ISSN: 2377-3634

International Journal of
Diabetes and Clinical Research

Research Article: Open Access

Endothelial Function in Obese and Overweight Patients: The Role of Olive Oil, Fish and Nuts

Marco Matteo Ciccone[1]*, Pietro Scicchitano[1], Francesca Cortese[1], Michele Gesualdo[1], Fara Fornarelli[1], Marco Sassara[1], Leonarda De Santis[2], Annapaola Zito[1], Roberta Riccardi[1], Maria Caterina Lovero[2], Lorenzo Ghiadoni[3], Rocco Lagioia[4], Domenico Scrutinio[4] and Giovanni De Pergola[2]

[1]Section of Cardiovascular Diseases, Department of Emergency and Organ Transplantation, University of Bari, School of Medicine, Italy
[2]Clinical Nutrition Outpatient Clinic, Clinical Oncology, Department of Internal Medicine and Clinical Oncology, University of Bari, School of Medicine, Italy
[3]Department of Internal Medicine, University of Pisa, Italy
[4]Section of Cardiovascular Diseases, IRCCS Fondazione Maugeri, Cassano Murge, Italy

***Corresponding author:** *Marco Matteo Ciccone, Section of Cardiovascular Diseases, Department of Emergency and Organ Transplantation, University of Bari, School of Medicine, Piazza G. Cesare 11 - 70124 Bari Italy, Tel: +39-080-5478791; Fax: +39-080-5478796; E-mail: marcomatteo.ciccone@uniba.it*

Abstract

Objective: To assess the effect of olive oil, non fried fish and nuts on endothelial function in overweight/obese patients.

Methods: 47 overweight/obese patients (24 men, mean age 54 ± 11 years) fed healthy diet (HD) defined according to the Nureta-PREDIMED study for 3 months; after this 3 months of HD, the patients were blindly divided into 4 groups: Controls (24: HD), Group A (8: HD+olive oil), Group B (8: HD+not-fried fish), Group C (7: HD+nuts). These four groups were also followed-up for further 15 months. All patients were evaluated at baseline, at 3 and 18 months for glucose and lipid profile, anthropometric measures and brachial artery Flow-Mediated Dilatation (FMD).

Results: HD alone significantly increased FMD at 3 and 18 months follow-up as compared to baseline. Olive oil or not-fried fish or nuts significantly increased FMD as compared to HD at 18 months follow-up. Waist circumference, body mass index significantly decreased after 3 and 18 months; lipid parameters at 18 months improved in all groups as compared to baseline. Fasting glycemia did not change after 18 months (baseline: 101 ± 19 mg/dl vs 18 months: 98 ± 12 mg/dl; p=ns).

Conclusions: HD is able to improve endothelial function after a short (3 months) period and olive oil, not fried fish and nuts adding seem to increase such an improvement at a longer (18 months) period. Thus, HD and some of its components can improve cardiovascular risk profile of individuals.

Keywords

Diet, Flow mediated dilation, Obesity, Overweight, Cardiovascular risk, Endothelial function

Introduction

Abdominal obesity increases the risk of cardiovascular diseases, since it is commonly associated with hypertension, dyslipidaemia, impaired fasting glucose, type 2 diabetes, metabolic syndrome, insulin resistance, systemic inflammation and endothelium dysfunction [1]. On this basis, central obesity is responsible for an increase of morbidity and mortality [2], and this finding is of particular interest if it is taken into account that obesity prevalence has increased in Europe since the 1990ies, and that predictions by 2015 suggest a possible further increase in European populations [3].

Endothelium function can be directly determined by the measurement of nitric oxide release that exerts vasodilatator, anti-proliferative, anti-inflammatory and anti-thrombotic effects [4]. However, it can be indirectly evaluated by the Flow Mediated Dilation (FMD) of brachial artery.

Abnormal endothelial function, expressed as lower vasodilatation in response to an increase in blood flow, is considered an index of subclinical atherosclerosis, and an early hallmark of cardiovascular disease, with a strong prognostic value for future cardiovascular events. Interestingly, lower FMD values are associated with most of risk factors for coronary artery disease [5,6].

