The innovative “Bio-Oil Spread” prevents metabolic disorders and mediates preconditioning-like cardioprotection in rats

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Abstract Background and aims: Obesity is often associated with an increased cardiovascular risk. The food industry and the associated research activities focus on formulating products that are a perfect mix between an adequate fat content and health. We evaluated whether a diet enriched with Bio-Oil Spread (SD), an olive oil-based innovative food, is cardioprotective in the presence of high-fat diet (HFD)-dependent obesity.Methods and Results: Rats were fed for 16 weeks with normolipidic diet (ND; fat: 6.2%), HFD (fat: 42%), and ND enriched with SD (6.2% of fat þ 35.8% of SD). Metabolic and anthropometric parameters were measured. Heart and liver structures were analyzed by histochemical examination. Ischemic susceptibility was evaluated on isolated and Langendorff-perfused cardiac preparations. Signaling was assessed by Western blotting. Compared to ND rats, HFD rats showed increased body weight and abdominal obesity, dyslipidemia, and impaired glucose tolerance. Morphological analyses showed that HFD is associated with heart and liver modifications (hypertrophy and steatosis, respectively), lesser evident in the SD group, together with metabolic and anthropometric alterations. In particular, IGF-1R immunodetection revealed a reduction of hypertrophy in SD heart sections. Notably, SD diet significantly reduced myocardial susceptibility against ischemia/reperfusion (I/R) with respect to HFD through the activation of survival signals (Akt, ERK1/2, and Bcl2). Systolic and diastolic performance was preserved in the SD group. Conclusions: We suggest that SD may contribute to the prevention of metabolic disorders and cardiovascular alterations typical of severe obesity induced by an HFD, including the increased ischemic susceptibility of the myocardium. Our results pave the way to evaluate the introduction of SD in human alimentary guidelines as a strategy to reduce saturated fat intake.

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Introduction

Men and women of all ages, races, and ethnic groups can be affected by obesity [1] and this is often accompanied by an increased morbidity and mortality due an increase of cardiovascular disease (CVD) risk factors [2,3]. Severe obesity causes permanent hemodynamic alterations that progressively impair cardiac structure and function. When comorbidities (i.e., systemic hypertension, sleep apnea, and hypoventilation) are present together with obesity, cardiac alterations progress to heart failure (HF) [4]. Metabolic syndrome (MetS) is a condition in which a high CVD risk is associated with obesity. It is characterized by disturbed glucose homeostasis, dyslipidemia, and hypertension [5] and is established in the presence of three of the following parameters: (i) waist circumference >40 (men) and >35 (women) inches; (ii) triglycerides >150 mg/dl; (iii) HDL (high-density lipoprotein) cholesterol <40 (men) and <50 (women) mg/dl; (iv) blood pressure >130/85 mm Hg; and (v) fasting glucose >100 mg/dl [6]. Currently, nutritional therapy is regarded as the best practice to prevent and/or reduce the cardiovascular damages associated with MetS [7].

According to the "lipid hypothesis," [8] a high intake of saturated fatty acids (SFAs) severely affects cardiovascular health. On the contrary, dietary polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) reduce cardiovascular morbidity and mortality in the presence of MetS [9]. In this regard, it is known that olive oil, largely present in Mediterranean diet, improves postprandial lipoprotein metabolism [10], reduces blood pressure in both normal and hypertensive subjects, and improves carbohydrate metabolism in healthy and type 2 diabetic subjects [11].

Therefore, food industry and the associated research activities focus on the formulation of alimentary products that perfectly combine adequate lipid contents and cardiovascular health. Bio-Oil Spread is an innovative food mainly based on olive oil. The food industry can use this hard fat phase as margarine, a shortening, or butter replacer for baker production, and/or as a ready-to-eat fat spread. Rheologically, it is a structured water-in-oil emulsion. The increased consistency of olive oil is not achieved by common techniques such as catalytic hydrogenation, transesterification, or chemical recombination of the starting oil, but is obtained by a novel technique, named organogelation [12]. Organo gels are gels based on an organic solvent (a vegetable oil) which reaches a solid-like consistency due to the self-assembly of "low molecular weight organogelator molecules" into a three-dimensional structure. This structure in turn entraps the solvent that, in the case of Bio-Oil Spread, is represented by the water droplets of the emulsion [13]. Therefore, the structured oil phase, that is, the organogel, produces a very consistent fat that, if necessary, can be emulsified for producing a spreadable material. This occurs without chemical changes in the inner structure of triglycerides, but by physical interactions between organogelator molecules; these molecules begin to crystallize at high temperatures and create a network at room temperature Based on these premises, we aimed to investigate whether and to which extent, compared with a standardized high-fat diet (HFD) used to induce MetS, a diet enriched with Bio-Oil Spread influences the rat metabolic and "anthropometric" parameters, associates with cardiac and hepatic structural changes, and influences myocardial ischemic susceptibility induced by ischemia/reperfusion (I/R) and post-conditioning (post-C) maneuvers.

