

Assessment of the effect of mixed nuts on glycemic control and coronary heart disease risk factors in type 2 diabetes

Avaliação do efeito de frutos gordos no controlo glicémico e factores de risco de doença coronária em diabéticos tipo 2

TRABALHO DE INVESTIGAÇÃO

Cláudia Patrícia Valadão Meneses

Orientadora: Professora Doutora Teresa Amaral

Co-Orientador: Dr. Cyril Kendall

Porto | 2009



FCNAUP

Faculdade de Ciências da Nutrição e Alimentação
Universidade do Porto

Assessment of the effect of mixed nuts on glycemic control and coronary heart disease risk factors in type 2 diabetes

Avaliação do efeito de frutos gordos no controlo glicémico e factores de risco de doença coronária em diabéticos tipo 2

TRABALHO DE INVESTIGAÇÃO

Cláudia Patrícia Valadão Meneses

Orientadora: Professora Doutora Teresa Amaral

Co-Orientador: Dr. Cyril Kendall

Acknowledgments

I would like to sincerely thank my two supervisors Dr. Cyril Kendall and Dr. Teresa Amaral for their guidance during the internship.

Thanks Dr. David Jenkins, Sandy, Kathy, Tina, Shahad, Stephanie, Kristie I especially appreciate their friendship, support and help.

Monica...I give you my heartfelt thanks

Contents

Acknowledgments	i
Abbreviations.....	v
Abstract	vii
Resumo	viii
1. Introduction.....	1
1.1 Overview of Diabetes.....	1
1.2 Symptoms of Diabetes.....	2
1.3 Complications of Diabetes	3
1.4 Diabetes and cardiovascular diseases.....	3
1.5 Managing of Type 2 Diabetes and prevention of CVD	4
1.6 Composition of nuts and its possible effects	5
1.7 Epidemiological and clinical evidence of nuts	7
1.8 Hypothesis	8
2. Objectives.....	8
3. Methods.....	9
4. Results	14
5. Discussion	21
6. Conclusion.....	27
7. References	29

Abbreviations

ADA – American Diabetes Association

ANOVA – Analysis of Variance

CHD – Coronary Heart Disease

CI – Confidence Interval

CVD – Cardiovascular Disease

DM – Diabetes Mellitus

FBG – Fasting Blood Glucose

HbA_{1c} – Glycated hemoglobin A_{1c}

HDL- C – High Density Lipoprotein Cholesterol

LDL - C – Low Density Lipoprotein Cholesterol

MUFAs – Monounsaturated Fatty Acids

NCEP ATP III - National Cholesterol Education Program and Adult Treatment

Panel II

PUFAs – Polyunsaturated Fatty Acids

SEM – Standard Error of the mean

SFAs – Saturated Fatty Acids

Abstract

Type 2 diabetes is a prevailing disease in the world and its burden of ill health is far broader than that resulting from diabetes complications alone. Cardiovascular disease is the major complication associated with diabetes. Nuts contain a variety of compounds that have already demonstrated benefits either in glycemic control or in the blood lipid profile. Therefore, the present study sought to evaluate the effect of mixed nuts on glycemic control and blood lipid profile in subjects with type 2 diabetes.

Ninety nine subjects were randomly assigned to one of the three treatments: a full-dose of mixed nuts, or a whole grain muffin or both mixed nuts and muffins for 12 weeks. Blood analysis, anthropometrical information and diet records were monitored at weeks 0, 2, 4, 8, 10 and 12.

Results showed that the group treated with a full-dose of the mixed nuts had their HbA_{1c} lowered after 12 weeks of treatment (-0.214% absolute HbA_{1c} units compared to baseline values; 95% CI, -0.826% to 0.004%; P=0.001). Control groups did not demonstrate significant improvements in their measures of glycemic control. Although the blood lipid profile did not significantly improve in all groups, the total:HDL cholesterol and LDL:HDL cholesterol had only decreased in the groups that consumed nuts.

In conclusion, the consumption of nuts significantly improved glycemic control in type 2 diabetic subjects and showed some benefits in terms of cardiovascular risk factors.

Key words: Nuts, glycemic control, blood lipid profile, type 2 diabetes

Resumo

A Diabetes é uma doença com elevada prevalência a nível mundial, em que as suas consequências não se limitam apenas às complicações que dela derivam. Sendo a doença cardiovascular uma das maiores preocupações associadas a esta doença. A composição nutricional dos frutos gordos abrange uma série de compostos que individualmente têm demonstrado efeitos benéficos ao nível do controlo glicémico e do perfil lipídico sanguíneo. O presente estudo pretendeu avaliar o efeito do consumo de frutos gordos no controlo glicémico e factores de risco de doença cardiovascular em diabéticos do tipo 2.

Um total de 99 indivíduos distribuídos aleatoriamente, consumiram um dos três suplementos durante 12 semanas: mistura de frutos gordos, “*muffins*” ou uma mistura de ambos. Ao longo deste período (semana 0, 2, 4, 8, 10, 12) foram realizadas análises sanguíneas e recolhidos dados antropométricos, assim como diários alimentares dos indivíduos em estudo.

Os resultados deste estudo demonstraram que o consumo de frutos gordos no grupo teste, melhorou a hemoglobina A_{1c} (-0.214% unidades absolutas HbA_{1c} relativamente aos valores iniciais; 95% IC, -0.826% to 0.004%; P=0.001), enquanto que os grupos controlo não demonstraram melhorias significativas no controlo glicémico. Embora as alterações do perfil lipídico não tenham melhorado significativamente em nenhum dos grupos, a razão colesterol total: HDL e LDL: HDL diminui apenas nos grupos que consumiram os frutos gordos.

Em suma, o consumo de frutos gordos em diabéticos do tipo 2 melhorou o controlo glicémico, demonstrando, possivelmente, ser benéfico relativamente aos factores de risco de doença cardiovascular.

x

Palavras-chave: Frutos gordos, controlo glicémico, perfil lipídico sanguíneo, diabetes tipo 2

1. Introduction

The rapid changes in diet and lifestyle that have occurred with industrialization, urbanization, economic development and market globalization have had a significant impact on the health and nutritional status of populations ⁽¹⁾. Chronic, non-communicable diseases including obesity, diabetes mellitus (DM), cardiovascular disease (CVD), hypertension, stroke, and some types of cancer are becoming increasingly significant causes of disability and premature death in both developing and developed countries ⁽¹⁾.

The number of people with diabetes was projected to double from 2000 to 2030⁽²⁾. However, these estimations have been only based on demographic changes where some risk factors considered, such as obesity or physical activity levels remained constant ⁽²⁾. Since people who are afflicted with diabetes are at an increased risk of cardiovascular, peripheral vascular and cerebrovascular disease, an expected increase in deaths, and increase in the prevalence of other diabetic health complications are likely to lead to enormous human and economic costs ^(2, 3).

1.1. Overview of Diabetes

Diabetes mellitus may be briefly described as a group of metabolic diseases characterized by hyperglycemia resulting from defects of either insulin secretion, insulin action, or a combination of both. Diabetes can be classified into different categories depending on its pathogenic nature.

Type 1 DM, also known as Insulin-dependent diabetes, is characterized by β -cell destruction usually leading to absolute insulin deficiency. This type of diabetes accounts for only 5-10% of those with diabetes, and may result from a cellular-

mediated autoimmune reaction against β -cells of the pancreas, or may be idiopathic.

The most common form of diabetes is type 2, accounting for approximately 90 to 95% of those affected by the disease, and can range from a predominant insulin resistance with relative insulin deficiency to a predominant insulin secretory defect with insulin resistance ^(2, 3). Initially and often throughout their lifetime, individuals with type 2 diabetes do not require insulin treatment to survive and therefore this form of diabetes is also referred to as non-insulin-dependent diabetes. Gestational diabetes mellitus occurs when hyperglycemia is first recognized during pregnancy ^(2, 3). Other types of diabetes may result from genetic defects of pancreatic β -cells, insulin action, diseases of the exocrine pancreas or endocrinopathies, or from drug or chemically-induced effects, infections, uncommon forms of immune-mediated diabetes and other genetic syndromes^(2, 3).

