Impact of the traditional Mediterranean diet on the Framingham risk score and the metabolic syndrome according to sex

Running head: Mediterranean diet and global cardiovascular risk

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Abstract

Background: The traditional Mediterranean diet (MedDiet) has been recognized as a food pattern with beneficial effects on cardiovascular health. However, even if sex-related differences in the cardiovascular response to diet have been previously highlighted, the existence of such differences in the impact of the MedDiet on the global cardiovascular risk has not been yet investigated. This study aimed at examining sex differences in the global cardiovascular impact of a 4-week isoenergetic controlled MedDiet using the Framingham risk score and the National Cholesterol Education Program (NCEP) metabolic syndrome criteria.

Methods: This study included 38 men and 32 premenopausal women (24-53 years) who had a slightly elevated LDL-C concentrations (between 3.4-4.9 mmol/l) or total cholesterol to HDL-C ratio ≥ 5.0. Cardiovascular risk factors were measured before and after the controlled MedDiet.

Results: A time effect (P=0.04) was found for the Framingham risk score, with both men and women showing a nonsignificant decrease in response to the MedDiet. No time effect was found for the prevalence of the metabolic syndrome and the number of metabolic syndrome criteria that were met by participants (P>0.05). However, a time effect was noted for the continuous metabolic syndrome score (P=0.008), with nonsignificant decreases in both men and women. No sex by time interaction was noted for any of variables studied (P>0.05).

Conclusions: Results from this study suggest that the global cardiovascular impact of the MedDiet, as assessed by the Framingham risk score and metabolic syndrome criteria, is not significantly different in men than in premenopausal women in isoenergetic conditions.

Introduction

Cardiovascular disease (CVD) is a major cause of disability and death in industrialized countries, accounting for respectively 33% [1] and 27% [2] of deaths in the United States and Canada. It is now widely known that sex-related differences exist in CVD incidence rate since premenopausal women have a lower risk of CVD compared to age-matched men [3]. This sex difference would be mainly ascribed to sex steroid hormones concentrations [4]. In fact, some evidence highlights that endogenous estrogens bring many beneficial effects related to cardiovascular risk factors. More specifically, endogenous estrogens have been identified as contributors of the lower visceral fat accumulation and blood pressure level, the lower triglyceride (TG) and cholesterol concentrations and the higher insulin sensitivity found in premenopausal women compared to age-matched men [5,6].

Since cardiovascular risk factors cluster and interact with each other [7], different tools combining some well-known cardiovascular risk factors have been created in order to estimate the global cardiovascular risk of individuals. Two well-recognized and frequently used tools are the Framingham risk score and metabolic syndrome criteria [8]. These tools have been demonstrated as good predictors of cardiovascular morbidity and mortality [9,10]. In prevention of CVD, the traditional Mediterranean diet (MedDiet) has been shown as a promising approach to globally improve cardiovascular health. In fact, the MedDiet has been associated with a reduction of the global 10-year CVD risk as estimated by the Framingham risk score [11,12]. Moreover, the adherence to the MedDiet has been associated with a reduced risk of 31% of the metabolic syndrome in a recent meta-analysis [13]. However, since most of the interventional studies have investigated MedDiet effects in

free-living conditions with concomitant weight loss, little is known about the global cardiovascular impact of the adoption of the MedDiet in controlled isoenergetic conditions without the confounding effect of body weight change.

Sex-related differences in the cardiovascular response to diet have been previously highlighted [14,15]; however the existence of such differences in the global cardiovascular risk response to the MedDiet has not been investigated yet in fully-controlled conditions. Nevertheless, the adoption of the traditional MedDiet has previously been found to reduce endogenous estrogen concentrations in postmenopausal women [16]. This raises the possibility that women may have limited cardiovascular benefits from the MedDiet compared to age-matched men due to a concomitant decrease in beneficial effects related to estrogens. Thus, the objective of this study was to investigate sex-related differences in the global cardiovascular impact of a 4-week controlled MedDiet using the Framingham risk score and the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III metabolic syndrome criteria in a sample of women and age-matched men characterized by a slightly deteriorated lipid profile. Since previous studies have highlighted that dietary food patterns affect in the same way but to a greater extent endogenous estrogen concentrations in premenopausal women compared to postmenopausal women [17,18], we decided in the present study to compare men and premenopausal women in order to maximize differences due to sex hormones.