We found that, with respect to rats fed with HFD, those fed with Bio-Oil Spread show a limited lipid accumulation, a reduction of body weight and abdominal circumference, and a lower ischemic susceptibility, revealed by a smaller infarct area and by a better postischemic recovery associated with pro-survival molecular cascade activation. Our results are of interest as they may open new perspectives in the use of innovative alimentary products able to contribute to prevent and/or treat HFD-dependent metabolic disorders and cardiovascular dysfunctions.

Methods

Animals

The study was performed on Sprague Dawley rats. The initial weights of rats fed with normolipidic diet (ND), HFD, and SD (Bio-Oil Spread diet) are, respectively, 357 _ 7, 359 _ 6, and 358 _ 5 g. Animals were housed one per cage, kept under controlled light and temperature conditions, and fed by free access to a normal chow diet and water. The scientific project was supervised and approved by the local ethical committee, and the experimental protocols were carried out according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (publication No. 85e23, revised 1996) and to the Italian law (DL 116/92 and DL 26/14).

Drugs and chemicals

Bio-Oil Spread

Bio-Oil Spread is a water-in-oil emulsion based on an oil phase mainly comprising extra-virgin olive oil (Gabro, Italy) for more than 60%w/w, cocoa butter (Icam, Italy), and Myverol (50% of glyceryl monostearate and 50% glyceryl monopalmitate, Kerry Group, Ireland). Distilled water is the aqueous phase of the final emulsion. Oil phase is produced by heating olive oil and mixing cocoa butter and monoglycerides; finally, emulsification is carried out at low temperatures with water. Further details can be found in the patent WO 2013111058 A1 by De Cindio et al. [14].

HFD, ND, and SD formula

Teklad diet TD.88137 purchased by Harlan (Udine, Italy) is a hypercaloric diet used as HFD (42% kcal fat, 15.2% kcal protein, and 42.7% kcal carbohydrate). On the contrary, Diet 2018 (Harlan, Italy; 6.2% kcal fat, 18.6% kcal protein, and 44.2% kcal carbohydrate) was used as ND. ND was enriched with SD, an olive oil emulsion, to obtain a fat percentage similar to that of HFD (35.8% kcal SD); SD was stored at 4 _C to avoid auto-oxidation of the fatty components. The anthropometric analysis of each rat, subjected to the diet, was checked weekly to evaluate the development of obesity [15,16].

Experimental protocols

Rats were divided into five groups (n Z 20 for each group) and fed as follows:

_ Group ND (fed with ND; n Z 20);
_ Group HFD (fed with HFD; n Z 20);
_ Group SD (fed with ND enriched with SD; n Z 20);
_ Group NDM1 (fed with ND enriched with 30 g/kg Myverol; n Z 5);
_ Group NDM2 (fed with ND enriched with 60 g/kg Myverol; n Z 5);

All the experimental groups were subjected to the diet for 16 weeks. At the end of treatment, all animals were subjected to the measurement of anthropometric parameters and plasma analysis. From the ND, HFD, and SD groups, 10 animals were assigned to I/R protocols, while 10 were randomly used for several immunohistochemistry and Western Blot analyses. NDM1 and NDM2 animals were assigned only to I/R protocols.

Anthropometric variables

Body weight, abdominal circumference, and length of each rat were measured weekly. The total amount of food and water consumed was recorded every day. The rat length was evaluated by measuring the noseeanus distance [17]. Body mass index (BMI) was calculated as reported by Panchal SK et al. [18] After the sacrifice, the abdominal fat tissue was removed and separately weighed.

Plasma analysis

Blood samples were collected both weekly and before the sacrifice, through the tail vein; the amount of blood was <1% of the body weight (e.g., 1 mL from a 250-g adult rat). Blood plasma was separated by centrifugation for 15 min at 1000 _ g at 2e8 _C within 30 min of collection. A plasma aliquot was refrigerated at 4 _C for glucose analysis, while the remaining plasma was frozen (_80 _C) for the subsequent biochemical analyses. The glucose levels were determined using a Glucometer (ACCUCHEK, Roche Diagnostics, Germany). Total cholesterol, HDL cholesterol, LDL cholesterol, and insulin levels were evaluated by commercial assay kits (enzyme-linked immunoassay, ELISA). The kit used for the determination of total cholesterol, HDL cholesterol, and LDL cholesterol levels is PKL_ POKLER ITALY S.r.l. The kit used for the determination of insulin levels is Millipore (St. Charles, MO, USA).