Although the tight adherence to pharmacological treatments (statin therapy, anti-hypertensive drugs use, antidiabetic drugs, etc) are fundamental in order to reduce the overall cardiovascular risk of individuals and their endothelial function, i.e. the early precursor of atherosclerotic disease, the daily diet is important in order to further ameliorate cardiovascular profile of individuals.

ClinMed
International Library

Citation: Ciccone MM, Scicchitano P, Cortese F, Gesualdo M, Fornarelli F (2014) Endothelial Function in Obese and Overweight Patients: The Role of Olive Oil, Fish and Nuts. Int J Diabetes Clin Res 1:004

Received: September 29, 2014: **Accepted:** October 18, 2014: **Published:** October 22, 2014

Changes in diet, level of physical activity and behavior are well known key elements to decrease the body weight. Concerning weight loss by diet, several strategies have been [7], but recent studies seem to show that Mediterranean diet pattern, characteristic of the populations bordering the Mediterranean sea, may be the best way in the long term to lose body weight or slow down age-related weight gain and simultaneously gain health status [8,9]. Several studies have established the beneficial role of its main components on cardiovascular risk and the occurrence of chronic degenerative diseases [10,11], and a recent review has even suggested that Mediterranean diet protects against the development of coronary heart disease not only because of its beneficial role on cardiovascular risk factors, but also through a possible effect on body weight and obesity [12].

At the best of our knowledge, the effect of a balanced diet and some foods such as olive oil, non fried fish or nuts on endothelial function in obese subjects has not been definitely established. Therefore, the aim of this study was to evaluate the impact of such dietary regimens on endothelium function, evaluated by FMD, in a group of obese and overweight subjects, for a short (3 months) or a longer (18 months) period.

Materials and Methods

Only overweight (Body Mass Index [BMI]: > 25.0 and < 30 kg/m^2) or obese (BMI: > 30.0 kg/m^2) patients, aged from 18 to 70 years, were enrolled in the study. They were outpatients, referred to the Section of Cardiovascular Diseases, Department of Emergency and Organ Transplantation, University of Bari, for periodic assessment of their own stable cardiovascular diseases, previously diagnosed: hypertension, chronic coronary heart disease, and hypertrophic and dilatative cardiomyopathies.

All subjects underwent a full examination by a physician in their first appointment, including evaluation of height, weight, Waist Circumference (WC) and blood pressure. Weight (Kg) and height (cm) were measured with subjects fasting and wearing only their undergarments, while WC (cm) was obtained at umbilicus level with subjects in the orthostatic position. Systolic and diastolic arterial blood pressures were measured by a mercury sphygmomanometer, with patients in the sitting position and in a quiet room after five minutes of rest.

Data about clinical history and drugs assumption were collected by a questionnaire, in order to design the basal characteristic of the study population. Exclusion criteria were: low left ventricular ejection fraction (LVEF < 50%), symptomatic cardiac disease in advanced stage or poorly controlled by medication, cerebral disorders, major liver and kidney diseases, cancer, excessive alcohol intake, and use of drugs addressed to lose weight. Therefore, 47 consecutive overweight and obese patients (24 men and 23 women, mean age 54 ± 11 years) were enrolled into the study. Cardiovascular risk factors were distributed as follows: 25 subjects (53% of total) were affected by arterial hypertension and were using antihypertensive drugs [13], 7 (15% of total) were affected by diabetes and were treated by oral antidiabetic medications [13], and 20 (42% of total) were dyslipidaemic and under statins therapy [13]. 11 patients (23% of total) were regularly smokers, since they had been smoking at least 5 cigarettes/day during the 3 months before the enrollment. All patients had WC in line with the diagnosis of abdominal obesity (WC > 88cm for women and > 102 cm for men) [13]. Moreover, all of them received full explanation of the design and the purpose of the study, and gave their written consent to participate to the study, that was approved by the Institutional Review Board of Bari University General Hospital and performed in accordance to the principles of the Helsinki Declaration.