Methods

Subjects

The study protocol has been extensively described in a previous publication [15]. Briefly, the study included 38 men and 32 premenopausal women (24-53 years) who had a slightly elevated LDL-C concentration (between 3.4 and 4.9 mmol/l) or total cholesterol to HDL-C ratio ≥ 5.0 and at least one of the four following cardiovascular risk factors [19]: waist circumference > 94 cm in men and > 80 cm in women [20]; TG > 1.7 mmol/l; fasting glycemia between 6.1 and 6.9 mmol/l and/or blood pressure concentrations ≥ 130 / 85 mm Hg. These inclusion criteria were fixed in order to have participants at risk of CVD in whom primary prevention should be started through the adoption of healthy dietary habits. In women, a follicle-stimulating hormone (FSH) measurement during the early follicular phase confirmed the premenopausal status (FSH < 20 IU/l). Exclusion criteria included significant weight change (> 2.5 kg) in the three months before the study, cardiovascular events and use of medication that could affect dependent variables under study (namely lipid-lowering, hypoglycemic, insulin sensitizers and anti hypertensive medication). Smokers, pregnant women and women using systemic hormonal contraceptives were also excluded from the study. All subjects gave written informed consent prior to inclusion in the study. All clinical investigations were conducted according to Declaration of Helsinki principles. All aspects of the study were approved by the Laval University Research Ethics Committee on human experimentation, Québec, Québec, Canada.

Study design

Before the experimental MedDiet intervention, participants went through a 4-week run-in period during which they had to comply with healthy eating according to the Canada's Food Guide [21] as instructed by a registered dietician.

The controlled phase was undertaken as a parallel design during which both men and women were assigned to a 4-week fully controlled experimental diet formulated to be concordant with the characteristics of the traditional MedDiet [22]. This study design permitted to evaluate precisely sex differences in response to the MedDiet. The controlled phase lasted 4 weeks since it has been shown that this duration under controlled conditions is sufficient to obtain significant changes in cardiometabolic variables in both sexes [23-25]. The experimental diet intervention and its nutritional composition have been previously described [15]. Briefly, the percentages of kilocalories derived from lipids, carbohydrates, proteins and alcohol were respectively of 32%, 46%, 17% and 5%. All foods and drinks were prepared by food technicians at the Clinical Investigation Unit (CIU) at the Institute of Nutraceuticals and Functional Foods (INAF; Laval University) and provided to participants according to a 7-day cyclic menu. Subjects were instructed to consume all foods and drinks provided. Body weight was measured on weekdays and energy intake was adjusted to keep each subject's body weight constant throughout the study. Participants were also instructed to maintain their usual physical activity level during this controlled intervention. In premenopausal women, since previous studies have suggested that the phase of the menstrual cycle influences the lipid and lipoprotein profile [26], glycemia, blood pressure and adiposity measurements, women's feeding was shortened or prolonged if needed in order to be able to carry out all tests in the early follicular phase of their menstrual cycle (from the third to the ninth day of the menstrual cycle; mean duration of the feeding period in women, 28·8 ± 4·3 days).

Dietary intakes

To determine whether dietary habits before the controlled MedDiet intervention were similar in men and women, a validated quantitative FFQ [27], containing ninety-one items and thirty-three subquestions, which inquires on food habits during the last month was administrated by a registered dietitian just before the controlled MedDiet intervention (i.e. after the run-in period), reflecting dietary habits during the entire run-in period. A Mediterranean score (MedScore) derived from the FFQ was then calculated as described by Goulet and colleagues [28]. The MedScore can vary between zero and forty-four points. A MedScore of forty-four would imply a food pattern which is perfectly concordant with the traditional MedDiet.

Cardiovascular risk factors assessments

Baseline cardiovascular risk factors were assessed before the controlled MedDiet intervention (i.e. after the run-in period). Cardiovascular risk factors were also measured after the controlled MedDiet intervention. Blood samples were collected from an antecubital vein into vacutainer tubes after a 12-h overnight fast. Assessment of total cholesterol, HDL-C and TG were performed according to previously described methods [28]. A blood sample was also collected into a vacutainer tube containing EDTA for the assessment of glucose concentrations. Plasma glucose concentrations were measured in a fasting state with the hexokinase-glucose-6-phosphate dehydrogenase method [29].

