0.86 (0.70, 1.05)</td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>38.4 (25.6-53.2)</td>
<td>334 (40.7)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>39.5 (28.6, 53.8)</td>
<td>486 (59.3)</td>
<td>1098 (50.0)</td>
<td>1.46 (1.24, 1.71)</td>
<td>1.33 (1.10, 1.61)</td>
</tr>
<tr>
<td>Whole grain bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;median</td>
<td>6.00 (0.00, 33.0)</td>
<td>493 (60.1)</td>
<td>1098 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥median</td>
<td>3.73 (0.0, 24.0)</td>
<td>327 (39.9)</td>
<td>1098 (50.0)</td>
<td>0.66 (0.56, 0.78)</td>
<td>0.74 (0.62, 0.90)</td>
</tr>
<tr>
<td>Wine (glasses/day) ⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or &gt;1 (women), 0 or &gt;2 (men)</td>
<td></td>
<td>663 (80.8)</td>
<td>1452 (66.1)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;0 to ≤1 (women), &gt;0 to ≤2 (men)</td>
<td></td>
<td>157 (19.2)</td>
<td>744 (33.9)</td>
<td>0.46 (0.38, 0.56)</td>
<td>0.56 (0.45, 0.70)</td>
</tr>
</tbody>
</table>

¹ Sex-specific medians in the controls were used as threshold for all food components, except wine.
² Median energy intake (kcal/day) (25th-75th P) in controls: women: 1992 (1661-2359); men: 2460 (2118-2917).
³ Unconditional logistic regression models adjusted for sex, age, education, total energy intake, fruits, refined cereals, white meat, smoking status, regular physical activity, family history of infarction, body mass index, and menopause and hormone replacement therapy in women.
⁴ Median wine consumption was 0 glass/day in women and approximately 1 glass/day in men.
OR: odds ratio; 95% CI: 95% confidence interval; 25th-75th P: percentile 25-percentile 75; AMI: acute myocardial infarction.
Figure 1. Regions in Northern Portugal and Northwest Spain where the Southern-European Atlantic diet is typically consumed.
General Discussion and Conclusions
In this dissertation, body fat distribution and diet were studied in relation to coronary outcomes. Inflammation showed to be a potential pathway by which adipose tissue and dietary factors could modulate the CHD risk. A gender-effect was present in many of the studied associations.

Different patterns of body fat distribution, identified by principal component analysis, were found to have opposing effects on hs-CRP levels and non-fatal AMI. As previously reported (58, 61, 63-65, 67, 73-77, 99-101), general and central patterns of body fat distribution were directly associated with the cardiovascular outcomes. The use of principal component analysis to create uncorrelated components of body fat distinguished two patterns based on the same classical anthropometric measures - BMI and WC, showing that they are not independent from each other and can aggregate in different ways. While the general pattern of body fat distribution showed a high correlation between BMI and WC, the central pattern was characterized by high WC, but low BMI, supporting that it is relatively difficult to distinguish between the effects of abdominal and total body fat using simple anthropometric measures. In that way, the identification of uncorrelated components of obesity could be a useful approach to assess the independent effect of fat depots on health outcomes.

While these two previously reported components increased the coronary risk, the peripheral fat pattern was inversely associated with both hs-CRP (paper I) and AMI (paper II) in women, supporting a possible role of inflammation as a link between obesity and CHD. Although scant information is available on the effect of peripheral fat on metabolic risk factors linked with coronary outcomes (71, 79-83, 297-298), its potential beneficial effect is supported by biological mechanisms related with a lower fatty acid turnover (93) and higher secretion of hormonal factors, such as adiponectin (94).

Of note is that, in men, the component characterized by the peripheral subcutaneous fat index showed no significant effects on hs-CRP levels, but predicted a higher risk for AMI; in that way yielding opposing associations by sex. Since men and women have different ranges of variation in levels of body fat (men have a larger proportion of visceral fat, while women have higher levels and variability of total and subcutaneous peripheral fat) (299-301), a gender-effect in these relations would be expected. Sex hormones could also hold important effects (32-33, 106) and contribute to some of the differences found, though statistical analyses were adjusted for hormone replacement therapy. The non-significant association between the peripheral
fat component and hs-CRP in men could be explained by the lower circulating CRP levels (100, 302) and weaker associations of adiposity with low-grade systemic inflammation frequently found in men than in women (100, 103).