Study Protocol

Diet: Each patient had a careful interview with a dietitian who provided a copy of a healthy diet which was defined according to the Nureta-PREDIMED study [14], with detailed explanations about how to adopt it, and the frequency and the approximate size of the meals. However, it is to note that subjects had not to weigh foods or to calculate kilocalories. Our suggested program included daily consumption of whole-wheat grains and derived products, 2 portions of dairy products with low-fat content, 2 salads (one of which should contain >1 tomato) and at least 3 fruits. Not more than 150 ml (1 glass) of red wine was permitted at each main meal. Patients were invited to eat at least 2 portions of not fried fish per week, whereas patients could have red meat not more than once per week. Olive oil was the only fat allowed (2-3 spoons per day). Whole grain pasta (spaghetti or rice) was proposed every day, but avoiding to eat other sources of carbohydrates such as bread and potatoes in the same occasion, whether pasta was eaten.

After 3 months of healthy diet, the study population was blindly assigned to 4 different harms: 24 patients continued to follow the healthy diet (control group); the remaining 23 were distributed among 3 groups: 8 subjects followed the healthy diet enriched with olive oil (5 spoons per day, group A), 8 individuals the healthy diet enriched with non-fried fish (at least 3 portion per week, group B), and 7 subjects the healthy diet enriched with nuts (15 nuts per week, group C).

Subjects were supervised by a dietitian, who weekly made a phone call to the patients, in order to evaluate the adherence to the proposed diet and to date them if adjustments in the diet were needed.

Apart from the dietary intervention, subjects were asked to maintain their lifestyle habits before the study and not to change their usual pharmacological treatment during the study.

Anthropometric parameters, metabolic variables and FMD were evaluated after 3 months and 18 months from the beginning of the study. However, all patients met the physician every 3 months.

Metabolic parameters: Metabolic parameters such as Total Cholesterol (TC), high density lipoprotein cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Triglycerides (TG), and fasting blood glucose were measured in all subjects before starting the protocol.

Plasma glucose levels were determined by the glucose-oxidase method (Sclavo, Siena, Italy). Plasma lipids (triglycerides, total cholesterol and HDL-cholesterol) were determined by an automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany).

Vascular ultrasound studies: The endothelial function of the enrolled patients was assessed by FMD of the brachial artery, the most common non - invasive system for this evaluation [15]. The study was performed by using an high resolution ultrasonograph (Philips Sonos 5500), connected to a system of image analysis certificated by the CNR of Pisa (MVE II), with a positivity value to the test chosen as less than 5% [15]. A long axis projection of the brachial artery at the level of the ante – cubital fossa was performed. The diameter of the brachial artery was measured at the onset of the R- wave on the electrocardiogram. All ultrasonographic assessments were performed by the same blinded physician. FMD was performed in a quiet and temperature controlled (22-24°C) room early in the morning. Electronic probe was positioned 4-5 cm above the elbow to obtain a longitudinal vascular scanning of the right brachial artery. When we obtained an optimal longitudinal B- mode scan of the brachial artery, the probe was maintained in right position by using a mechanic arm. The baseline diameter of the brachial artery was measured from the anterior to the posterior intima for 1 minute. After 1 minute of resting period in the supine position, a sphygmomanometer cuff was put near to the imaging transducer on the upper arm and then inflated to a pressure of 50 mmHg above systolic blood pressure (200-220 mmHg) for exactly 5 min. When the blood pressure cuff was rapidly deflated a reactive hyperemia in the brachial artery started and the diameter of the artery had been recording until for 3 minutes. In this condition the patient rested for 5 minutes and the reactive hyperemia was confirmed measuring the arterial blood flow using pulse-wave Doppler. The maximum flow velocity in the brachial artery was obtained with pulse–wave Doppler with the sample volume in the

ISSN: 2377-3634

Table 1: Study population characteristics at baseline and at 18 months.

N=47	Baseline	At 18 months	p
Diastolic blood pressure (mmHg)	78 ± 9	80 ± 7	ns
Systolic blood pressure (mmHg)	126 ± 12	127 ± 13	ns
HDL-C (mg/dL)	44 ± 9	52 ± 13	**<0.001**
LDL-C (mg/dL)	124 ± 35	105 ± 31	**<0.001**
TC (mg/dL)	200 ± 42	180 ± 32	**<0.001**
TG (mg/dL)	129 ± 50	119 ± 40	ns
Fasting glycaemia (mg/dL)	101 ± 19	98 ± 12	ns

Values expressed as mean ± standard deviation, numbers and percentages; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TC: Total cholesterol; TG: Triglycerides; p<0,05 statistically significant.