Calculation of insulin sensitivity index (HOMA-IR)

The measurement of basal insulin sensitivity, an index of insulin resistance (HOMA-IR; homeostatic model assessment-estimated insulin resistance), was performed as reported by Matthews et al. [19] Assuming that the control young adult rats have an average HOMA-IR of 1, analogous to the assumptions applied in the development of HOMA-IR in humans, this index was calculated as the product of the fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels, divided by a constant [20]. The equation is HOMA-IR Z (FPG(mUI/ml) _ FPI(mg/dl))/2.430.

Cardiovascular structure and function After 16 weeks of diet, the rats were anesthetized by ethyl carbamate (2 g/kg body weight, intraperitoneal) and were sacrificed, and the heart was assigned to the specific protocol.

Light microscopy

After sacrifice, hearts (n Z 4 for each group), removed from the pericardial cavity and flushed with phosphate buffered saline (PBS; pH: 7.6), were blocked in diastole with an excess of KCl (0.5 g/L). The liver was collected from the same animal (n Z 3 for each group) and dissected into several sections. Both heart and liver were treated as follows: tissues were fixed in MAW fixative (methanoleacetoneewater, 2:2:1), dehydrated in graded ethanol, embedded in paraplast (Sherwood, St. Louis, MO, USA), and serially sectioned at 8 mm. Sections were placed onto Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany). The sections were stained either with hematoxylin and eosin for a general assessment of tissue structure, or with Sirius red for collagen fiber detection.

Immunohistochemistry

In parallel with the evaluation of the localization pattern of the IGF-1R (insulin-like growth factor-1 receptor), deparaffinized and rehydrated heart sections were processed by immunohistochemical stain using an HRP/DAB (3, 3-diaminobenzidine) detection kit (Abcam, Cambridge, MA, USA). Briefly, the sections in PBS were pretreated with H_2O_2 to remove endogenous peroxidase activity, and then incubated for 1 h with Protein Block and overnight with mouse polyclonal IGF receptor antibody (1:100) at 4 _C. The slides were than washed in PBS and incubated with biotinylated goat anti-mouse IgG (immunoglobulin G) and finally with streptavidineperoxidase complex. The signal was visualized using diaminobenzidine (DAB) as the final chromogen. The sections were observed using a ZEISS AXIOSKOP microscope and the images were digitalized by Axiocam 105 color, ZEISS.

Cardiac function

Cardiac function was evaluated with the Langendorff method. After sacrifice, the heart was rapidly excised, placed in ice-cold perfusion buffer, cannulated through the aorta, and perfused at constant retrograde flow (12 ml/ min) and constant temperature (37 _C). The perfusion medium used was a modified KrebseHenseleit solution (KHs; pH 7.4) gassed with 95% O_2 and 5% CO_2 containing (in mmol/l) 113.0 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.8 $CaCl_2$, 11.0 glucose, 1.1 mannitol, and Naepyruvate 5 [21]. The left ventricular pressure (LVP) was measured through a latex water-filled balloon inserted into the left ventricle through the mitral valve, connected to a pressure transducer (BLPR gauge, WRI, Inc. USA), and set to obtain a left ventricular end-diastolic pressure (LVEDP) of 5e8 mm Hg [21].

Ischemia/reperfusion (I/R) and ischemic postconditioning (Post-C) After 40 min of stabilization, the baseline parameters were recorded and each heart was randomly assigned to one of the experimental groups described below.

1) ND I/R group (n Z 10): after 30 min of global noflow ischemia, hearts were subjected to 120 min of reperfusion;

2) HFD I/R group (n Z 10): after 30 min of global noflow ischemia, n Z 5 hearts were subjected to 120 min of reperfusion, while n Z 5 hearts were subjected to five cycles of 10 s of reperfusion and 10 s of ischemia and then to 120 min of reperfusion [22];

3) SD I/R group (n Z 10): after 30 min of global no-flow ischemia, n Z 5 hearts were subjected to 120 min of reperfusion, while n Z 5 hearts were subjected to five cycles of 10 s of reperfusion and 10 s of ischemia and then to 120 min of reperfusion [22];

4) Myverol (30 mg/kg) group (n Z 5): hearts were stabilized and subjected to 120 min of perfusion;

5) Myverol (60 mg/kg) group (n Z 5): hearts were stabilized and subjected to 120 min of perfusion.