Previous studies using more accurate measures of peripheral body fat (estimated by dual-energy X-ray absorptiometry) (71, 79, 81-83, 297-298) have focused only in women (71, 79, 83) or men (298) or not taking into account gender differences (82, 297), hampering direct comparisons between sexes. These studies systematically concluded that peripheral body fat is inversely related to cardiovascular risk in women (71, 79, 81, 83). In men, direct associations of peripheral fat with lipoprotein concentrations, blood pressure and insulin levels (297-298) have been reported.

Further metabolic studies to clarify how sex hormones may modulate the way in which fat is accumulated and stored and how it could influence the production of adipokines are warranted (303). Also, longitudinal research is needed to confirm this potential sex effect on the relation of peripheral fat with coronary risk (protective in women and adverse in men).

Since it seems that the effect of body fat on coronary risk could reflect the separate and opposing metabolic effects of central and peripheral adiposity, practical implications could arise from the identification of individuals at high cardiovascular risk only based on WC measurements. Moreover, sustained physical activity programs promoting the loss of fat mass within the peripheral depots must be under public eye discussion.

In the present investigation, surrogate measures of adiposity based on body circumferences and skinfold thickness were used due to logistical limitations; however, the correlation between indirect and direct measures of body fat is reported to be reasonably high (301, 304). Moreover, because we are not using it as an absolute measure, but we are classifying individuals into quartiles of the skinfold composite measure, the problem of differential misclassification would be minimized. In addition, anthropometrics were performed by trained interviewers, according to standardized procedures, which tend to reduce measurement variability.

Obesity can also modulate the effect of diet on CHD risk. The consumption of fruit and vegetables, antioxidant vitamins and dietary fiber was inversely associated with hs-CRP levels in men, whereas in women no associations were reported (paper III), which we have mainly attributed to the high level of body fat present in women than in men. Therefore, in women the adipose tissue could be the most important source of
proinflammatory cytokines, and the isolate effect of nutrients and foods would not be enough to be detected. The impact of sex hormones on circulating levels of inflammatory cytokines (32-33, 106) could also be neglecting the overall effect of diet on hs-CRP levels.

Under the same thought, among men, stronger associations between the dietary factors and hs-CRP levels were found in non-overweight individuals. Although we did not find a statistical BMI modification effect, it is possible that, in individuals with a high accumulation of fat, the latter is the most important source of inflammation, and therefore the dietary effect on hs-CRP levels is overcome.

Also, alcohol intake seems to hold different associations with sub-clinical inflammation, by sex (paper IV). In women, alcohol intake showed a J-shaped relation with hs-CRP levels, as corroborated by previous studies (267-268), but a positive linear-shaped relation was found in men, which probably reflects the drinking patterns of our country. Portugal, within the context of the European Union, presents a high level of alcohol consumption (270), particularly in men (271). In fact, more than 40% of men in the present investigation reported an intake of alcohol above 30 g/day, which is the current American Heart Association recommended threshold for those who drink (160). Therefore, it seems that, among men, excessive alcohol intake was responsible for a right-skewed effect, producing an overall significant linear association with hs-CRP.

Similarly to what happened in the previous analysis of the effect of fruit and vegetables, antioxidant vitamins and fiber intake on hs-CRP levels, the reported associations were weaker in overweight individuals. In most studies, body fat is seen as a confounder of the relation between alcohol intake and cardiovascular risk markers; in the present investigation, however, a modification effect of the study associations by BMI categories were found. The J-shaped association between alcohol intake and hs-CRP was found only in normal weight women; among overweight women, excess body weight might counteract the effect of alcohol intake on hs-CRP levels. In contrast, in men a positive linear association between alcohol and hs-CRP was found only among overweight individuals. This finding could be explained by two main reasons: the lower variability of hs-CRP among non-overweight men which could be insufficient to find an association; and the higher alcohol intake of overweight men compared with normal weight men and women, which offset the effect of body fat on inflammatory markers.
In this study, the relatively small sample size among men could have lead to less precise estimates than in women. Therefore, future research in high-alcohol intake populations with larger sample sizes should confirm the reported associations, particularly for men. Noted that mostly alcohol intake in the study population is from wine consumption; thus beverage-specific analyses were not possible. However, there is evidence that ethanol itself could be responsible for the anti-inflammatory effects of moderate consumption of alcoholic beverages (274-276).