Table 2: TC, LDL-C and HDL-C values at baseline and at 18 months follow-up in group A (diet enriched with olive oil), B (enriched with non fried fish), C (enriched with nuts) and in control group (healthy Mediterranean diet).

Variable		Group A (n=8)	Group B (n=8)	Group C (n=7)	Control group (n=24)	p
TC	**At baseline** **At 18 months**	196 ± 46 165 ± 46	207 ± 39 186 ± 27	203 ± 39 180 ± 25	194 ± 44 179 ± 35	ns ns
	p	**0.04**	**0.04**	**0.04**	**<0.001**	
LDL-C	**At baseline** **At 18 months**	115 ± 28 88 ± 41	126 ± 31 99 ± 30	123 ± 23 101 ± 26	125 ± 41 110 ± 30	ns ns
	p	Ns	**0.02**	**0.02**	**0.02**	
HDL-C	**At baseline** **At 18 months**	47 ± 8 56 ± 12	51 ± 9 58 ± 15	42 ± 10 54 ± 10	42 ± 9 47 ± 12	ns ns
	p	**0.02**	**0.04**	**0.03**	**0.04**	

Values expressed as mean ± standard deviation, numbers and percentages; TC: Total Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; TG: Triglycerides; p<0,05 statistically significant.

Table 3: Anthropometric variables (BMI, WC and weight) and FMD values at baseline, and at 3 and 18 months of diet in the overall population.

N=47	Baseline	At 3 months	At 18 months	p
BMI (Kg/m²)	33.9 ± 5.1	33.3 ± 3.9*	31.6 ± 3.8**	**<0.001**
WC (cm)	111 ± 13	109 ± 10§	107 ± 12§§	**<0.001**
Weight (Kg)	93.4 ± 14.3	88.5 ± 17.2#	84.9 ± 11.9##	**<0.001**
FMD (%)	4.8 ± 2.4	5.9 ± 2.3 ^	6.4 ± 1.9 ^^	**<0.001**

Values expressed as mean ± standard deviation. BMI: Body Mass Index (kg/m²); WC: Waist Circumference; FMD: Flow-Mediated Dilatation. p<0.05 statistically significant

*p<0.01 vs. baseline; **p= 0.03 vs. 3 months

§ p<0.001 vs. baseline; §§ p< 0.01 vs. 3 months

# p<0.001 vs. baseline, ## p= 0.04 vs. 3 months

^ p<0.001 vs. baseline; ^^ p<0.01 vs. 3 months

centre of the artery and a correction angle at 70°, at rest, and during the first 15 seconds after the cuff deflation, taking the average of 3 measurements. The maximum velocities considered normal were 50–70 cm/sec. Reactive hyperemia was calculated as the ratio between the maximal velocity and the baseline [15]. The maximum value of recovery FMD was calculated as the ratio between the change in diameter (maximum – baseline) and the baseline value.

Statistical analysis

Values of continuous variables are expressed as mean ± standard deviation. Continuous variables were compared using Wilcoxon matched- pairs test or Mann-Whitney U-test for independent samples. Kruskal-Wallis ANOVA was used for multiple comparisons. A p < 0.05 was considered statistically significant.

Results

Table 1 shows the general characteristic of the study population at the moment of the enrollment and after 18 months follow-up.

At the start of the study all patients had blood pressure levels under control and this parameter did not change at the end of the study (Table 1).

Table 4: BMI, WC and Weight values at 3 and at 18 months of dietary treatment in groups A,B, C and in control group.

Variable		Group A (n=8)	Group B (n=8)	Group C (n=7)	Control group (n=24)	p
BMI (Kg/m²)	**At 3 months** **At 18 months**	36 ± 3.43 31.6 ± 4.1	33.1 ± 4.8 33.3 ± 4.2	30.3 ± 3.6 30.9 ± 3.9	32.9 ± 4.0 31.9 ± 3.6	ns ns
	p	0.05	ns	ns	ns	
WC (cm)	**At 3 months** **At 18 months**	111.3 ± 6.3 109 ± 17.6	106.4 ± 14.3 104.6 ± 9.7	103.4 ± 12.6 103.2 ± 11.3	109.4 ± 3.43 107.3 ± 4.1	ns ns
	p	ns	ns	ns	**<0.001**	
Weight (Kg)	**At 3 months** **At 18 months**	91.8 ± 14.3 88.4 ± 14.7	89.8 ± 16.3 84 ± 12.4	82.7 ± 11.4 82 ± 10.4	90.4 ± 12.9 85.7 ± 12.1	ns ns
	p	ns	**0.03**	ns	ns	

BMI: Body Mass Index (kg/m²); WC: Waist Circumference.