Waist circumference measurement was taken directly on the skin at the mid-distance between the last rib and the top of the iliac crest after a normal expiration. Waist circumference was determined as the mean of three measurements. Systolic and diastolic

blood pressures were measured on the right arm using an automated blood pressure monitor (BPM 300-BpTRU: Vital Signs Monitor) after a 10 min rest in the sitting position. Blood pressure was computed as the mean of three readings.

Global cardiovascular risk tools

The Framingham 10-year CVD risk score was calculated using age, total cholesterol, HDL-C, systolic blood pressure and smoking habits as described in the NCEP-ATP III final report [8].

The metabolic syndrome was defined according to the revised NCEP-ATP III criteria [30]. Subjects with three or more of the following criteria were defined as having the metabolic syndrome: 1) waist circumference > 102 cm in men and > 88 cm in women; 2) TG > 1.7 mmol/L; 3) HDL-C < 1.03 mmol/L in men and < 1.29 mmol/L in women; 4) fasting glucose ≥ 5.6 mmol/L and 5) blood pressure ≥ 130 / 85 mm Hg. We chose the NCEP-ATP III definition since it is the most frequently used definition in clinical trials investigating the effects of the MedDiet on the metabolic syndrome [13], thereby facilitating the comparison between our results and the literature.

We also calculated a continuous metabolic syndrome score as used in other studies [31,32]. A continuous score was calculated for each metabolic syndrome component using difference between individual subject data and the NCEP-ATP III criterion (numerator) and standard deviation (denominator) as suggested by Johnson and collaborators [31]. A continuous metabolic syndrome score was created as a sum of the five individual scores. A decrease in the score indicates a reduced CVD risk. We decided to include a continuous score in our

analyses because 1) the CVD risk increases proportionally with levels of metabolic syndrome components [33] and 2) a continuous metabolic syndrome score is more sensitive to overall metabolic changes found in response to lifestyle interventions than a dichotomous approach [33,34].

Statistical analyses

Data were analyzed by using SAS statistical package version 9.2 (SAS Institute Inc., Cary, NC, USA). Data were collected before (i.e. after the run-in period) and after the controlled MedDiet intervention and results are expressed as means and standard deviation (SD) or standard errors of the mean (SEM). For variables not normally distributed, a transformation was performed in order to obtain a normal distribution. Differences in anthropometric and metabolic variables between men and premenopausal women before the controlled MedDiet intervention were assessed using the Student's t-test. To compare the prevalence of the metabolic syndrome and its components between men and women before the controlled MedDiet intervention, chi-square test was used for analyses with five or more participants per cell; otherwise Fisher's exact test was used. The Cochran-Mantel-Haenszel test was used in order to determine whether sex differences exist in changes in the prevalence of the metabolic syndrome in response to the isoenergetic MedDiet. Chi-square test was used to evaluate the effect of the MedDiet on the prevalence of the metabolic syndrome in men and premenopausal women taken separately. MIXED procedures for repeated measurements were used to evaluate time and sex by time interaction effects on the Framingham 10-year CVD risk score, the number of metabolic syndrome criteria and the continuous metabolic syndrome score in response to the MedDiet. Tukey-Kramer tests were used to determine

precisely the location of significant differences. Results were all adjusted for the slight but significant body weight loss which occurred during the controlled MedDiet intervention (from 91.8±14.0 to 90.6±13.7 kg in men (-1.2 kg or -1.3% of initial body weight; $P<0.0001$) and from 78.0±14.7 to 77.5±14.3 kg in women (-0.5 kg or -0.7%; $P=0.03$). Differences were considered statistically significant at $P < 0.05$ (two-sided). One man was excluded of our analyses due to illness, which led to a significant reduction of food intake during several days at the end of the controlled MedDiet intervention. Thus, 37 men and 32 premenopausal women were included in the analyses. A sample size of 69 participants allowed to detect a difference of 1.9% in change in the Framingham risk score and a difference of 2.2 in change in the continuous metabolic syndrome score between sexes, considering a standard deviation of respectively 2.8% and 3.2 with an alpha risk of 0.05 and a beta risk of 0.20 in two-sided contrasts.