1.2. Symptoms of Diabetes

Diabetes may present a range of symptoms that may include the following: polyuria (increased urine output), polydipsia (excessive thirst), weight loss, polyphagia (excessive hunger) and blurred vision. In poorly controlled diabetes individuals may experience ketoacidosis or nonketotic hyperosmolar syndrome, which may ultimately lead to coma and death^(3, 4). In type 2 diabetes, hyperglycemia develops gradually and is not usually detectable in the early stages because patients do not present with symptoms. Therefore, diabetes may be undiagnosed for many years until symptoms gradually become apparent in these patients^(3, 4).

1.3. Complications of Diabetes

Chronic hyperglycemia in diabetic patients may result in microvascular complications, such as retinopathy, neuropathy and nephropathy^(3, 4). Long-term hyperglycemia may also relate to other several disorders and among those, atherosclerotic cardiovascular, peripheral arterial and cerebrovascular diseases⁽⁴⁾ are markedly increased in patients with diabetes^(3, 4). The diabetic condition is itself considered as a coronary heart disease (CHD) equivalent by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III)⁽⁵⁾.

1.4. Diabetes and Cardiovascular disease

Conventional cardiovascular disease risk factors are amplified⁽⁶⁻⁸⁾ in diabetic patients, increasing the risk of a cardiovascular event by a factor of two to four in comparison with non-diabetic patients⁽⁸⁻¹²⁾. Glycated proteins, (such as haemoglobin A1c (HbA_{1c}) or serum fructosamine) are markers of hyperglycemia, and may be a central cause of the increased vascular disease associated with type 2 DM. These markers may relate to increases in oxidative stress⁽¹³⁻¹⁵⁾, inflammation⁽¹⁶⁾, alterations in nitric oxide generation^(17, 18) and vascular reactivity^(19, 20).

In a prospective study of 18 years⁽²¹⁾, women with type 2 diabetes had a worse prognosis for CHD as an outcome than non diabetic subjects with prior CHD. Furthermore, the United Kingdom Prospective Diabetes Study (UKPDS)⁽²²⁾ found that an improvement of 0.9 % in HbA_{1c} from intensive treatment by pharmacological agents (sulphonylureas and insulin) resulted in a reduction of 25% in microvascular complications.

Despite the controversial association between HbA_{1c} and cardiovascular events, there is evidence from epidemiological studies that similar reductions in HbA_{1c} may also reduce the risk of cardiovascular disease in people with diabetes ^(12, 22). Selvin *et al* found that some risk factors for CVD (such as waist-to-hip ratio, plasma triglycerides and Low Density Lipoprotein (LDL - C) and High Density Lipoprotein cholesterol (HDL-C) levels) remained significantly associated with HbA_{1c} after multivariate adjustment ⁽²³⁾. The same study reported that HbA_{1c} was also strongly associated with atherosclerosis. Accordingly, studies conducted in diabetic subjects demonstrated that the lipid-lowering drug, simvastatin, reduced the risk and occurrence of major coronary events ^(24, 25).

Blood pressure control was shown to also be an important factor to reduce the risk of cardiovascular complications in type 2 diabetics as well as in prevention of retinopathy and nephropathy ⁽²⁶⁾.

1.5. Managing of Type 2 Diabetes and prevention of CVD

The most recent recommendations from the American Diabetes Association (ADA) claim that Medical Nutrition therapy is an important factor in preventing and managing diabetes, and possibly slowing the development of diabetic complications ⁽²⁷⁾.

Due to the coexistence of many CVD risk factors in individuals with type 2 diabetes, some essential recommendations were published jointly by ADA and American Heart Association (AHA) ⁽²⁸⁾. Lifestyle measures to prevent CVD in people with diabetes include reducing fat and total energy intake and increasing regular physical activity. These two recommendations can be effective tools to achieve body weight goals and improve glycemic control ^(27, 28). Beside these

measures, a reduction of saturated fat and cholesterol intake and an increase in dietary fiber, is also recommended due to the improvements that have been demonstrated in the lipid profile in diabetic participants ^(27, 28). As part of medical nutrition therapy in diabetics for primary prevention of CVD, the total amount of dietary fat should range from 25-35% and mainly be constituted of monounsaturated or polyunsaturated fat ⁽²⁸⁾. A reduction in sodium intake is recommended for lowering blood pressure, as it has been shown to reduce the risk of cardiovascular complications in type 2 diabetic subjects ^(27, 28).

Fiber has been shown to decrease insulin resistance and demand⁽²⁹⁾. In addition to these effects it is also inversely associated with the risk of type 2 diabetes ⁽³⁰⁻³²⁾. Similar effects were also demonstrated with magnesium intake ^(33, 34). Some other food components have also shown to improve diabetes and its complications. One of those components is a monounsaturated fat, where a diet rich in this kind of fat was shown to improve lipoprotein profile as well as insulin sensitivity and glycemic control in type 2 diabetics ⁽³⁵⁻³⁸⁾. Jointly, vitamin E has been well documented in its ability to reduce oxidative stress, improve insulin action and reduce the risk of coronary heart disease ^(39, 40). Considering that diabetes is a key factor in development of CVD, a significant improvement in the lipid profile by soy protein intake in a meta-analysis may therefore be a highly relevant benefit⁽⁴¹⁾. Also, intake of flavonoids was significantly and inversely associated with CHD mortality in epidemiological studies.

1.6. Composition of Nuts and its possible effects

One aspect of diet related strategies may include nut consumption. Nuts have a favourable fatty acid profile as they are a rich source of monounsaturated fatty

acids (MUFAs) and polyunsaturated fatty acids (PUFAs) and are low in saturated fatty acids (SFAs)⁽⁴²⁾. Several bioactive compounds such as plant protein (high content of arginine), fiber, antioxidant vitamins (e.g. vitamin E), various minerals (e. g. magnesium and copper) and other constituents have also been found in nuts⁽⁴³⁾.

However, interventional studies investigating nut consumption have not demonstrate considerable benefits in glycemic control ⁽⁴⁴⁾. Despite these studies, an evaluation by the Nurses' Health Study⁽⁴⁵⁾ found that nut and peanut butter consumption was inversely associated with risk of type 2 diabetes after adjusting for other several risk factors. Even with the high fat content of nuts, some studies demonstrated that nuts may be helpful in terms of controlling body weight and improving insulin resistance. Since obesity and being overweight are major contributors to developing insulin resistance, diabetes and its complications, nuts might also be advantageous at this point.

More recently, It has been hypothesized that the magnitude of the glycemic fluctuations is a more relevant determinant of oxidative damage rather than ambient glucose levels, reflected in HbA1c levels ⁽⁴⁶⁾. Furthermore, acarbose (which effectively decreases postprandial glycemia) reduced the progression of diabetes but also improved the risk of CHD ^(47, 48).

Nuts in general do not have considerable amounts of available carbohydrate and therefore contribute little to the postprandial glycemic response. A study conducted by Josse *et al* ⁽⁴⁹⁾ demonstrated that almonds reduced glycemic response when fed with bread in a dose-dependent fashion compared to bread alone. This effect was possibly attributed to dietary components of almonds such as fat, protein and phytochemicals (found in almond skin). When an assessment of oxidative stress

was conducted, almonds decreased protein thiol oxidation, suggesting that almonds protect against meal-induced oxidative damage⁽⁵⁰⁾. Consumption of almonds may also protect against the atherogenic process since almond consumption had significantly decreased oxidized LDL-C (considered to be a more relevant marker of atherogenesis than native LDL-C) in hyperlipidemic subjects^(51, 52). Furthermore, flavonoids and vitamin E in nuts may help to counter the elevated oxidative stress and inflammation experienced by diabetics.