Table 5: FMD values at 3 and 18 months follow-up in groups A,B, C and in control group.

		Group A (n=8)	Group B (n=8)	Group C (n=7)	Control group (n=24)	p
FMD	**At 3 months**	5.4 ± 0.4	5.8 ± 2.2	5.8 ± 2.2	5.7 ± 2.4	ns
	At 18 months	7.1 ± 1.0*	7.3 ± 2.1**	7.6 ± 2.0***	6.3 ± 1.5	0.04
	p	0.02	0.04	0.02	0.04	

Values expressed as mean ± standard deviation; FMD: Flow Mediated Dilation; p< 0.05 statistically significant. *A vs Control p= 0.04; **B vs Control p= <0.01; ***C vs Control p=0.04.

After 18 months of dietary treatment, we observed a significant improvement of the lipid profile, with a significant increase of HDL-C, and a simultaneous decrease of TC and LDL-C. However, no differences in plasma concentrations of TG and fasting glucose were detected.

The addition *per se* of olive oil or non fried fish or nuts to the healthy diet did not influence the lipid profile, since TC, HDL-C and LDL-C levels of groups A, B and C were not significant different from those of the control group (Table 2).

Table 3 shows anthropometric parameters (BMI, WC and weight) and FMD data at the start of the study and after 3 and 18 months of diet. Anthropometric parameters showed a significant decrease and FMD showed a significant increase after 3 months of healthy diet as compared to baseline levels. A further significant improvement of all anthropometric parameters and FMD was present at 18 months, as compared to 3 months levels. The addition *per se* of olive oil or non fried fish or nuts to the healthy diet did not influence anthropometric parameters, since BMI, WC and body weight of groups A, B and C were not significant different from those of the control group (Table 4).

It is noteworthy to note that FMD increased only after 18 months of diet, rather than 3 months, i.e. after adding olive oil or non fried fish or nuts to the healthy diet (Table 5).

At 18 months, the FMD levels of groups A, B, and C were significantly higher than those of control group (Table 5).

Discussion

The present study, performed in overweight and obese patients, showed that three months of a healthy diet significantly improves endothelial function (evaluated by FMD) with a further significant improvement after 18 months. Moreover, this study showed that the addition of olive oil or fish or nuts induced an even more favorable effect on endothelial function. Olive oil, non fried fish and nuts are already known to induce beneficial effects on endothelial function [16,17]. Olive oil has several beneficial effects on endothelial function, due to oleic acid, phenolic compounds and antioxidant vitamins [18]. In addition, phenolic compounds are strong antioxidants and radical scavengers, increasing bioavailability of nitric oxide [19]. Fish has favorable effects on health, since it is the primary source of dietary omega-3 poly-unsaturated fatty acids (particularly eicosapentaenoic and docosahexaenoic acids), with demonstrated protective effects against coronary artery disease and heart failure [20]. Although randomized trials have demonstrated that short term intake of fish oil was able to improve endothelial function evaluated by brachial artery FMD, few studies investigated the long-term effects of fish consumption on endothelial function [21,22]. Some epidemiological studies have suggested the important role of nut intake in decreasing the risk to develop cardiovascular diseases [23,24]. In fact, nuts have important effects on the inflammatory process related to atherosclerosis [25], and are now included in the dietary guidelines of the American Heart Association for cardiovascular health [26]. The healthy effects of nuts are possibly due to their unique composition: high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, phenolic compounds, and the interaction among these bioactive elements [27].

An interesting overview about the beneficial effects of nuts on cardiovascular system is offered by Casas-Agustench et al. [28]. The authors pointed out the positive effects of nuts on endothelial function and, therefore on hypertension. They considered the possible role of the main molecular components of nuts such as electrolytes quantities (magnesium, potassium and calcium), antioxidants and low sodium concentrations in amelioration of blood pressure control and endothelial function.

