



## Applied nutritional investigation

## Study of the effects of a diet supplemented with active components on lipid and glycemc profiles

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## ABSTRACT

**Objectives:** Currently, there are numerous studies on risk factors for cardiovascular disease and the potential for functional foods to bring benefits or improve people's health. However, most of these studies are conducted with middle-aged individuals. The aim of this study was to evaluate the effects of supplementing a typical diet with some functional components, which are substances that when consumed in small quantities can improve people's welfare.

**Methods:** The participants in this study were young; slightly overweight; had normal glucose tolerance; and had lipid values indicating dyslipidemia or close dyslipidemia. Following a 4-wk run-in phase, participants followed either a diet containing foods enriched with  $\omega$ -3 fatty acids,  $\beta$ -glucans, phytosterols, and vitamin E or an isoenergetic diet without the active components. Sixteen individuals (age range 20 to 37 y) were randomly assigned to one of two groups. At the end of treatment, while fasting, plasma concentrations of triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol were measured. Furthermore, blood glucose was evaluated after fasting and after a meal enriched with  $\beta$ -glucans.

**Results:** There was a statistically significant reduction ( $\alpha < 0.05$ ) across the lipid profile. A meal rich in  $\beta$ -glucans produced a glycemc response significantly lower than the nonenriched meal.

**Conclusion:** The dietary supplements used in this work, based on the integration of functional components into the usual diet of the population, have proved effective in reducing peak levels of postprandial glucose and the risk for dyslipidemia. Therefore, these functional components proved a valuable aid in the prevention of cardiovascular diseases and metabolic disorders.

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## Introduction

Dyslipidemia is one of the most important risk factors for the development of atherosclerotic vascular and cardiovascular diseases (CVD) and remains, despite numerous prevention campaigns, the leading cause of death and disability in developed countries [1]. Numerous studies have shown that in combination with a healthy lifestyle, functional foods can furnish important contributions to health [2]. During the past few years, there has been an increase in both scientific and public interest in the role of  $\omega$ -3 fatty acids found in fish and fish oils in the prevention and management of CVD. The  $\omega$ -3 fatty acids that are of particular

interest for cardiovascular care are eicosapentaenoic acid and docosahexaenoic acid, which are found predominantly in fish and fish oils [3,4].

Observational studies and clinical trials have shown that high consumption of dietary fiber is associated with important benefits for the lipid profile, in particular, benefits for total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and, consequently, the reduction of cardiovascular risk [5]. This effect appears to be greater for soluble fiber or gel-forming fiber (pectins, gums,  $\beta$ -glucans, mucilages, and the remaining hemicellulose), the main dietary sources of which are cereals, such as oats and barley (and the products derived from them) and legumes. The intake of 5 to 10 g/d of soluble fiber, such as  $\beta$ -glucans, glucomannan, guar, or psyllium, induces a  $\sim$ 5% reduction in LDL-C [6]. Moreover, the  $\beta$ -glucans have a positive effect on blood glucose, as demonstrated by clinical studies that have shown significant reductions in postprandial glycemc peak [7,8].

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It is now known that phytosterols reduce serum cholesterol by modulating the absorption of dietary cholesterol and cholesterol of biliary origin in the intestine and by competing for some of the selective mechanisms of absorption of the same cholesterol [9,10]. Accordingly, fiber may be considered a functional component that is safe and effective at reducing plasma cholesterol (in particular LDL-C) and risk for CVD [11,12].

Vitamin E is the family of substances comprising two classes of compounds: tocopherols and tocotrienols. The importance of this vitamin is linked to the fact that its deficiency exposes the body to risk for major diseases. Numerous studies have attempted to investigate vitamin E's prophylactic and therapeutic mechanisms and to confirm the increasing interest in this family of substances. These compounds have shown, in human studies, the ability to improve clinical atherosclerosis and prevent the atherogenic process and act as a therapeutic tool suitable for the prevention of CVD and myocardial infarction [13].

In the effective management of hyperlipidemia, there are a number of dietary approaches that can achieve good results. These include reducing the amount of saturated fat in the diet, increasing the intake of  $\omega$ -3 polyunsaturated fatty acids, and increasing the intake of dietary fiber. It is important that the introduction of novel dietary strategies does not adversely affect the overall dietary profile [14,15]. Dietary modifications that can be carried out to reduce cardiovascular risk typically recommend increasing the consumption of fruits and vegetables in a balanced diet as well as regular exercise. It is not advisable to tell the general population that consuming foods containing functional components will be the solution to all problems. However, teaching people the characteristics of a balanced, varied, and sufficient diet should be a public health priority. Within this context, foods containing functional components, such as  $\omega$ -3 fatty acids,  $\beta$ -glucans, phytosterols, and vitamin E, offer an interesting weapon in the fight against CVD. Therefore, the objective of this study was to evaluate the effects of supplementing the typical diet of individuals between the ages of 20 and 37 y, with some functional components, such as  $\omega$ -3 fatty acids,  $\beta$ -glucans, phytosterols, and vitamin E, on the plasma concentration of triglycerides (TGs) high-density lipoprotein cholesterol (HDL-C), LDL-C, and TC after fasting. Additionally, glycemia was assessed after fasting and after a meal of foods enriched in  $\beta$ -glucans and then compared with a nonenriched meal.