1.7. Epidemiological and clinical evidence of nuts

In a prospective cohort study⁽⁴⁵⁾, women who consumed a higher amount of nuts and peanut butter had a lower risk of type 2 diabetes. Furthermore, evidence from epidemiological studies and clinical trials have consistently demonstrated benefits of nut and peanut consumption on CHD risk and associated risk factors. A multivariate analysis of the Adventist Health Study, the Nurses' Health Study, the Physicians' Health Study and the Iowa Women's Health Study demonstrated that in subjects with the highest intake of nuts, the relative risk for total CHD was 0.65 (Confidence Interval (CI): 0.47-0.89)⁽⁵³⁻⁵⁷⁾. In the Nurses' Health Study⁽⁵⁶⁾, peanut consumption was shown to be independently associated with lower relative risk of CHD. These favourable effects of nuts observed may be supported by its effects in blood lipids.

A meta analysis of clinical studies (with different degrees of dietary control and comparison diets) describes that a regular consumption of tree nuts has consistently shown beneficial effects on lipid and lipoprotein levels, and above all, a decrease in LDL - C in healthy subjects^(51, 58).

Beneficial changes in blood lipids by nut consumption have also been seen in type 2 diabetes ^(44, 59, 60) however, the same studies have not demonstrated improvements in glycemic control.

1.8. Hypothesis

A variety of nutrients and bioactive substances found in nuts, have already shown to be associated with healthy benefits in CHD risk factors and glycemic response. Therefore, a beneficial effect of nuts in the management of diabetes and associated cardiovascular disease complication is hypothesized.

2. Objectives

The present study was conducted to determine the effect of mixed nuts (peanuts and tree nuts) consumption on glycemic control and serum lipid profile in individuals with type 2 diabetes.

3. Methods

Study design

A 12 week parallel, randomized, controlled trial was conducted (study design and measurements are shown in Figure 1) to assess the effect of three different treatments. Randomization was stratified on participants' sex and HbA1c level.

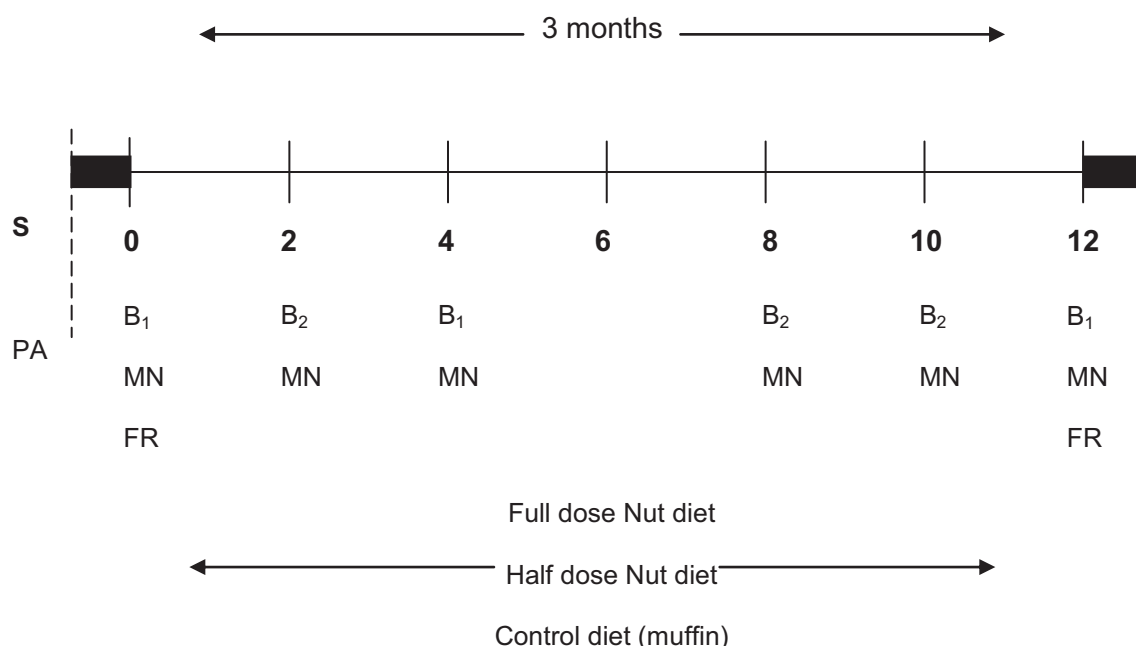


Figure 1: Study design and measurements.

Legend: B₁ – Hb A1c, fasting blood glucose, lipids (cholesterol, triglyceride, HDL-C, LDL-C); B₂ – Hb A1c, fasting blood glucose, electrolytes (sodium, potassium, chloride); MN – Medical notes (weight, waist to hip ratio, blood pressure, smoke and alcohol habits, medications, activity level, gastrointestinal symptoms, satiety and palatability); FR – 7 days food record; PA – Previous analyses (HbA1c, fasting blood glucose, lipids, electrolytes, liver function, urea, creatinine, food record)

Participants attended clinic visits at baseline and weeks 2, 4, 8, 10 and 12 at the Clinical Nutrition and Risk factor Modification Center. Seven-day weighed diet records were obtained before visits at all study weeks. All subjects included in the study were instructed to weigh all consumed foods using a self-tarring digital scale that was provided to participants before starting the study.

After overnight fasts (12 to 14 hours), participants attended study visits where measurements⁽⁶¹⁾ of height, weight, waist and hip circumference, blood pressure and a fasting blood sample were taken at all center visits. Information on

medication use, smoking habit, alcohol intake and exercise pattern were recorded at each visit. Participants were also asked what their overall feeling of satiety was using a 9-point bipolar semantic scale and assessed palatability of the supplements on a semantic scale of zero to 10 (0, very distasteful; 5 neutral; and 10 very appetizing).

Subjects

A total of 99 men and post menopausal women with type 2 completed this study. The subjects age mean was 62 ± 0.9 years old. They were recruited by newspaper advertisement, physician referral, and the diabetic clinic. Eligible diabetic subjects were to be diagnosed at least 6 months prior to randomization and taking oral hypoglycaemic agents other than acarbose in a stable dosage for the previous 3 months. The inclusion range of HbA1c was between 6.5 and 8.0% and the inclusion range for weight was less than 45 Kg/m^2 for BMI. Patients with known nut allergies, clinically significant gastroparesis, treatment with insulin or acarbose, treatment with steroids, gastro intestinal disease, or presence of major debilitating disorder such as liver disease (cirrhosis, infectious hepatitis and transaminases $>130 \text{ IU/L}$), renal failure (creatinine $>150 \text{ mmol/L}$), elevated serum triglyceride ($>6 \text{ mmol/L}$) or cancer were excluded. Subjects were accepted after surgery or myocardial infarction providing an event-free 6 month period had elapsed prior to the study.

Subjects were asked to maintain their habitual level of physical activity, dose of diabetes medications and other medications throughout the study.

Treatments

One of three different treatments was added as supplements to the subject's usual diet. Subjects took either mixed tree nuts (almonds, hazelnuts, pistachios, macadamia nuts, pecans, walnuts and cashews) with peanuts (test group), or muffins (control) or both nuts and muffins (half dose nut group). The amount of supplement intake was based on the subjects' estimated daily energy requirement⁽⁶²⁾. For individuals requiring less than 1600 Kcal daily, the full dose of nuts, half dose of nuts and muffins were 50g/day of nuts, 25g/day of nuts plus 1 muffin/day, or 2 muffins/day (approximately 300 Kcal/supplements) respectively. Subjects with calorie needs between 1600 and 2400 Kcal daily received either: 75g/day of nuts, 37.5g/day of nuts and 1 ½ muffin/day or 3 muffins/day (approximately 450 Kcal/supplements), according to their treatment. Full dose supplement (needs of 2400 Kcal or greater daily) were: 100g/day of nuts, or 50g/day of nuts plus 2 muffins/day or four muffins/day (approximately 600 Kcal/supplement group). The muffins were prepared conform to an NCEP Step 2 diet, made from whole-wheat flour, safflower and coconut oil, skim milk powder and egg white. Supplements were provided at biweekly intervals and the subjects were advised to consume the mixed nuts with a source of starch. The muffins were stored in a freezer until being consumed.