Results

Age and BMI were similar in men and women before the controlled MedDiet intervention (Table 1). Men had higher baseline values for body weight, waist circumference, TG, systolic and diastolic blood pressures and fasting glucose and lower baseline values for HDL-C than premenopausal women. No sex difference was observed regarding the MedDiet score. The baseline Framingham risk score was significantly higher in men than in women. The prevalence of the metabolic syndrome, the number of metabolic syndrome criteria and the continuous metabolic syndrome score were not significantly different between sexes before the controlled MedDiet intervention. With regards to components of the metabolic syndrome, no difference was found for HDL-C and blood pressure criteria prevalence whereas a greater proportion of men had TG and fasting glucose criteria and a lower proportion of men had the waist circumference criterion compared to premenopausal women.

A time effect (P=0.04) was observed for the Framingham 10-year CVD risk score (mean change: -0.28 ± 0.13 %) and a nonsignificant decrease was found in both men and women (respectively -0.38 ± 0.23 %; P=0.15 and -0.16 ± 0.08 %; P=0.84) (Figure 1, a). No sex by time interaction was noted for this variable (P=0.39). Results were similar after adjustment for the baseline Framingham risk score (results not shown).

Men experienced a nonsignificant decrease in the prevalence of the metabolic syndrome (from 46.0% before the controlled MedDiet intervention to 32.4% after the controlled intervention; P=0.23) whereas women showed a nonsignificant increase (from 34.4% to 37.5%; P=0.79). However, no significant sex difference was observed for the change in the prevalence of the metabolic syndrome in response to the MedDiet (P=0.32). Moreover, no

time (P=0.17) and sex by time interaction (P=0.27) effects were found for the number of metabolic syndrome criteria (Figure 1, b). However, a time effect (P=0.008) was observed for the continuous metabolic syndrome score and a decrease was noted for this variable when the whole sample was considered (Figure 1, c). This is indicative of a reduced risk after the MedDiet (changes ranged from -5.23 to 1.75 for all participants; mean change: -0.49±0.18). When men and women were analysed separately, changes observed were of similar magnitude as those for the total sample but did not reach statistical significance (respectively -0.46 ± 0.26, P=0.23 and -0.51 ± 0.24, P=0.21). Finally, no sex by time interaction was noted for the continuous metabolic syndrome score (P=0.91).

Discussion

Our results showed for the first time that the impact of the MedDiet on the global cardiovascular risk, as determined by the Framingham risk score and metabolic syndrome criteria, is not different in men than in premenopausal women in an isoenergetic context. This conclusion is supported by the absence of significant sex by time interaction for all variables studied. More precisely, we found a significant decrease in the Framingham risk score in response to the MedDiet. Moreover, a decrease in the continuous metabolic syndrome score occurred, indicating a beneficial effect of the MedDiet on the cardiovascular risk factors included in the metabolic syndrome definition; however this beneficial effect was not reflected by significant decreases in the prevalence of the metabolic syndrome and in the number of criteria that were met by participants. These global beneficial effects, along with the previously reported decrease in LDL-C found in response to the MedDiet in both men and women [15], support the fact that the MedDiet has beneficial effects on the cardiovascular health, independently of the sex.

Endogenous estrogens have a beneficial impact on total cholesterol and HDL-C concentrations, blood pressure level and glucose/insulin homeostasis [4,6]. Interestingly, all these risk factors are included in the calculation of the Framingham 10-year CVD risk score. Thus, because of the decrease in endogenous estrogen concentrations previously found by Carruba and collaborators in women in response to the adoption of the MedDiet [16], we initially hypothesized that premenopausal women could have a less important beneficial impact on the cardiovascular risk as assessed by the Framingham risk score than age-matched men. However, our results showed a decrease in the Framingham risk score in

response to the MedDiet when the whole sample was considered with no sex difference for this variable. Taken together, our results are consistent with observational and uncontrolled interventional studies which highlighted that the adoption of the MedDiet is associated with a global cardiovascular risk reduction as measured by the Framingham risk score in samples including both men and women [11,12,35]. Moreover, our results are in line with those of a well-controlled study which showed a decrease in the Framingham risk score in response to an isoenergetic MedDiet in a sample of men with the metabolic syndrome [36]. However, our study brings additional useful information to the previous literature since it is the first to be designed to directly compare the response to the MedDiet between men and premenopausal women with a maximum of control over confounding variables and therefore allowing to demonstrate that the impact of the MedDiet on cardiovascular risk, as measured by the Framingham risk score, is not different in men and in premenopausal women.