The previous findings support the hypothesis that the effect of single dietary factors on coronary risk could be sustained, at least in part, through an inflammatory pathway, beyond them widely recognized effects on blood lipids.

These previous dietary factors (fruit and vegetables and alcohol) seem to cluster in the Portuguese population. Individuals with a higher consumption of red meat and alcohol, and simultaneously lower intake of vegetables and dairy products were aggregated into the same dietary pattern (paper V). This pattern, compared to a “healthy” reference, showed to increase the AMI risk and was also associated with a worse cardiovascular biomarker profile, namely with higher hs-CRP levels, thus also supporting the importance of inflammation as an intermediate step in the causal chain between diet and CHD.

Actually, take into account the cumulative effects of nutrients and foods seem to be a more suitable and fruitful way of understanding the effect of diet on CHD (117, 119), for which multiple dietary factors are established. Moreover, the effect of a single food can be confounded by population-specific dietary behaviors, and the clustering of food components could vary across populations reflecting their own cultural traditions, as it happens in the case of alcohol.

Though moderate alcohol consumption is usually considered a healthy behavior (125, 135, 150), and particularly in Southern-European countries is usually associated with healthier food habits (namely consumption during meals) (305), the high intake levels of our population placed alcohol in the high-risk pattern. Of note is that, in most a posteriori dietary patterns described in the literature, alcohol consumption was seldom identified as a main food component. One reason might be the insufficient variation in alcohol intake in Northern American populations, where most studies were conducted. Therefore, in our particular population and probably also in others with similar drinking and eating patterns, recommendations on alcohol intake must be extremely careful, since alcohol clusters with red meat intake and is inversely associated with other
healthier foods, such as fruit and vegetables; the overall pattern resulting in an increased AMI risk.

Curiously, this same dietary pattern combined foods which frequently are described has belonging to opposing patterns – the “Prudent”, corresponding to a high intake of fruit and vegetables, and the “Western” pattern, reflecting a high intake of red meat and fats (110, 195-197).

When using a dietary pattern approach, BMI or other anthropometric factors did not modify the associations between diet and AMI risk, as reported with the single dietary factors. Also, the inclusion of BMI as a covariate in the multivariate models led to a small attenuating effect, suggesting that this relation could be independent of body fat, as reported elsewhere (195-196, 306). In the case of dietary patterns, we could therefore assume that the relation between the different foods and nutrients are strong enough to sustain a significantly association with CHD risk, beyond the body fat effects.

An additional methodological approach of studying diet was applied to the current study population. Based upon a conceptual definition of an Atlantic diet, proposed in international meetings over the last years (24, 199-202), a Southern-European Atlantic diet (SEAD) index was created for the first time in the literature. The Atlantic diet is believed to be the traditional diet of regions with a straight link with the Atlantic Ocean (particularly Northern Portugal and Galicia, Spain), which share a specific high consumption of fish and other sea foods, red meats, vegetables and whole foods, and wine consumption during meals.

A higher adherence to the SEAD index was associated with a lower risk of non-fatal AMI. These findings suggest that SEAD might contribute to the low coronary mortality rates traditionally registered in Northern Portugal and Galicia. Therefore, other dietary patterns, beyond the Mediterranean diet, might have a beneficial effect on CHD outcomes. The investigation of the effect of adherence to the SEAD on other important disorders, such as stroke or gastric cancer, which represent a large disease burden in Portugal and Spain, would be of public health interest.