Subjects were instructed on reducing carbohydrate intake to allow supplements to be taken without increasing total energy intake. Nuts and peanut butter were excluded from the background diet of people on muffins treatment during the study. Also, fish oil supplements were excluded from the background diet of all the patients.

Food records were reviewed by a study dietician in presence of the patient for consistency and to record supplement intake. Compliance was also assessed by the returned wrappers, which were counted.

Analyses

The main outcome measures of this study were markers of glycemic control, such as fasting blood glucose (FBG) and HbA1c. Secondary outcome measures of the study were the serum lipid profile: total-cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (study design and measurements are shown in Figure 1).

Blood glucose was measured in the routine hospital analytical laboratory. Diets were assessed for macronutrients, fatty acids, cholesterol, fiber and glycemic index using ESHA, a computer program based on USDA data and international glycemic index tables with glucose as the standard.

Statistical analyses

Results are expressed either as means $\pm$ SEM or means with 95% confidence intervals (CI). Analyses included all subjects who completed the study (n=99) including those who changed their medication (n=6). The normal distribution of the variables was tested against the Kolmogorov-Smirnov test. According to normality of the data distribution, both t-test and Wilcoxon were used to assess the significance of the difference between the means at the beginning and end of the study.

Values of means were compared with the ANOVA test to evaluate differences between the treatments data with a Tukey adjustment for multiple means

comparisons. For the non normal distribution variables, the difference of the means between groups was assessed with the Kruskal-Wallis H test. Significant results were considered when $P < 0.05$.

All statistical analyses were carried out using the Software Package for Social Sciences for Windows, version 14.0 (SPSS Inc. Chicago, IL, U.S.A.).

4. Results

At baseline there were no treatment differences in participants' characteristics (Table 1) as well as in the nutritional profile of their diet (Table 2). The greatest change in MUFAs was an increase seen in the nut (test) group over the week 12 period (from $12.3 \pm 0.6\%$ to $19.0 \pm 0.3\%$; $P < 0.001$) by comparison with the half-dose nut group (from $12.6 \pm 0.5\%$ to $15.0 \pm 0.5\%$; $P < 0.001$) and a decrease in MUFAs was observed in the muffin (control) group (from 13.2 ± 0.5 to 10.8 ± 0.5 ; $P = 0.001$). The increase in MUFAs in the nut and half-dose nut groups indicated adherence to the mixed nut supplement (Table 2). At the end of the treatment period the carbohydrate and saturated fat, both expressed as percentages, were significantly different between the test and control group (Table 2).

The change in satiety rating (scale, +4 to -4) was not different among the 3 groups at the end of the study (nuts, 0.2 ± 0.3 , $P = 0.361$; half-dose, -0.1 ± 0.3 , $P = 0.770$; control 0.2 ± 0.3 , $P = 0.417$) and satiety was also similar between treatments (nuts, 1.1 ± 0.2 ; half-dose, 0.8 ± 0.2 ; muffins/control, 1.1 ± 0.1 ; $P = 0.596$).

The nuts' and muffins' perception of palatability was not different between groups at week 12 ($P = 0.972$ and $P = 0.463$, respectively) and did not change in the groups that ate only 1 type of supplements (nuts, from 8.4 ± 0.2 to 8.3 ± 0.5 , $P = 0.763$; muffins from 6.4 ± 0.4 to 6.2 ± 0.6 , $P = 0.763$).

	Nuts (n=37)			Both (n=32)			Muffin (n=30)		
	Week 0	Week 12	P	Week 0	Week 12	P	Week 0	Week 12	P
Body weight, kg	84.7±15.1	81.8±14.0	0.435	81.0±14.8	83.4±16.4	0.537	78.8±14.1	79.8±13.9	0.752
BMI	29.6±4.8	28.5±4.5	0.325	29.1±4.4	29.7±4.7	0.525	28.3±4.1	29.2±5.1	0.677
Glycemic Control									
HbA _{1c} , %	7.09±0.59	6.68±1.30	0.000	7.00±0.51	6.95±0.68	0.485	7.09±0.43	7.07±0.69	0.858
Glucose, mg/dL	128.6±29.8	131.6±24.0	0.433	126.0±22.6	137.1±41.9	0.045	127.4±28.8	130.7±35.1	0.416
Blood Lipids, m/dL									
Total cholesterol	166.3±48.1	159.8±45.9	0.144	148.8±24.8	144.9±40.8	0.526	164.0±39.7	170.7±39.2	0.047
LDL-C	95.4±46.1	87.5±40.9	0.124	84.2±21.8	79.0±28.0	0.241	88.5±34.7	96.7±32.0	0.106
HDL-C	41.7±9.2	42.4±9.5	0.463	41.0±11.2	40.4±13.8	0.601	44.7±12.1	45.5±13.5	0.362
Triglycerides	138.0±81.6	144.1±98.9	0.892	119.0±67.3	128.3±70.7	0.220	137.0±84.7	144.1±77.5	0.523
Total cholesterol:HDL-C ratio	4.1±1.1	3.8±1.0	0.148	3.8±1.1	3.8±1.1	0.929	3.9±1.1	4.0±1.2	0.171
LDL-C:HDL-C ratio	2.3±1.1	2.1±1.0	0.175	2.1±0.8	2.1±0.9	0.503	2.1±0.9	2.3±0.9	0.163
Blood Pressure, mm Hg									
Systolic	125±15	121±12	0.200	124±13	125±13	0.692	122±12	124±12	0.438
Diastolic	71±8	70±8	0.501	72±11	71±10	0.478	70±10	72±10	0.422

Table 1: Baseline (week 0) and final (week 12) characteristics of the subjects. Values are mean ± SD. BMI, calculated as weight in kilograms divided by height in meters squared.

		Week 0			Week 12				
		Nuts (n=36)	Half-dose (n=32)	Muffins (n=30)	P value	Nuts (n=36)	Half-dose (n=32)	Muffins (n=30)	P value
Energy, Kcal/d		2032±92	2077±100	1925±74	0.496	2116±62	2144±111	1945±93	0.256
Total protein, %		19.4±0.7	19.3±0.6	19.7±0.6	0.923	17.6±0.5	18.7±0.5	18.6±0.6	0.265
Carbohydrate, %		43.0±1.6	43.3±1.3	41.6±1.3	0.671	37.7±1.1†	39.6±1.0	42.6±1.0	0.006
Total Dietary fiber, g/1000 Kcal		15.9±1.0	15.4±1.2	12.9±0.6	0.089	14.8±0.8	14.3±0.7	12.9±0.5	0.154
Total fat, %		31.5±1.2	31.6±1.0	33.6±1.1	0.320	40.2±0.7†	37.1±0.9†	34.7±0.7	0.000
SFA		9.7±0.4	9.6±0.5	10.3±0.5	0.599	9.0±0.3†	9.8±0.4	10.6±0.4	0.012
MUFA		12.3±0.6	12.6±0.5	13.2±0.6	0.528	19.0±0.3†	15.0±0.5†§	10.8±0.5†	0.000
PUFA		6.0±0.3	6.1±0.3	6.5±0.2	0.514	9.0±0.3	9.2±0.3	9.6±0.4	0.450

Table 2: Macronutrient profile on the different groups at baseline and at the end of the study.