Similarly to the Framingham risk score, the metabolic syndrome definition includes cardiovascular risk factors, which can be influenced by estrogen levels. In the present study, we found that, in addition to observing no sex-related differences, participants had no benefit related to the metabolic syndrome from the adoption of an isoenergetic MedDiet when dichotomous criteria were used. Results from Richard and collaborators are in line with our results since they reported that a controlled isoenergetic MedDiet had no effect on the metabolic syndrome prevalence in men [36]. However, our results are inconsistent with those from Esposito and collaborators which have suggested that the adoption of the MedDiet may decrease the number of the metabolic syndrome criteria even after adjustment for the weight loss which occurred during their "real life" intervention [37].

Differences in inclusion criteria and study design may explain this divergence between their results and ours. It is relevant to highlight that, in our study, the metabolic syndrome criterion which was the most prevalent in men before the MedDiet intervention was fasting glucose while it was waist circumference in women. Since these criteria are particularly sensitive to weight change [38] and since the adoption of the MedDiet in uncontrolled conditions usually leads to weight loss, it is therefore possible to suggest that, in a free-living context, the MedDiet could have a significant impact on these dichotomous criteria of the metabolic syndrome. On the other hand, even if no change in metabolic syndrome was observed when dichotomous criteria were used, we noted a decrease in the continuous metabolic syndrome score, a more sensitive tool to reflect changes observed in response to lifestyle interventions than dichotomous criteria. Thus, our results suggest that the MedDiet has a global beneficial effect on metabolic syndrome factors. However, this beneficial effect was not reflected in our study by significant decreases in the prevalence of the metabolic syndrome and in the number of criteria that were met by participants.

It is essential to highlight that participants included in this study were characterized by a slightly deteriorated LDL-C or total cholesterol to HDL-C ratio, and are therefore individuals in whom primary prevention should be started in clinical practice through the adoption of healthy dietary habits [8,39]. Therefore results from this study are highly clinically relevant since they demonstrated that, among these at-risk individuals, the sex has no influence on the effects of the traditional MedDiet on the global cardiovascular risk. A strength of this study is the well-controlled design of the MedDiet intervention in which all foods and drinks were provided to participants and compliance was closely monitored each day. This well-controlled context allowed us to precisely evaluate sex-related differences in

response to the MedDiet. However, some limitations should be noted. First the study's 'single strand before and after' design does not allow comparisons to a control diet and therefore non-specific treatment effects that are not attributable to the MedDiet can not be ruled out. In fact, since the cardioprotective effects of the MedDiet are well known and have been widely documented [11-13], it is important to highlight that this study had as a main objective to investigate sex-related differences in response to the MedDiet, which was possible due to the parallel design with men and women and the fully controlled nature of the nutritional intervention. Nevertheless, since our study included only premenopausal women, our results can not be generalized to all women. Moreover, premenopausal women were characterized by a very low Framingham risk score before the controlled intervention, which may have limited the impact of the MedDiet on this global cardiovascular risk assessment tool in women. The nonsignificant effect of the MedDiet on the prevalence of the metabolic syndrome and its criteria may be due to the small sample size of our fully controlled nutritional intervention. Therefore, additional studies with a larger sample size are needed to confirm these results. Finally, sex steroid hormones were not measured in our study, and thus it is not possible to determine whether a decrease in estrogen levels occurred.