Cardiac performance before and after ischemia was evaluated by analyzing LVP, as an index of the recovery in contractile activity, and LVEDP as an index of contracture (i.e., myocardial tissue damage), defined as an increase in LVEDP of 4 mm Hg above the baseline level [23].

Assessment of myocardial injury

In order to assess infarct area extension, after each experimental protocol, hearts were rapidly removed from the perfusion apparatus and the left ventricles were dissected into 2- to 3-mm circumferential sections. After incubation at 37 _C for 20 min in a phosphate buffer plus

0.1% of nitro blue tetrazolium, unstained necrotic tissue was carefully separated from the stained tissue and weighed. The infarct size was expressed as a percentage of the total left ventricular mass, including septum [23].

Western blotting

Hearts (n Z 3 for each group) were immediately frozen in liquid nitrogen at the end of I/R protocols and stored at _80 _C until their homogenization in RIPA (radioimmunoprecipitation assay) buffer. Equal amounts of total protein (40e100 mg), for each sample, were loaded on an 8e12% sodium dodecyl sulfate (SDS)epolyacrylamide gel and then transferred to a PVDF (polyvinylidene difluoride) membrane. Blots were blocked for 1 h by a 5% nonfat milk solution and then incubated overnight at 4 _C with primary antibodies (diluted 1:1000) recognizing pAKT, p-ERK1/2, Bcl2, and GAPDH (glyceraldehyde 3-phosphate dehydrogenase; all antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies rabbit anti-mouse (1:10.000) or goat anti-rabbit (1:10.000; Stressgen, Victoria, BC, Canada). GAPDH was used as the loading control. The level of proteins and phosphoproteins was detected using an ECL_ (Enhanced chemiluminescence) System (GE Healthcare, Milan, Italy). Autoradiographs were obtained by exposure to X-ray films, immunoblots were digitalized, and the densitometric analysis of the bands was carried out using NIH IMAGE 1.6 for a Macintosh computer based on 256 gray values (0 Z white; 256 Z black).

Lactate dehydrogenase

Lactate dehydrogenase (LDH) release, which is known to be reduced by post-C [22], was evaluated in coronary effluent in each experimental group. Perfusate samples were collected during the reperfusion through a catheter inserted into the right ventricle via the pulmonary artery. LDH concentration was measured by kit for rat LDH ELISA assay (E02L0006 Blue Gene). The data were expressed as cumulative values for the entire reperfusion period.

Statistics

Statistical analysis was performed by using the GraphPad Prism Software_ (version 5.0; Graph Pad Software, San Diego, CA, USA). Data were expressed as the mean _ SEM (standard error of mean). One-way ANOVA (analysis of variance) test and nonparametric KruskaleWallis test followed by Dunn's multiple comparison test (for post-ANOVA comparisons) were used when appropriate. Statistically significant differences: $p < 0.05$.

Results

Anthropometric variables

As shown in Table 1, with respect to ND-fed animals, rats fed for 16 weeks with HFD showed a significant increase in BMI, waist circumference, abdominal fat, and food intake (*$p < 0.05$). A decrease in all the above parameters was observed in SD animals. Notably, with respect to the HFD group, SD rats showed a significant decrease in abdominal fat and food intake (x$p < 0.05$). All groups showed similar water intake.

Metabolic variables

Rats fed with HFD for 16 weeks showed a higher blood glucose level than ND, thus suggesting a diet-dependent glucose intolerance in HFD ()$p < 0.05$). In the SD group, the blood glucose level was significantly lower with respect to HFD and higher than that detected in the ND group (_, x$p < 0.05$). In addition, the total cholesterol level was significantly higher in HFD than in SD rats ($p < 0.05$), but it was significantly lower in the ND group compared with the HFD and SD groups (_, x$p < 0.05$). Similar changes were also observed for HDL and LDL (see Table 2). HOMAIR, calculated as the product of fasting plasma glucose and fasting plasma insulin levels, assuming that the ND group has an index of 1.0, was 2.60 in the HFD group, while it was 1.31 in the SD group.