† P ≤ 0.05, significance of the difference of Nut (test) and half-dose vs Muffins (control); § P ≤ 0.05, significance of the difference of Nut (test) vs half-dose treatment.

Glycemic control and body weight

At the end of the study, the main outcome, HbA_{1c}, decreased by -0.214% absolute HbA_{1c} units (95% CI, -0.826% to 0.004%; P=0.001) in the test group compared with -0.056% absolute HbA_{1c} units (95%CI, -0.106% to 0.219; P=0.485) and -0.020% absolute HbA_{1c} units (95% CI, -0.207% to 0.247%; P=0.858) in the half-dose and control treatment, respectively (Table 1). The difference in HbA_{1c} in the half-dose treatment almost reached significance after 8 weeks (P=0.082) (Figure 2) and in the test group the reduction in HbA_{1c} was significant after 8 weeks (Figure 2).

Regarding fasting blood glucose, the test group showed a trend to reduce FBG until week 10 where it approaches significance (P=0.077) (Figure 2). However, in the group that took both muffins and nuts, glucose levels significantly increased at the end of the study (11.1 mg/dL, 95% CI 0.3 mg/dL to 21.9 mg/dL; P=0.045).

No difference between treatments was noticed at the end of the study for both the measures of glycemic control (HbA_{1c}, P=0.480; FBG, P=0.718). Moreover, any significantly difference between the changes in HbA_{1c} and FBG (week12-week 0) were found between treatments.

Differences in body weight changes were not significantly different for any treatment neither at the end nor through the study (Figure 2).

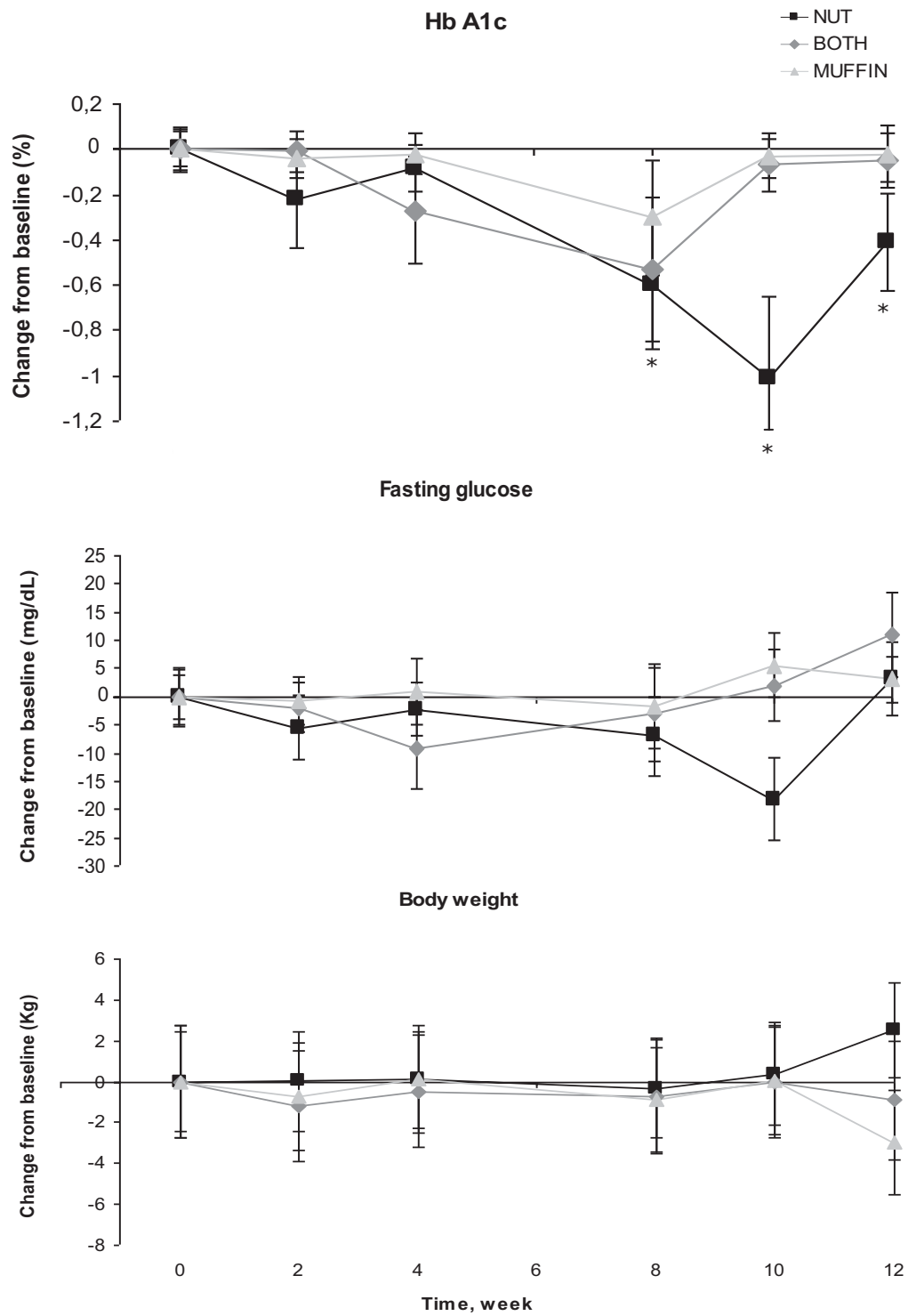


Figure 2: Change from baseline in HbA_{1c}, Fasting blood glucose and body weight between on test, half-dose and control groups. * Denotes P<0.05. Values are mean±SEM.

Blood Lipids

When compared with baseline values, a non-significant reduction in total cholesterol and LDL-C was observed at week 12 in the test group (LDL-C, -8.2 ± 4.4 mg/dL, $P=0.071$; Total cholesterol, -6.5 ± 4.4 mg/dL, $P=0.144$) (Table 1). Otherwise, the group that received muffins shown a significantly increase in total cholesterol at week 4 and week 12 (12.4 ± 4.3 , $P=0.007$; 6.8 ± 3.3 , $P=0.047$, respectively). Both control and test group shown an increase trend in HDL-C, but none that reached significance (Figure 2). The total cholesterol: HDL-C and LDL-C: HDL-C ratios declined through the study in the groups that ate nuts (Figure 2). However, in the control group, both the ratios increase comparing with baseline value and even the total cholesterol: HDL-C reaches significance at week 4 ($P=0.012$) (Figure 2). Triglycerides increased in all groups at the end of the study but it just reach significance on the test group at week 4 ($P=0.03$). No significantly differences among groups were found for the changes on blood lipids between the beginning and the end of the study.

Blood pressure

Considering the baseline values, systolic and diastolic blood pressure dropped in the treatments with nuts and ascended in the test group. These changes were in a small extend and were not significant at the end of the study (Table 1).