In this study, it was also found that some dietary changes, namely decreasing the red meat and potatoes consumption, might increase even more the benefit of SEAD on CHD. However, more research is needed on this issue, because dietary interventions based on a few food components might lead to compensatory diet changes, whose overall health effects are as yet unknown.
It is noteworthy the similarities between some of the identified patterns by a posteriori methods and the SEAD index. In particular, the “fish” pattern in men, characterized by the highest intake of fish, but also higher intakes of red meat, vegetables, particularly vegetable soup, holds important features of the Atlantic Diet.

These findings enhance the conviction that different approaches of studying diet (single nutrients or foods and dietary patterns identified by different methodologies) represent complementary advances in the clear understanding of the multiple and complex diet-disease relationships.

Lastly, some limitations and strengths of this research should be discussed. Although they were described in detail across the several manuscripts, three main points should be emphasized.

The present investigation was partially based in a cross-sectional evaluation of the EPIPorto cohort study, which apparently could be seen as a constraint; however it seems unlikely that hs-CRP of healthy individuals would affect body fat distribution or self-reported dietary intake (i.e. reverse causality). Moreover, since only sub-clinical inflammatory levels are of interest, the problem of variability from acute infection or other short-term insult is discarded. In the study population, the mean difference between hs-CRP levels determined in the two evaluations of the cohort was almost zero, which has hampered a longitudinal analysis. Also, the conduction of a case-control study to evaluate the effect of diet on AMI could be seen as less suitable than a prospective cohort study. However, considering that the atherosclerotic process is not seen anymore as the single result of long-term effect of exposures and that exposures with a role in the inflammatory process, namely diet, could wield its effects on a relatively short-period of time, the case-control design might be appropriate. Additionally, since it is a population-based study, the association’s estimates are expected to be similar to the true relative risk.

An important limitation could be the assessment of hs-CRP, which was not planned at the beginning of the participants’ evaluation. Thus, although hs-CRP measurements were performed consecutively, they are only available for a subset of participants. Trying to predict the effect from this on risk estimates, comparisons between the characteristics of participants with and without hs-CRP measurements were stated across the several papers. Regarding the magnitude of the differences between participants and non-participants characteristics, it seems not likely to affect the results.
Dietary intake was assessed by a food frequency questionnaire, which has shown good reproducibility and validity in this specific population (288-289). Additionally, data collection was conducted by trained personnel in face-to-face interviews, using a photograph album to help with the selection of portion sizes; this might have minimized the expected underestimation of intake, which is frequent in food consumption studies. Previous research has shown that dietary patterns are fairly stable over time (206, 307), which supports a single assessment of dietary intake, as we have. The use of different methodologies of studying diet, namely the application of new statistical methods to identify dietary patterns represents for sure an important strength of this research.

In a few words, six main conclusions could be drawn from this dissertation:

- Different patterns of body fat distribution, identified by principal component analysis, were found to have opposing effects on hs-CRP levels: while a central pattern of fat distribution was directly associated with hs-CRP levels in men, a high proportion of peripheral subcutaneous fat in the arms seemed to be inversely associated with hs-CRP, but only in women.

- The same anthropometric factors were found to be associated with non-fatal AMI, but while the peripheral subcutaneous fat index predicted a lower risk of AMI in women, a higher risk was found in men.

- The consumption of fruit and vegetables (with exception of vegetable-soup), vitamin C, E and fiber were inversely associated with hs-CRP levels in men, whereas in women no associations were reported. The associations tended to be weaker in overweight individuals.

- Alcohol intake showed a J-shaped relation with hs-CRP levels in women, and a positive linear-shaped relation in men. Uric acid levels increased with increasing alcohol intake in each sex. BMI modified these associations, so that they only held in normal weight women and in overweight men for hs-CRP, and in normal weight individuals for uric acid.
• Individuals with a higher consumption of red meat and alcohol, and lower intake of vegetables and dairy products, had an increased risk of AMI and a worse cardiovascular biomarker profile than those with a "healthy" dietary pattern.

• A higher adherence to the South-European Atlantic diet (SEAD index), a highly palatable diet which is culturally rooted in Northern Portugal and Galicia, was associated with a lower risk of non-fatal AMI events.
References


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