Morphological experiments

Heart

The sections of the left ventricle from rats fed with HFD showed a higher infiltration of inflammatory cells(Fig. 1D) and collagen deposition (Fig. 1) compared to the ND rats (Fig. 1A, B, and C). These changes were attenuated in the left ventricle of the rats fed with SD with respect to the HFD group (Fig. 1G, H, and I). The analysis of the ventricular structure revealed a higher percentage of myocardium per unit of area in the HFD-fed rats (86.23%), while the percentage in the SD group was similar to that measured in the ND group (77.9% and 80.51%, respectively). Immunohistochemical analysis, using anti-IGF-1 receptor antibody as a marker of hypertrophy, revealed a major expression of this molecule in HFD with respect to ND heart, suggesting a higher degree of myocardial hypertrophy. On the contrary, the IGF-1R expression in SD heart was reduced with respect to HFD heart and was similar to ND heart (Fig. 2).

Liver

The liver from HFD rats showed a higher infiltration of inflammatory cells (Fig. 3A and B) along with the presence of fat vacuoles (Fig. 3B) and portal fibrosis (Fig. 3E), which were of a lesser extent in liver from ND (Fig. 3A and D) and, in particular, in liver from SD rats (Fig. 3C and F).

Cardiovascular function

Basal cardiac parameters

Cardiac parameters, obtained after 20-min equilibration, are given in Table 3. Endurance and stability of the preparations for each heart, analyzed by measuring the performance variables every 10 min, was stable up to 180 min. Under basal conditions, a significant increase in CP (circulatory power) was observed in HFD with respect to ND ()$p < 0.05$). In addition, rats fed with HFD diet showed a significant increase in heart weight with respect to the SD and ND groups (), _, x$p < 0.05$).

Postischemic cardiac function

Systolic function is represented by the value of developed LVP recovery (dLVP; i.e., inotropic activity). As shown in Fig. 4A, in the postischemic phase, at the end of the reperfusion, HFD post-C dLVP was significantly higher than HFD (x$p < 0.05$). Notably, in SD and SD post-C, the systolic recovery is higher than in HFD and ND (x$p < 0.05$). LVEDP (i.e., contracture state) represents the diastolic

function in the postischemic phase. In the ND and HFD groups, LVEDP values indicate the presence of contracture. On the contrary, in HFD post-C, the SD and SD post-C LVEDP values were lower when compared to their respective ND counterpart (xp < 0.05; Fig. 4B).

Infarct size (IS)

Infarct area was similar in ND and HFD hearts (w67% of risk area; Fig. 5A). In HFD post-C, IS was significantly lower (w55% of risk area) compared to HFD and ND hearts(xp < 0.05). Of note, in SD and SD post-C, IS was reduced atw48% and w42% of risk area, respectively (xp < 0.05). In all groups, the cardioprotective profile of LDH release was similar to IS (Fig. 5B).

Myverol group

In order to exclude the possible influence of the additive Myverol on rat anthropometric and metabolic parameters and on cardiac performance, two groups were fed with ND enriched with Myverol at lower (30 gr/kg of diet, i.e., 2% of Myverol) and higher (60 gr/kg of diet, i.e. 5% of Myverol) concentrations. The systolic and diastolic functions and IS and LDH levels in these groups are similar to those in the ND group (data not shown).

Involvement of RISK pathway

In heart homogenates from all groups, the expression of the phosphorylated kinases ERK1/2 and AKT was evaluated. Densitometric analysis of the WB bands (Fig. 6) showed that the expression of p-AKT was higher in the SD and SD post-C groups ()p < 0.05) than in the HFD, HFD post-C, and ND groups. ERK1/2 phosphorylation was higher in the HFD post-C, SD, and SD post-C groups ()p < 0.05) than in the HFD and ND groups.

Involvement of anti-apoptotic protein

Western blotting experiments in ND, HFD, HFD post-C, SD, and SD post-C hearts showed that the levels of the antiapoptotic protein Bcl2 were significantly increased in HFD post-C, SD, and SD post-C hearts groups, with respect to the HFD and ND groups ()p < 0.05; Fig. 7).