Three months healthy diet significantly decreased body weight, BMI and waist circumference, and a further decrease of body weight, WC and BMI was obtained after 18 months of follow-up. In our opinion, the effect on anthropometric parameters is of particular interest since weight loss was not one of the aim of our study, and subjects had not to weight foods or count calories, and were educated just to prefer healthy food and reduce or abolish unhealthy food [14].

We have to emphasize the role of the dietitians, who called or met the patients every week to verify the correct adherence to the diet. This aspect has not to be underestimated, since previous studies clearly showed that a close supervision by a dietitian improves weight-loss success and may reduce the Framingham risk [29].

It is well known that weight loss improves endothelial function in obesity, but it is not known whether this result is related to weight loss *per se* or to the effect of some component of the diet responsible for the decrease of body weight [30]. In this study, at variance with the effect on endothelium, the adding of olive oil or fish or nuts to a healthy diet did not induce more favorable effects on anthropometric parameters. The adopted healthy diet induced significant favorable effects on lipid profile. In fact, after 18 months, HDL-C was significantly increased, whereas TC and LDL-cholesterol were significantly decreased. Olive oil or fish or nuts adding did not induce more favorable effects on the lipid profile. This behavior is in contrast with some literature data about the favorable role of olive oil and nuts on lipids levels [31,32]. Nevertheless, we believe that the small sample size can account for this discrepancy. Further trials are needed in order to confirm or not such results [33].

The plasma concentrations of triglycerides and fasting glucose were not affected by the healthy diet. In particular, we showed no significant effects (positive or negative) on fasting blood glucose, either in patients with or without diabetes.

In the end, our study has limitations. The most important one is the small sample size which reduces the strength of the results. We are planning to develop a more complex protocol able to involve a greater number of patients. This will overcome another limitation of our study: the wide range of ages of the patients which ranges from 18 to 70 years old. The inclusion of a great number of patients will allow us to make the groups more homogenous according to age and reduce the influence of such a confounding factor. Furthermore, it was not possible to evaluate the approximate weight (in grams) of nuts ingested by the patients and, therefore, we could not fully compare our data with literature one. The same is for phenolics content of the specific olive oil studied and the nutritional components of the nuts.

In conclusion, this study showed that a healthy diet induces after 18 months a significant improvement of endothelial function, a decrease of BMI, waist circumference, TC and LDL-C and an increase of HDL-C. Olive oil, not fried fish and nuts seem to be of particular interest for the health of endothelium and the amelioration of cardiovascular risk profile of obese/overweight individuals.