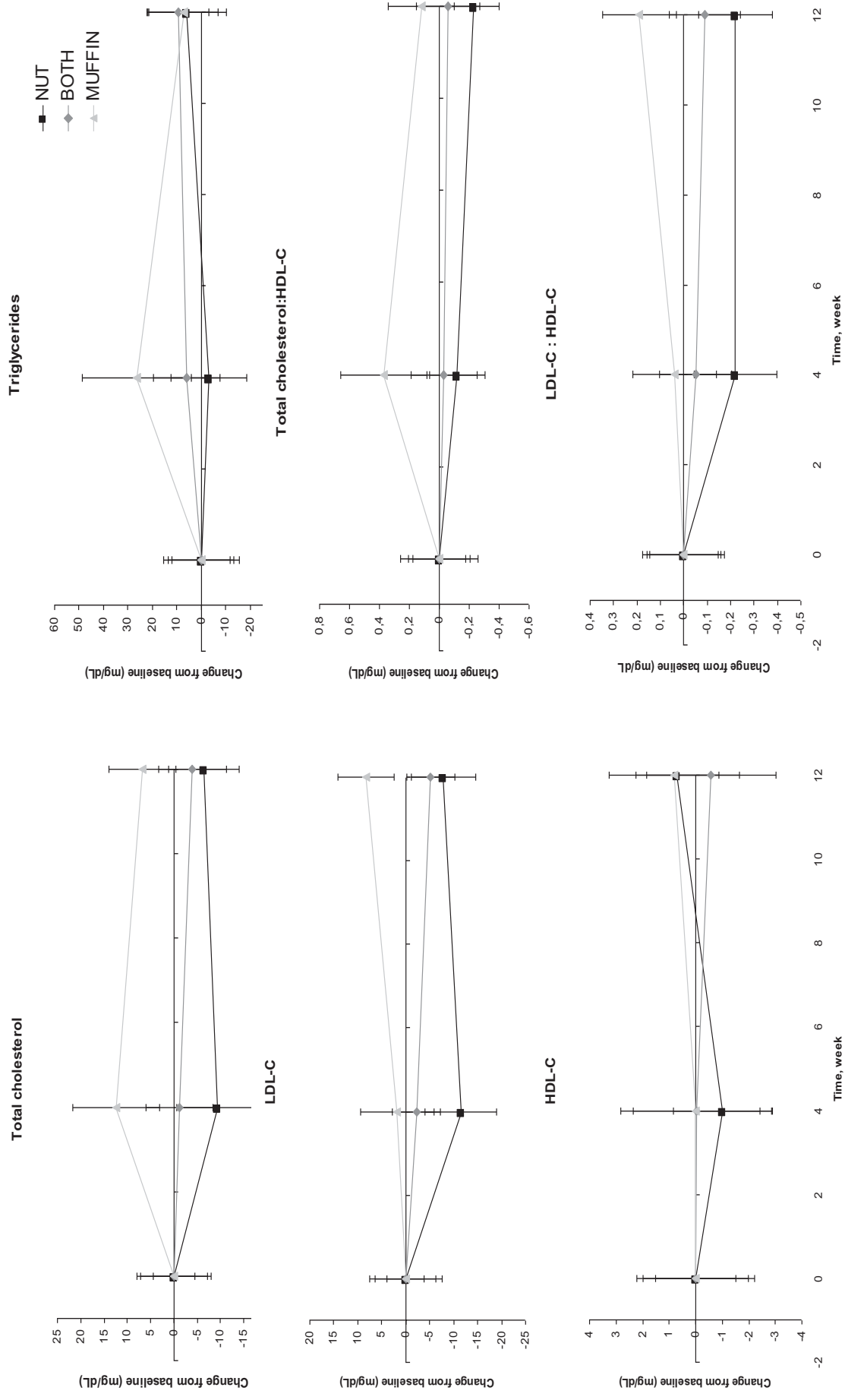


Figure 3: Change from baseline in Total Cholesterol, LDL-C, HDL-C, Triglycerides, Total:HDL CholesterolHbA_{1c}, LDL:HDL Cholesterol ratio on test, half-dose and control groups

5. Discussion

The inclusion of a mixture of nuts as a supplement in the diet of type 2 diabetic subjects was shown to improve glycemic control. Comparing with baseline values, all groups had reductions in glycated hemoglobin A_{1c} at the end of the study, but the group that consumed the full dose of nuts had the greatest reduction, that remained stable over the last three visits.

The same result in HbA1c reduction was not obtained in a study where type 2 diabetes subjects were fed with almonds during a 4 week period⁽⁴⁴⁾. However, the lack of a glycemic effect (HbA1c reduction) may have been due to the short duration of the study as HbA1c is a long term indicator of the average plasma glucose concentrations over a 3 months period⁽⁶³⁾.

Despite the results in the almond study⁽⁴⁴⁾, a prospective cohort of the Nurses' Health Study⁽⁵⁴⁾ found that nut and peanut butter consumption was inversely associated with the risk of type 2 diabetes, even after adjusting for several other risk factors. In their study, those who ate nuts at least 5 times per week compared with those who rarely or never ate nuts had a relative risk of developing diabetes reduced by 27%.

The significant reduction in HbA1c seen by the end of the present study (from week 8 to week 12) suggests that adding nuts to a diabetic diet may be an alternative method to manage and improve glycemic control in type 2 diabetes. Furthermore a reduction in HbA1c of 1% and 0.67%, respectively, in the UKPDS⁽²²⁾ and Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified

Release Controlled Evaluation (ADVANCE) ⁽⁶⁴⁾ studies have shown reductions by 37% and 21%, respectively, in microvascular complications of diabetes.

Although fasting blood glucose had a similar pattern as HbA1c in the test group, the reduction was not significant and the trend changes at week 12 even went beyond the baseline values. Nevertheless, blood glucose which reflects the physiology of glucose in the extracellular space is considered as a measure more vulnerable to the recent factors that could affect glucose concentrations. This could partially explain the unexpected increases in glucose but not in HbA1c levels.

A more recent hypothesis has suggested that glycemic fluctuations determine oxidative damage rather than constant hyperglycemia ⁽⁶⁵⁾, and this effect was independent of the total amount of carbohydrate tested. The mechanism through which oscillating glucose levels may be more deleterious than chronic hyperglycemia is still incompletely undefined, but the effects of the simultaneous administration of vitamin C demonstrated that the generation of radicals leading to oxidative stress could be the major factor.

In the same study, vitamin C was shown to normalise endothelial function and oxidative stress in diabetic subjects after a glucose peak while in healthy subjects, this effect was demonstrated only during the period of hyperglycemia. A possible explanation for this finding is that only the simultaneous control of hyperglycemia together with vitamin C could be effective in normalizing the endothelial function in diabetes had been already described⁽⁶⁶⁾.

A study conducted by Josse *et al*⁽⁴⁹⁾ demonstrated that almonds were advantageous at normalizing glucose fluctuations as they reduced glycemic response when fed with bread in a dose-dependent fashion. Moreover, when an

assessment of oxidative stress was conducted, almonds were shown to reduce meal induced oxidative damage in healthy subjects ⁽⁵¹⁾. Therefore, the deleterious effect of oscillating glucose levels leading to oxidative stress and endothelial dysfunction, were improved by almonds^(51, 67, 68).

Although data is limited in the phenolic content of different nuts and on the antioxidant activity of the nuts, almonds (especially in their seed coats) were described as having approximately 30 different antioxidant compounds that may help to counter the elevated oxidative stress and inflammation experienced by diabetics⁽⁶⁹⁻⁷¹⁾. Additionally, nuts are also a good source of magnesium, which is an essential cofactor for multiple enzymes involved in glucose metabolism and is therefore thought to be a contributor to glucose homeostasis, insulin action, and development of type 2 diabetes^(72, 73).

Nuts also contain fat, vegetable protein and fiber that may also contribute to a reduced postprandial glycemia and consequently, a decrease in reactive oxygen species production ⁽⁵⁰⁾. The consumption of fiber by itself has been shown to improve glycemic control, decrease hyperinsulinemia, and lower plasma lipid concentration in patients with type 2 diabetes ⁽²⁹⁾. Furthermore, data from the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) showed that acarbose, the α -glucosidase inhibitor (which effectively slowed the rate of carbohydrate absorption) lowered the risk of CHD in individuals with type 2 diabetes^(47, 48).

The high content in unsaturated fatty acids in nuts might be one of most important factors that contributes to the beneficial associations of frequent nut intake observed in epidemiologic studies (prevention of CHD, DM, for example), effects

in short-term feeding trials (i.e. cholesterol lowering effect), and decreases in other risk factors.

With respect to insulin sensitivity, while a meta-analysis revealed an effect of a high monounsaturated fat diet in lowering the fasting plasma glucose, a study carried by Lovejoy *et al* failed to show any improvement when almonds were consumed by either healthy and diabetic subjects^(44, 60). However, the improvement in insulin sensitivity in the meta-analysis may be due to the reduction in the carbohydrate load instead of the effect of the high intake of MUFAs.