Discussion

Proper nutrition is a major determinant for prevention and treatment of many diseases. This becomes even more important in the presence of obesity, a severe problem in the Western society, often associated with fatal diseases [24]. Although genetic factors may increase the propensity to obesity, epidemiological evidence identified a strong correlation with a diet rich in fat [25], which is often associated with a significant increase in CV risk [26]. In this regard, the industrial research on nutrition is constantly attempting to formulate innovative products that are a perfect balance between fat content and health. Bio-Oil Spread, containing olive oil as the main ingredient, is a recently developed innovative alimentary material. The present work is the first to analyze the longterm metabolic and cardiac effects induced by the dietary supplementation with Bio-Oil Spread on rats. The results showed that, with respect to animals fed with HFD, those fed with this novel product show changes in anthropometric and metabolic parameters. Their heart is preserved by obesity-dependent morpho-functional alterations and is protected against ischemic injury through the activation of pro-survival pathways such as RISK and SAFE and antiapoptotic proteins. We here observed that rats fed with a diet enriched with Bio-Oil Spread showed reduced anthropometric index, in particular abdominal fat, with respect to HFD-fed animals. As largely shown in animal models and in humans, these parameters are closely associated with dyslipidemia, disorders in glucose metabolism, and severe cardiovascular dysfunctions, which remarkably enhance the risk of myocardial infarction [27,35,36]. Consistent with these metabolic alterations, we found in HFD rats a lipid profile indicative of dyslipidemia. By contrast, SD animals showed an opposite behavior, as they were characterized by HDL values that were higher than those measured before the dietary treatment. At the same time, blood glucose and insulin levels, which were higher in HFD-fed rats, were reduced in SD rats. Insulin resistance plays a role in the pathogenesis of diabetes and metabolic syndrome. Accordingly, it is important to determine, in vivo, the insulin sensitivity, both in human and in animal models [28]. In human studies, a number of simple indexes, derived from fasting glucose and insulin levels, have been obtained and validated, for example, HOMA-IR index. An increased HOMA-IR index was suggested to be indicative of insulin resistance typically in the early stages of diabetes or steatohepatitis [29]. Based on these premises, in the present study, we adapted this index to the rat, a widely used model to study insulin resistance. HOMA-IR index was used on the basis of biochemical parameters (glucose and insulin) that can be obtained in a single sample of fasting blood. Our data showed that HOMA-IR in the SD group is reduced with respect to the HFD group, thereby suggesting that the Spread Bio Oil diet did not determine insulin resistance. High blood glucose and insulin levels are typical traits of obesity and are responsible for glucotoxicity [30]. This determines cardiac injury through direct and indirect effects on cardiac myocytes, fibroblasts, endothelial cells, via overproduction of reactive oxygen species, and apoptosis [30]. Hyperglycemia also contributes to alteration of cardiac structure and function through advanced glycation end products and posttranslational modification of extracellular matrix proteins, leading to an altered expression/function of intramyocellular calcium channels. These processes contribute to systolic and diastolic cardiac dysfunction [31,32]. In obesity, the cardiovascular condition is aggravated by the detrimental role of the excessive adipose tissue and fat-free mass, which results in a higher metabolic demand. This leads to hyperdynamic circulation, increased blood volume, and cardiac output [33]. Heart weight and body weight exhibit a linear relationship; peripheral resistances increase, which enhance left ventricular afterload [34]. In long-standing obesity, these changes are associated with left ventricular hypertrophy [35]. Interestingly, we found that rats fed with Bio-Oil Spread showed a heart weight similar to that of ND animals. This is opposite to the observations in HFD rats. Moreover, the cardiac tissue was characterized by a scarce infiltration of inflammatory cells, the absence of ventricular hypertrophy, and a limited collagen deposition, indicative of a reduced ventricular stiffness. In addition, IGF1R, an established factor in cardiac hypertrophy, is overexpressed in hypertrophic heart mediating the hypertrophic pathology process. In particular, it has been shown that the short-term activation of IGF signaling pathway promotes physiologic growth, whereas sustained hyperactivation leads to the development of pathologic hypertrophy and HF [36,37]. Interestingly, we observed that the expression of IGF-1R and myocardial hypertrophy in SD heart, are lower than those of HDF heart, suggesting that SD diet protects the heart from obesity dependent hypertrophy. These observations are of interest as they may necessitate a preserved heart performance in the presence of a diet enriched with Bio-Oil Spread. Of note, we observed in SD rats that, although body weight increased, abdominal fat decreased. Together with the ameliorated metabolic profile, this is indicative of a reduced risk of myocardial infarction, as suggested by the direct relationship described between abdominal obesity and the increased myocardial risk [38e40]. Consistent with this, the heart of rat fed with Bio-Oil Spread showed a higher

protection against I/R injury with respect to HFD animals in the presence of ischemic Post-C maneuvers. This was indicated by a significant reduction of IS and LDH release and by a marked improvement of the postischemic contractile function expressed as an increase in LVP and a decrease of contracture development. It is known that cardiac protection is mediated by the activation of the pro-survival RISK pathway [41]. This cascade involves PI3K/Akt, PKCε, and ERK1/2, which require the opening of mitoKATP channels [42e44]. Here we observed that, compared to HFD-fed rats, cardioprotection induced by SD diet increased the expression of p-Akt and p-ERK. This agrees with the involvement of these kinases in ischemic pharmacological cardioprotection and Post-C maneuvers [45,46]. In line with an SD-associated protective role, the heart of SD-fed rats showed an increased expression of the anti-apoptotic protein Bcl-2 which correlates with the reduction of IS.