In conclusion, results from this fully controlled-feeding study suggest that no sex-related difference exists in the global cardiovascular response to an isoenergetic MedDiet with regards to the Framingham risk score and the metabolic syndrome criteria. Since the MedDiet is now widely recommended in prevention of CVD by many organizations [40-42], this study gives additional useful information about the effectiveness of this food pattern,

which is independent of the sex, in order to reduce the global cardiovascular risk even in absence of weight loss.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Centers for Disease Control and Prevention. Vital Statistics Public Use Data Files - 2008 Mortality Multiple Cause Files. Available at http://www.cdc.gov/nchs/data_access/Vitalstatsonline.htm#Mortality_Multiple. Accessed July 16, 2012.

2. Canadian Cancer Society. Canadian Cancer Statistics 2010. Available at http://www.cancer.ca. Accessed July 16, 2012.

3. Barrett-Connor E. Sex differences in coronary heart disease - Why are women so superior? The 1995 Ancel Keys lecture. *Circulation* 1997;95:252-264.

4. Vitale C, Fini M, Speziale G, et al. Gender differences in the cardiovascular effects of sex hormones. *Fundam Clin Pharmacol* 2010;24:675-685.

5. Guarner-Lans V, Rubio-Ruiz ME, Perez-Torres I, et al. Relation of aging and sex hormones to metabolic syndrome and cardiovascular disease. *Exp Gerontol* 2011;46:517-523.

6. Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. *Am J Cardiol* 2002;89:12E-17E.

7. D'Agostino RB, Russell MW, Huse DM, et al. Primary and subsequent coronary risk appraisal: New results from The Framingham Study. *Am Heart J* 2000;139:272-281.

8. National Cholesterol Education Program (NCEP) Expert Panel. 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) final report. *Circulation* 2002;106:3143-3421.

9. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837-1847.

10. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: A meta-analysis. *Am J Med* 2006;119:812-819.

11. Lerman RH, Minich DM, Darland G, et al. Enhancement of a modified Mediterranean-style, low glycemic load diet with specific phytochemicals improves cardiometabolic risk factors in subjects with metabolic syndrome and hypercholesterolemia in a randomized trial. *Nutr Metab* 2008;5:29.

12. Panagiotakos D, Sitara M, Pitsavos C, et al. Estimating the 10-year risk of cardiovascular disease and its economic consequences, by the level of adherence to the Mediterranean diet: the ATTICA study. *J Med Food* 2007;10:239-243.

13. Kastorini CM, Milionis HJ, Esposito K, et al. The Effect of Mediterranean Diet on Metabolic Syndrome and its Components A Meta-Analysis of 50 Studies and 534,906 Individuals. *J Am Coll Cardiol* 2011;57:1299-1313.

14. Knopp RH, Paramsothy P, Retzlaff BM, et al. Gender differences in lipoprotein metabolism and dietary response: basis in hormonal differences and implications for cardiovascular disease. *Curr Atheroscler Rep* 2005;7:472-479.

15. Bédard A, Riverin M, Dodin S, et al. Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. *Br J Nutr* 2012;8:1428-1434.

16. Carruba G, Granata OM, Pala V, et al. A traditional Mediterranean diet decreases endogenous estrogens in healthy postmenopausal women. *Nutr Cancer* 2006;56:253-259.

17. Goldin BR, Gorbach SL. Effect of Diet on the Plasma-Levels, Metabolism, and Excretion of Estrogens. *Am J Clin Nutr* 1988;48:787-790.

18. Goldin BR, Adlercreutz H, Gorbach SL, et al. The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women. *Am J Clin Nutr* 1986;44:945-953.

19. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009;120:1640-1645.

20. International Diabetes Federation. IDF worldwide definition of the metabolic syndrome. Available at http://www.idf.org/webdata/docs/IDF_Meta_def_final.pdf. Accessed July 16, 2012.

21. Minister of Health Canada. Eating Well with Canada's Food Guide. Available at http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/food-guide-aliment/view_eatwell_vue_bienmang-eng.pdf. Accessed July 16, 2012.

22. Willett WC, Sacks F, Trichopoulou A, et al. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 1995;61:1402S-1406S.

23. Beauchesne-Rondeau E, Gascon A, Bergeron J, et al. Plasma lipids and lipoproteins in hypercholesterolemic men fed a lipid-lowering diet containing lean beef, lean fish, or poultry. *Am J Clin Nutr* 2003;77:587-593.