Despite the fact that nuts in the present study did not show a significant improvement in fasting glucose, current literature suggests that their effect could be at least beneficial in postprandial glycemia in the subjects eating a meal, for example.

The potential benefits of nuts may be due to their content in a variety of bioactive compounds that have been already described to have positive effects on health. Nevertheless, real benefits of nuts could be attributed to their complex food matrix composition that might produce a synergistic effect and maximize their strength than by themselves alone.

Several large prospective cohort studies found an inverse association between nut consumption and CHD risk, suggesting that frequent nut consumption may have a protective effect⁽⁵⁵⁻⁵⁸⁾. Unsaturated fatty acids, which are in high content in nuts, are associated with improved lipid profiles and a lower potency of intermediate biomarkers of atherosclerosis and lesser incidence of cardiovascular disease⁽⁷⁴⁾. A beneficial effect of nuts on blood lipid profiles in humans was examined in a systematic review, which found that a consumption of nuts (in particular, almonds, walnuts, pecans and peanuts) of 1.5 – 3.5 servings $\geq$ 5 times per week was

associated with a significantly lower total cholesterol and LDL cholesterol in normal and hyperlipidemic subjects⁽⁷⁵⁾. Similar results were obtained in type 2 diabetic subjects, however in these studies glycemic control was not improved.

The effect of nuts on glycemic control found in the present study might be taken into account in relation to the strong association between DM and the development of CVD,. Moreover, despite the differences which were not significant, the test group improved total cholesterol, LDL-C, HDL-C and the ratios total: HDL cholesterol and LDL: HDL cholesterol which is in line with another study showing that the effect of almonds improved some CHD risk factors⁽⁵¹⁾. Although, the development of CVD involves several mechanisms (such as oxidative stress, inflammation and vascular reactivity, for example), the nutritional composition of nuts have a variety of compounds that might be effective against some of the risks of CHD.

The increased body weight seen in test group, although not significant, did not match the trend seen in other studies where adding nuts in the diet did not result in weight gain over time^(76, 77). The reason why the nuts may not contribute to weight gain, even with their recognized energy density, might result from their effect on satiety and therefore displacing unhealthy foods, or their poor nutrient bioaccessibility due to their cell wall structures ^(78, 79). Furthermore, the non significant reduction seen in the control groups (half-dose nuts and muffins) is not explained by their difference in satiety ratings when compared to the test group. Moreover, even with the unfavourable increase in body weight at the end of the study, the test group was the only group that significantly improved their glycemic control, reflected in the HbA1c level observed.

Although the randomized control design of the study was a strength, uncontrolled bias may still have occurred. Unfortunately, even with the effort by the dietitians, there was a limited method to reinforce compliance by the subjects. While palatability did not change in the groups that just ate 1 type of supplement (mixed nuts or muffins), the routine consumption of the supplements could decrease the enthusiasm of the patients through 12 week.

Considering the complex metabolic alterations that occur in diabetes, the lack of significance in the benefits of the cardiovascular risk factors in these patients, might be due to the length of the study, which was not long enough to sufficiently improve these measures.

6. Conclusion

In conclusion, using nuts as a supplement in the diabetic diet has been shown to be a potential dietary method to improve glycemic control in subjects with type 2 diabetes, by reducing HbA1c levels. However, in order to improve other metabolic disorders associated with type 2 diabetes, nut consumption itself may be incorporated in a healthy lifestyle.

7. References

1. World Health Organization, Joint WHO/FAO Expert Consultation on Diet NatPoCD. Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. Geneva: WHO; 2003.
2. Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004; 27(5):1047-53.
3. World Health Organization, Department of Noncommunicable disease Surveillance. Definition, diagnosis and classification of Diabetes Mellitus and its complications: report of a WHO consultation
Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva: World Health Organization; 1999.
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2008; 31 Suppl 1:S55-60.
5. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001; 285(19):2486-97.
6. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998; 339(4):229-34.
7. Haffner SM. Coronary heart disease in patients with diabetes. *N Engl J Med*. 2000; 342(14):1040-2.

8. Stamler J, Vaccaro O, Neaton JD, Wentworth D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care*. 1993; 16(2):434-44.
9. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care*. 1979; 2(2):120-6.
10. Wingard DL, Barrett-Connor EL, Ferrara A. Is insulin really a heart disease risk factor. *Diabetes Care*. 1995; 18(9):1299-304.
11. Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev*. 1987; 3(2):463-524.
12. Laakso M. Cardiovascular disease in type 2 diabetes: challenge for treatment and prevention. *J Intern Med*. 2001; 249(3):225-35.
13. Ceriello A, Bortolotti N, Motz E, Pieri C, Marra M, Tonutti L, et al. Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: the possible role of hyperglycemia. *Metabolism*. 1999; 48(12):1503-8.
14. West IC. Radicals and oxidative stress in diabetes. *Diabet Med*. 2000; 17(3):171-80.
15. Ceriello A. The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. *Diabetes Metab Res Rev*. 2000; 16(2):125-32.
16. Schalkwijk CG, Poland DC, van Dijk W, Kok A, Emeis JJ, Drager AM, et al. Plasma concentration of C-reactive protein is increased in type I diabetic patients without clinical macroangiopathy and correlates with markers of endothelial dysfunction: evidence for chronic inflammation. *Diabetologia*. 1999; 42(3):351-7.
17. Baron AD. Vascular reactivity. *Am J Cardiol*. 1999; 84(1A):25J-27J.

18. Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol.* 1998; 31(3):552-7.
19. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes.* 1999; 48(9):1856-62.
20. Vavuranakis M, Stefanadis C, Triandaphyllidi E, Toutouzas K, Toutouzas P. Coronary artery distensibility in diabetic patients with simultaneous measurements of luminal area and intracoronary pressure: evidence of impaired reactivity to nitroglycerin. *J Am Coll Cardiol.* 1999; 34(4):1075-81.
21. Juutilainen A, Lehto S, Ronnema T, Pyorala K, Laakso M. Type 2 diabetes as a "coronary heart disease equivalent": an 18-year prospective population-based study in Finnish subjects. *Diabetes Care.* 2005; 28(12):2901-7.
22. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998; 352(9131):837-53.
23. Selvin E, Coresh J, Golden SH, Boland LL, Brancati FL, Steffes MW. Glycemic control, atherosclerosis, and risk factors for cardiovascular disease in individuals with diabetes: the atherosclerosis risk in communities study. *Diabetes Care.* 2005; 28(8):1965-73.
24. Collins R, Armitage J, Parish S, Sleight P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet.* 2003; 361(9374):2005-16.

25. Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). *Diabetes Care*. 1997; 20(4):614-20.
26. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ*. 1998; 317(7160):703-13.
27. Bantle JP, Wylie-Rosett J, Albright AL, Apovian CM, Clark NG, Franz MJ, et al. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care*. 2008; 31 Suppl 1:S61-78.
28. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, et al. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care*. 2007; 30(1):162-72.
29. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med*. 2000; 342(19):1392-8.
30. Rivellese A, Riccardi G, Giacco A, Pacioni D, Genovese S, Mattioli PL, et al. Effect of dietary fibre on glucose control and serum lipoproteins in diabetic patients. *Lancet*. 1980; 2(8192):447-50.
31. Simpson HC, Simpson RW, Lousley S, Carter RD, Geekie M, Hockaday TD, et al. A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. *Lancet*. 1981; 1(8210):1-5.