Human application

In the last years, food industry is focusing on formulating healthy products for consumers, also forced by new legislative requirements. This is encouraging research for increasing quality and wholesomeness of food materials. Bio-Oil Spread is a healthy alternative to conventional margarines or shortenings. For instance, margarines can be used as a ready-to-eat product (fat spread) or as ingredients for leavened and baked foodstuffs. Independent of the intended use, their production process always involves different steps aimed at producing W/O emulsions. The specific rheological characteristics (hard gel-like properties) are given by the saturated fats of the oil phase, which form an interacting network surrounding water droplets [47]. Oil-phase structuring is achieved through two alternative methods. The first is the addition of natural hard fats (saturated fats), mainly palm oil derivatives, to the oil phase. However, at present, intensive use of palm oil in the food industry is discouraged because of problems associated not only with rainforest destruction but also with the high palmitic acid content, considered responsible for the hypercholesterolemic effect [48]. The second alternative is the partial hydrogenation of vegetable oils [49] with a catalytic three-phase process [50] to saturate carbone carbon double bonds. This implies that the movement of double bonds in their positions on the fatty acid carbon chain, producing isomers and trans-fatty acids (TFAs) has several negative effects on consumers' health [51,52]. Based on these premises, Bio-Oil Spread could be addressed to human nutrition because it is characterized by rheological properties very similar to those of spreads or margarines, for which it could be considered a substituent. However, the oil phase is structured by organogelation, and therefore, without chemical recombination of the molecules within raw materials. Bio-Oil Spread composition also includes cocoa butter in its formulation.

Therefore, its lipid profile contains stearic acid (principally present in cocoa butter) and high-oleic acid oils (i.e. olive oil). This is very intriguing because of the neutral effects of stearic acid on cholesterol levels and the beneficial effects of oleic acid on human health [52].

Strengths and limitations of the study

This study is the first to highlight the benefits of the dietary consumption of Bio-Oil Spread, a novel olive oil-based food. It also provides indications concerning the impact of this alimentary product on health, comparing its effects with those of the classic hyperlipidic diet. The use of an animal model represents a limitation as it reveals aspects that cannot be directly used for applicative purposes and require further efforts to be translated into the human setting. At the same time, this is an advantage as it allows the study of aspects of the complex networks of physiological and pathological processes that are activated in the presence of obesity. Animal studies represent, even today, a fundamental preclinical step to investigate, at the organ and molecular level, the mechanisms related to the onset and progression of diseases. In this regard, the results provided by the present study represent a stimulating scientific base for further investigations focused on the clarification of the applicative potential of Bio-Oil Spread in human nutrition with the purpose to reduce the negative impact of a high lipid diet on cardiovascular health.

Conclusion

This study provides first evidence that long-term dietary supplementation with the innovative alimentary product, Bio-Oil Spread, may contribute to prevent metabolic disorders and cardiovascular alterations typical of severe obesity induced by an HFD, including the increased ischemic susceptibility of the myocardium. Our results enrich the knowledge on the beneficial effects of olive oil on the cardiovascular health and pave the way to evaluate the introduction of Bio-Oil Spread in human alimentary guidelines as a strategy to reduce saturated fat intake.

Disclosure statement

The authors have nothing to disclose.

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Table 1 Anthropometric variables in rats fed with ND (normolipidic diet) or HFD (high fat diet) or SD (Spread-oil diet) diets for 16 weeks. Values are mean ± SEM, $n = 20$. Significance of difference (nonparametric Kruskal–Wallis test followed by Dunn's multiple comparison test): $p < 0.05$.* = HFD versus ND; § = SD versus HFD; ° = SD versus ND.

Anthropometric variables	ND	HFD	SD
Body weight, g	530 ± 10	630 ± 16	557 ± 11
Water intake, ml/day	26.80 ± 2.1	29.60 ± 4.13	28.33 ± 5
Food intake, g/day	13.33 ± 1.4	15.51 ± 1.7*	15.91 ± 1.06§
BMI, Kg/m^2	6.64 ± 0.1	7.99 ± 0.2*	7.07 ± 0.14
Waist circumference, cm	19.07 ± 0.2	23.33 ± 1*	20.14 ± 0.3
Abdominal fat, g	7.40 ± 0.1	15.03 ± 0.3*	8.42 ± 0.2§

Table 2 Metabolic variables in rats fed with ND (normolipidic diet) or HFD (high fat diet) or SD (Spread-oil diet) for 16 weeks. Values are mean ± SEM, $n = 20$. Significance of difference (nonparametric Kruskal–Wallis test followed by Dunn's multiple comparison test): $p < 0.05$. * = HFD versus ND; § = SD versus HFD; ° = SD versus ND.