References

1. Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, et al. (2008) The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med 168: 1617-1624.
2. Hotchkiss JW, Leyland AH (2011) The relationship between body size and mortality in the linked Scottish Health Surveys: cross-sectional surveys with follow-up. Int J Obes (Lond) 35: 838-851.
3. von Ruesten A, Steffen A, Floegel A, van der A DL, Masala G, et al. (2011) Trend in obesity prevalence in European adult cohort populations during follow-up since 1996 and their predictions to 2015. PLoS One 6: e27455.
4. Félétou M, Vanhoutte PM (2006) Endothelium-derived hyperpolarizing factor: where are we now? Arterioscler Thromb Vasc Biol 26: 1215-1225.
5. Jambrik Z, Venneri L, Varga A, Rigo F, Borges A, et al. (2004) Peripheral vascular endothelial function testing for the diagnosis of coronary artery disease. Am Heart J 148: 684-689.
6. Yoshida T, Kawano H, Miyamoto S, Motoyama T, Fukushima H, et al. (2006) Prognostic value of flow-mediated dilation of the brachial artery in patients with cardiovascular disease. Intern Med 45: 575-579.
7. Serdula MK, Mokdad AH, Williamson DF, Galuska DA, Mendlein JM, et al. (1999) Prevalence of attempting weight loss and strategies for controlling weight. JAMA 282: 1353-1358.
8. Issa C, Darmon N, Salameh P, Maillot M, Batal M, et al. (2011) A Mediterranean diet pattern with low consumption of liquid sweets and refined cereals is negatively associated with adiposity in adults from rural Lebanon. Int J Obes (Lond) 35: 251-258.
9. Romaguera D1, Norat T, Vergnaud AC, Mouw T, May AM, et al. (2010) Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. Am J Clin Nutr 92: 912-921.
10. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, et al. (1999) Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon diet heart study. Circulation 99: 779-785.
11. Robertson RM, Smaha L (2001) Can a Mediterranean-style diet reduce heart disease? Circulation 103: 1821-1822.
12. Kastorini CM, Milionis HJ, Goudevenos JA, Panagiotakos DB (2010) Mediterranean diet and coronary heart disease: is obesity a link? - A systematic review. Nutr Metab Cardiovasc Dis 20: 536-551.
13. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, et al. (2012) European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 33: 1635-1701.
14. Babio N, Bulló M, Basora J, Martínez-González MA, Fernández-Ballart J, et al. (2009) Adherence to the Mediterranean diet and risk of metabolic syndrome and its components. Nutr Metab Cardiovasc Dis 19: 563-570.
15. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, et al. (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 39: 257-265.
16. Perona JS, Cabello-Moruno R, Ruiz-Gutierrez V (2006) The role of virgin olive oil components in the modulation of endothelial function. J Nutr Biochem 17: 429-445.
17. Whelan AP, Sutherland WH, McCormick MP, Yeoman DJ, de Jong SA, et al. (2004) Effects of white and red wine on endothelial function in subjects with coronary artery disease. Intern Med J 34: 224-228.
18. Massaro M, Basta G, Lazzerini G, Carluccio MA, Bosetti F, et al. (2002) Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. Thromb Haemost 88: 335-344.
19. Tuck KL, Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem 13: 636-644.
20. Levitan EB, Wolk A, Mittleman MA (2009) Fish consumption, marine omega-3 fatty acids, and incidence of heart failure: a population-based prospective

study of middle-aged and elderly men. Eur Heart J 30: 1495-1500.

21. Anderson JS, Nettleton JA, Herrington DM, Johnson WC, Tsai MY, et al. (2010) Relation of omega-3 fatty acid and dietary fish intake with brachial artery flow-mediated vasodilation in the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr 92: 1204-1213.
22. Shah AP, Ichiuji AM, Han JK, Traina M, El-Bialy A, et al. (2007) Cardiovascular and endothelial effects of fish oil supplementation in healthy volunteers. J Cardiovasc Pharmacol Ther 12: 213-219.
23. Kelly JH Jr, Sabaté J (2006) Nuts and coronary heart disease: an epidemiological perspective. Br J Nutr 96 Suppl 2: S61-67.
24. Sabaté J, Ang Y (2009) Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr 89: 1643S-1648S.
25. Urpi-Sarda M, Casas R, Chiva-Blanch G, Romero-Mamani ES, Valderas-Martínez P, et al. (2012) Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomakers related to atherosclerosis. Pharmacol Res 65: 577-583.
26. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, et al. (2010) Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation 121: 586-613.
27. Ros E (2010) Health benefits of nut consumption. Nutrients 2: 652-682.
28. Casas-Agustench P, López-Uriarte P, Ros E, Bulló M, Salas-Salvadó J (2011) Nuts, hypertension and endothelial function. Nutr Metab Cardiovasc Dis 21 Suppl 1: S21-33.
29. Raatz SK, Wimmer JK, Kwong CA, Sibley SD (2008) Intensive diet instruction by registered dietitians improves weight-loss success. J Am Diet Assoc 108: 110-113.
30. Hamdy O (2005) Lifestyle modification and endothelial function in obese subjects. Expert Rev Cardiovasc Ther 3: 231-241.
31. Pérez-Jiménez F, Ruano J, Perez-Martinez P, Lopez-Segura F, Lopez-Miranda J (2007) The influence of olive oil on human health: not a question of fat alone. Mol Nutr Food Res 51: 1199-1208.
32. Ros E, Núñez I, Pérez-Heras A, Serra M, Gilabert R, et al. (2004) A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation 109: 1609-1614.
33. Mendez MA, Covas MI, Marrugat J, Vila J, Schröder H (2009) Glycemic load, glycemic index, and body mass index in Spanish adults. Am J Clin Nutr 89: 316-322.