## Materials and methods

### Study design

We used a randomized, crossover design. The study lasted 8 wk during which time, each participant acted as his or her own control. The randomization increased the likelihood that other variables not considered in the study design were distributed uniformly in the experimental group and the control group. In this way, the possible differences observed between the two groups can be attributed to the treatment. The characteristics required of participants in the study were age between 20 and 40 y, slightly overweight (body mass index [BMI] min 25 kg/m<sup>2</sup>, max 29.9 kg/m<sup>2</sup>), normal glucose tolerance (<60 mmol/L, <100 mg/dL), slight dyslipidemia (cholesterol 200–239 mg/dL, HDL 40–59 mg/dL, LDL 130–159 mg/dL, TGs 70–170 mg/dL), and absence of diseases, such as CVD and diabetes. During treatment, all participants followed their usual diet with the appropriate supplements required by the study. They also were required to fill out a food diary for the duration of the study period. Moreover, for a better evaluation of the results during recruitment, participants with similar eating habits were selected (data not shown, but this was verified by consulting the food diary completed by the participants). Initially, 96 individuals were identified as potentially suitable (selected by age, body weight, and absence of disease) to participate in the study. Participants were then asked to complete a lifestyle questionnaire before being screened for fasting TG, HDL-C, LDL-C, and TC to determine their eligibility. After screening, only 28 individuals were found to be

suitable after baseline blood tests. Another 12 left the study for a variety of reasons. At the end of recruitment, the study included 16 overweight volunteers who were ages 20 to 37 y old with a BMI  $\geq$ 25 kg/m<sup>2</sup> [16], normal glucose tolerance, slight dyslipidemia, and no history of diabetes, hypertension, or CVD. After being informed about the design of the study, the different tests, and the obligation to complete the food diary, all volunteers signed the informed consent form.

Following a 4-wk run-in phase, eligible participants were randomly assigned to one of the two groups. Group 1 (case group) was given a diet containing foods enriched with  $\omega$ -3 fatty acids, phytosterols, and vitamin E. Group 2 (control group) received a similar diet (isoenergetic) without these active components. Participants followed the assigned intervention for 4 wk, and after a 2-wk washout period, they were crossed over to the other intervention group (Fig. 1).

At the end of 8-wk in each intervention and the 2-wk washout period, glycemia was assessed after fasting and after a meal of foods enriched in  $\beta$ -glucans (case) and compared with glycemia after fasting and after an isoenergetic meal without fiber (control). A validated questionnaire was used to check food diaries [17]. Participants were interviewed and their food diaries were assessed to monitor compliance with the study's protocol. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Federico II University Ethics Committee. The baseline characteristics of the study participants are provided in Table 1.

### Participants

The weight and height of participants were measured by means of an anthropometric balance (a stand measuring height in cm from 80 to 210, with divisions of 0.5 cm, and measuring weight up to 200 kg, with divisions of 100 g). The colorimetric method (Trinder end point method) was used for the determination of plasma TC and TG. A direct enzymatic method was used for assays of HDL-C and LDL. Capillary blood glucose tests were performed with finger samples using a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Participants were given a food diary and asked to record any omission of food and the reason for omitting the food. The functional components were consumed daily for a total quantity of 3915 mg of  $\omega$ -3 fatty acids [3,4], 130.7 mg vitamin E [13], 1.6 g of phytosterols [9], and 3.74 g of  $\beta$ -glucans [7]. The functional components were provided through milk and margarine enriched with  $\omega$ -3, fish oil, pasta, biscuits, and crackers enriched with  $\beta$ -glucans and yogurt enriched with phytosterols (Table 2).

All of the demographic characteristics of the participants were collected at the beginning of the study, such as age and height and weight, from which BMI was calculated. Moreover, TG, LDL-C, HDL-C, TC, and blood glucose were measured after fasting. Thereafter, to evaluate the effects of the administration of  $\beta$ -glucans on the glycemic response, blood glucose was evaluated after fasting and after a meal enriched in  $\beta$ -glucans. In detail, the individuals in group 1 first consumed the diet with functional compounds (case), and group 2 participants followed their usual diet without any supplementation (control). The treatment was carried out for 4 wk, after which blood parameters were measured (TG and cholesterol). After 6 wk (4wk + 2 wk washout), the treatment was reversed. That is, for another 4 wk, group 2 participants consumed the diet with functional compounds (case), and those in group 1 followed their usual diet without any supplementation (control). At the end of this second period, blood parameters were determined again. After a 2-wk washout period, the same groups were used to assess the benefits of the  $\beta$ -glucans on blood glucose. The participants in the case group had their blood glucose measured at fasting and 40, 80, and 120 min after consuming a meal consisting of a portion of 80 g of spaghetti enriched with  $\beta$ -glucans and 200 mL of a beverage enriched with  $\beta$ -glucans. Similarly, those in the control group 2 consumed the same amount of spaghetti and drink (isoenergetic meal) but without  $\beta$ -glucan supplementation.

### Statistical analysis

Data are expressed as the mean  $\pm$  SD. The differences between the dietary treatments were evaluated by Student's *t* test as a one-tailed paired sample (only for the HDL-C was the alternative hypothesis of opposite sign compared with the other variables). A significance level of  $P < 0.05$  was used. Statistical analysis was performed using the data analysis add-on component of Microsoft Excel software.

## Results

Figure 2 shows the descriptive statistics (in mean  $\pm$  SD) of the groups, both before and after treatment. The results obtained show a significant reduction of TG, LDL-C, and TC and a significant increase of HDL-C (Fig. 2A, group 1: participants treated for 4 wk with foods enriched with functional

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