32. Anderson JW, Gustafson NJ, Bryant CA, Tietzen-Clark J. Dietary fiber and diabetes: a comprehensive review and practical application. *J Am Diet Assoc.* 1987; 87(9):1189-97.
33. Paolisso G, Sgambato S, Pizza G, Passariello N, Varricchio M, D'Onofrio F. Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. *Diabetes Care.* 1989; 12(4):265-9.
34. Paolisso G, Sgambato S, Gambardella A, Pizza G, Tesaro P, Varricchio M, et al. Daily magnesium supplements improve glucose handling in elderly subjects. *Am J Clin Nutr.* 1992; 55(6):1161-7.
35. Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am J Clin Nutr.* 1998; 67(3 Suppl):577S-82S.
36. Sarkkinen E, Schwab U, Niskanen L, Hannuksela M, Savolainen M, Kervinen K, et al. The effects of monounsaturated-fat enriched diet and polyunsaturated-fat enriched diet on lipid and glucose metabolism in subjects with impaired glucose tolerance. *Eur J Clin Nutr.* 1996; 50(9):592-8.
37. McIntosh M, Miller C. A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. *Nutr Rev.* 2001; 59(2):52-5.
38. Parillo M, Rivellese AA, Ciardullo AV, Capaldo B, Giacco A, Genovese S, et al. A high-monounsaturated-fat/low-carbohydrate diet improves peripheral insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism.* 1992; 41(12):1373-8.
39. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. *Am J Clin Nutr.* 1993; 57(5):650-6.

40. Rimm EB, Stampfer MJ. The role of antioxidants in preventive cardiology. *Curr Opin Cardiol*. 1997; 12(2):188-94.
41. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med*. 1995; 333(5):276-82.
42. International Tree Nut Council Nutrition Research & Education Foundation, [Homepage]. [citado em: 2008 Dec 14]. Disponível em: <http://www.nuthealth.org/>.
43. Kris-Etherton PM, Yu-Poth S, Sabate J, Ratcliffe HE, Zhao G, Etherton TD. Nuts and their bioactive constituents: effects on serum lipids and other factors that affect disease risk. *American Journal of Clinical Nutrition*. 1999; 70(3 Suppl):504S-11S.
44. Lovejoy JC, Most MM, Lefevre M, Greenway FL, Rood JC. Effect of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. *Am J Clin Nutr*. 2002; 76(5):1000-6.
45. Jiang R, Manson JE, Stampfer MJ, Liu S, Willett WC, Hu FB. Nut and peanut butter consumption and risk of type 2 diabetes in women. *JAMA*. 2002; 288(20):2554-60.
46. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*. 2006; 295(14):1681-7.
47. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA*. 2003; 290(4):486-94.

48. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet*. 2002; 359(9323):2072-7.
49. Josse AR, Kendall CW, Augustin LS, Ellis PR, Jenkins DJ. Almonds and postprandial glycemia-a dose-response study. *Metabolism*. 2007; 56(3):400-4.
50. Jenkins DJ, Kendall CW, Josse AR, Salvatore S, Brighenti F, Augustin LS, et al. Almonds decrease postprandial glycemia, insulinemia, and oxidative damage in healthy individuals. *J Nutr*. 2006; 136(12):2987-92.
51. Jenkins DJ, Kendall CW, Marchie A, Parker TL, Connelly PW, Qian W, et al. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. *Circulation*. 2002; 106(11):1327-32.
52. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med*. 1989; 320(14):915-24.
53. Albert CM, Gaziano JM, Willett WC, Manson JE. Nut consumption and decreased risk of sudden cardiac death in the Physicians' Health Study. *Arch Intern Med*. 2002; 162(12):1382-7.
54. Kris-Etherton PM, Hu FB, Ros E, Sabate J. The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *J Nutr*. 2008; 138(9):1746S-51S.
55. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch Intern Med*. 1992; 152(7):1416-24.

56. Hu FB, Stampfer MJ, Manson JE, Rimm EB, Colditz GA, Rosner BA, et al. Frequent nut consumption and risk of coronary heart disease in women: prospective cohort study. *BMJ*. 1998; 317(7169):1341-5.
57. Ellsworth JL, Kushi LH, Folsom AR. Frequent nut intake and risk of death from coronary heart disease and all causes in postmenopausal women: the Iowa Women's Health Study. *Nutr Metab Cardiovasc Dis*. 2001; 11(6):372-7.
58. Griel AE, Kris-Etherton PM. Tree nuts and the lipid profile: a review of clinical studies. *Br J Nutr*. 2006; 96 Suppl 2:S68-78.
59. Tapsell LC, Gillen LJ, Patch CS, Batterham M, Owen A, Bare M, et al. Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol ratios in patients with type 2 diabetes. *Diabetes Care*. 2004; 27(12):2777-83.
60. Scott LW, Balasubramanyam A, Kimball KT, Aherns AK, Fordis CM, Jr., Ballantyne CM. Long-term, randomized clinical trial of two diets in the metabolic syndrome and type 2 diabetes. *Diabetes Care*. 2003; 26(8):2481-2.
61. Marfell MJ, Olds T, Stewart A, Carter L. International Standards for anthropometric assessment. ISAK: Potchefstroom; 2006.
62. Harris JA, Benedict TG. Biometric studies of basal metabolism in man. Carnegie Institution of Washington; 1919.
63. Pecoraro RE, Chen MS, Porte D, Jr. Glycosylated hemoglobin and fasting plasma glucose in the assessment of outpatient glycemic control in NIDDM. *Diabetes Care*. 1982; 5(6):592-9.
64. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2008; 358(24):2560-72.

65. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. 2008; 57(5):1349-54.
66. Ceriello A, Kumar S, Piconi L, Esposito K, Giugliano D. Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type 1 diabetes. *Diabetes Care*. 2007; 30(3):649-54.
67. Pennathur S, Heinecke JW. Mechanisms for oxidative stress in diabetic cardiovascular disease. *Antioxid Redox Signal*. 2007; 9(7):955-69.
68. Pennathur S, Heinecke JW. Oxidative stress and endothelial dysfunction in vascular disease. *Curr Diab Rep*. 2007; 7(4):257-64.
69. Chen CY, Milbury PE, Lapsley K, Blumberg JB. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. *J Nutr*. 2005; 135(6):1366-73.
70. Dourado F, Barros A, Mota M, Coimbra MA, Gama FM. Anatomy and cell wall polysaccharides of almond (*Prunus dulcis* D. A. Webb) seeds. *J Agric Food Chem*. 2004; 52(5):1364-70.
71. Sang S, Lapsley K, Jeong WS, Lachance PA, Ho CT, Rosen RT. Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch). *J Agric Food Chem*. 2002; 50(8):2459-63.
72. Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, et al. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med*. 2003; 24(1-3):39-52.
73. Paolisso G, Scheen A, D'Onofrio F, Lefebvre P. Magnesium and glucose homeostasis. *Diabetologia*. 1990; 33(9):511-4.

74. Sturmer T, Hasselbach P, Amelang M. Personality, lifestyle, and risk of cardiovascular disease and cancer: follow-up of population based cohort. *BMJ*. 2006; 332(7554):1359.
75. Zibaeenezhad MJ, Shamsnia SJ, Khorasani M. Walnut consumption in hyperlipidemic patients. *Angiology*. 2005; 56(5):581-3.
76. Wien MA, Sabate JM, Ikle DN, Cole SE, Kandeel FR. Almonds vs complex carbohydrates in a weight reduction program. *Int J Obes Relat Metab Disord*. 2003; 27(11):1365-72.
77. Jaceldo-Siegl K, Sabate J, Rajaram S, Fraser GE. Long-term almond supplementation without advice on food replacement induces favourable nutrient modifications to the habitual diets of free-living individuals. *Br J Nutr*. 2004; 92(3):533-40.
78. Kirkmeyer SV, Mattes RD. Effects of food attributes on hunger and food intake. *Int J Obes Relat Metab Disord*. 2000; 24(9):1167-75.
79. Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, et al. Role of cell walls in the bioaccessibility of lipids in almond seeds. *Am J Clin Nutr*. 2004; 80(3):604-13.