24. Chisholm A, Mann J, Skeaff M, et al. A diet rich in walnuts favourably influences plasma fatty acid profile in moderately hyperlipidaemic subjects. *Eur J Clin Nutr* 1998;52:12-16.

25. Gerhard GT, Connor SL, Wander RC, et al. Plasma lipid and lipoprotein responsiveness to dietary fat and cholesterol in premenopausal African American and white women. *Am J Clin Nutr* 2000;72:56-63.

26. Muesing RA, Forman MR, Graubard BI, et al. Cyclic changes in lipoprotein and apolipoprotein levels during the menstrual cycle in healthy premenopausal women on a controlled diet. *J Clin Endocrinol Metab* 1996;81:3599-3603.

27. Goulet J, Nadeau G, Lapointe A, et al. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. *Nutr J* 2004;3:13.

28. Goulet J, Lamarche B, Nadeau G, et al. Effect of a nutritional intervention promoting the Mediterranean food pattern on plasma lipids, lipoproteins and body weight in healthy French-Canadian women. *Atherosclerosis* 2003;170:115-124.

29. Richterich R, Dauwalder H. [Determination of plasma glucose by hexokinase-glucose-6-phosphate dehydrogenase method]. *Schweiz Med Wochenschr* 1971;101:615-618.

30. Grundy SM, Brewer HB, Cleeman JI, et al. Definition of metabolic syndrome - Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Circulation* 2004;109:433-438.

31. Johnson JL, Slentz CA, Houmard JA, et al. Exercise training amount and intensity effects on metabolic syndrome (from studies of a targeted risk reduction intervention through defined exercise). *Am J Cardiol* 2007;100:1759-1766.

32. Franks PW, Ekelund U, Brage S, et al. Does the association of habitual physical activity with the metabolic syndrome differ by level of cardiorespiratory fitness? *Diabetes Care* 2004;27:1187-1193.

33. Kahn R, Buse J, Ferrannini E, et al. The metabolic syndrome: Time for a critical appraisal: Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2005;28:2289-2304.

34. Ragland DR. Dichotomizing Continuous Outcome Variables - Dependence of the Magnitude of Association and Statistical Power on the Cutpoint. *Epidemiology* 1992;3:434-440.

35. Panagiotakos DB, Pitsavos C, Arvaniti F, et al. Adherence to the Mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. *Prev Med* 2007;44:335-340.

36. Richard C, Couture P, Desroches S, et al. Effect of the Mediterranean diet with and without weight loss on cardiovascular risk factors in men with the metabolic syndrome. *Nutr Metab Cardiovasc Dis* 2011;21:628-635.

37. Esposito K, Marfella R, Ciotola M, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440-1446.

38. Manco M, Mingrone G. Effects of weight loss and calorie restriction on carbohydrate metabolism. *Curr Opin Clin Nutr Metab Care* 2005;8:431-439.

39. Genest J, McPherson R, Frohlich J, et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. *Can J Cardiol* 2009;25:567-579.

40. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 2006;114:82-96.

41. World Health Organization Study Group. Diet, nutrition, and the prevention of chronic diseases. Available at http://whqlibdoc.who.int/trs/who_trs_916.pdf. Accessed July 16, 2012.

42. Kris-Etherton P, Eckel RH, Howard BV, et al. AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. *Circulation* 2001;103:1823-1825.

Table 1: Characteristics of men and women before the controlled Mediterranean diet intervention

	Men (n=37)		Women (n=32)	
	Mean	SD	Mean	SD
Age (years)	42.6	7.3	41.2	7.3
Body weight (kg) †	91.8	14.0	78.0 *	14.7
BMI (kg/m^2) †	29.2	3.2	29.6	5.4
Waist circumference (cm) †	102.6	10.7	96.4 *	10.5
TG (mmol/l) †	1.86	1.17	1.36 *	0.63
HDL-cholesterol (mmol/l) †	1.09	0.31	1.30 *	0.26
Systolic blood pressure (mm Hg)	117.1	12.6	108.6 *	10.4
Diastolic blood pressure (mm Hg)	80.3	9.0	73.5 *	9.0
Fasting glucose (mmol/l) †	5.89	0.37	5.68 *	0.63
Mediterranean score	24.8	5.9	24.6	4.4
Framingham risk score (%)	3.4	3.2	0.3 *	0.4
Metabolic syndrome score	-0.16	2.89	-0.90	3.44
Number of metabolic syndrome criteria	2.5	1.2	2.0	1.5
Metabolic syndrome (n (%)) ‡	17 (45.9)		11 (34.4)	
Waist circumference (n (%))	15 (40.5)		**23 (71.9) **	
TG (n (%))	18 (48.6)		**8 (25.0) **	

HDL-cholesterol (n (%))	18 (48.6)	15 (46.9)
Blood pressure (n (%))	12 (32.4)	4 (12.5)
Fasting glucose (n (%))	29 (78.4)	**14 (43.8) **

† Analysis was performed on transformed values

Mean values were significantly different between sexes before the controlled Mediterranean diet intervention by Student's t-test; * $P<0.05$

‡ Metabolic syndrome is defined according to the revised National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III criteria

Prevalence was significantly different between sexes before the controlled Mediterranean diet intervention by Chi-square test; ** $P<0.05$

Figure 1.

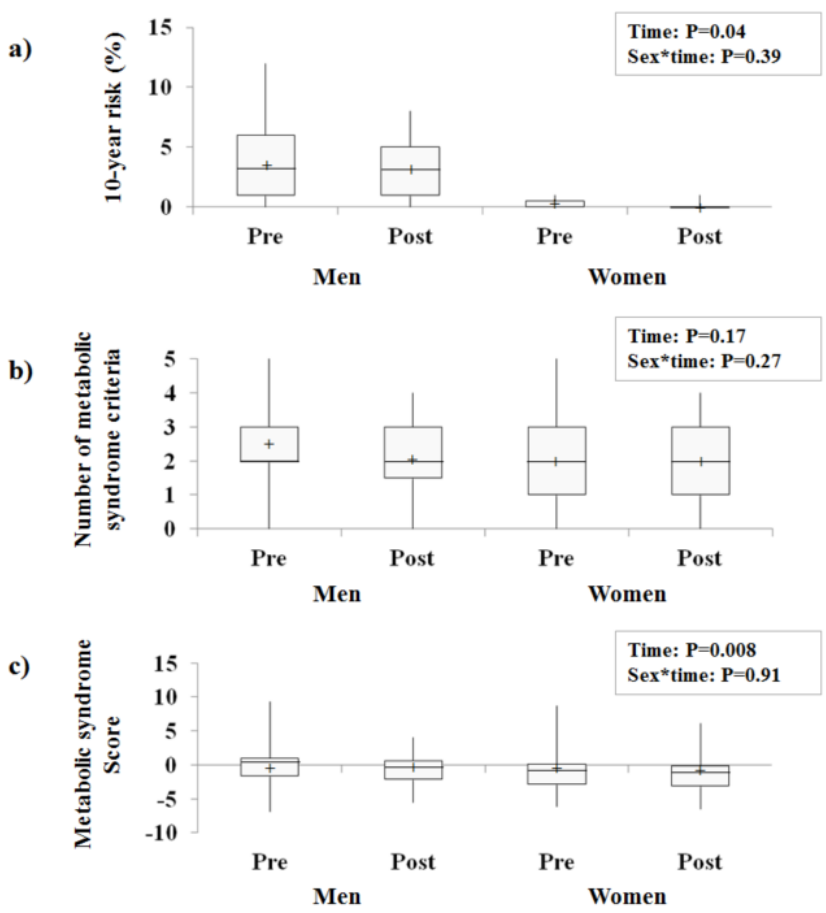

Figure 1. Box plots of the distributions of the 10-year risk of total cardiovascular disease (Framingham risk score) (a), the number of metabolic syndrome criteria that were met by participants (b) and the continuous metabolic syndrome score (c) in men and women before and after the controlled MedDiet intervention. Data are presented as the median and interquartile range; with solid lines extend to the extremes of the data. The cross indicates the mean of the data. Metabolic syndrome is defined according to the revised National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) III criteria. MIXED procedures for repeated measurements were used to evaluate time and sex by time interaction effects.