Metabolic variables	ND	HFD	SD
Basal blood glucose, mg/dl	85.90 ± 6	191.35 ± 14*	102 ± 6°,§
Plasma total cholesterol, mg/dl	151.35 ± 18	207 ± 22*	181.9 ± 16°,§
Plasma LDL cholesterol, mg/dl	35.77 ± 2	96.43 ± 2*	46.57 ± 2°,§
Plasma HDL cholesterol, mg/dl	70.21 ± 1	39.30 ± 1.8*	54.59 ± 1.9°,§
Plasma insulin, μUI/ml	83.2 ± 24	97.2 ± 25	92.3 ± 24

Table 3 Basal cardiac parameters in ND (a), HFD (b) and SD (c) rats. Values were calculated as means ± SEM ($n = 10$ for each group). Significance of difference (nonparametric Kruskal–Wallis test followed by Dunn's multiple comparison test): $p < 0.05$. * = HFD versus ND; § = SD versus HFD; ° = SD versus ND. For abbreviation, see Material and Methods.

LVP (mm Hg)	HR (beats min^{-1})	LVEDP (mm Hg)	CP (mm Hg)	Heart weight (g)	Pressure perfusion (mm Hg)
(a) 76 ± 3.7	265 ± 11	5–8	59 ± 1.9	1.41 ± 0.04	100
(b) 96 ± 5.1	295 ± 14	5–8	86 ± 2.5*	2.59 ± 0.08*	100
(c) 83 ± 3.7	282 ± 10	5–8	71 ± 2.5	1.67 ± 0.03°,§	100

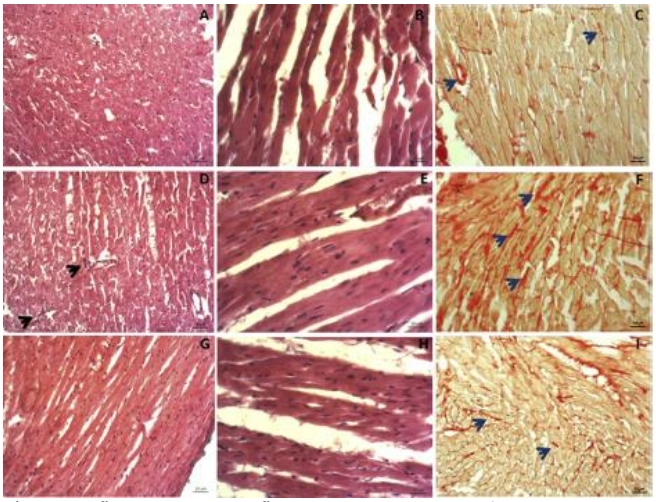

Figure 1 Inflammation (D) and fibrosis (C, F, I) in the heart from ND (A, B, C), HFD (D, E, F), and SD (G, H, I) rats (n Z 4 for each group). Hematoxylineeosin staining (A, B, D, E, G, and H) of LV showed infiltration of inflammatory cells in HFD rats (D black arrows). Hypertrophied cardiomyocytes are evident in HFD (E) respect to ND (B) and SD (H) rats. Picrosirius red staining of LV (C, F, and I) showed collagen deposition (blue arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

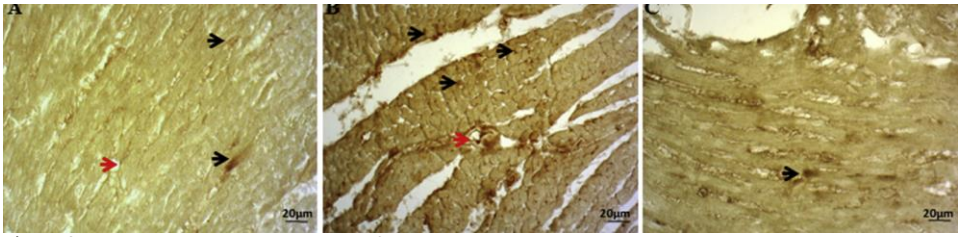

Figure 2 Immunohistochemical localization of the IGF 1R in the ventricular cardiomyocytes (black arrows) and vascular endothelium (red arrows) of ND (A), HFD (B), and SD (C) rats. A higher signal was detected in HFD (B) with respect to ND (A) and SD (C) heart sections. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

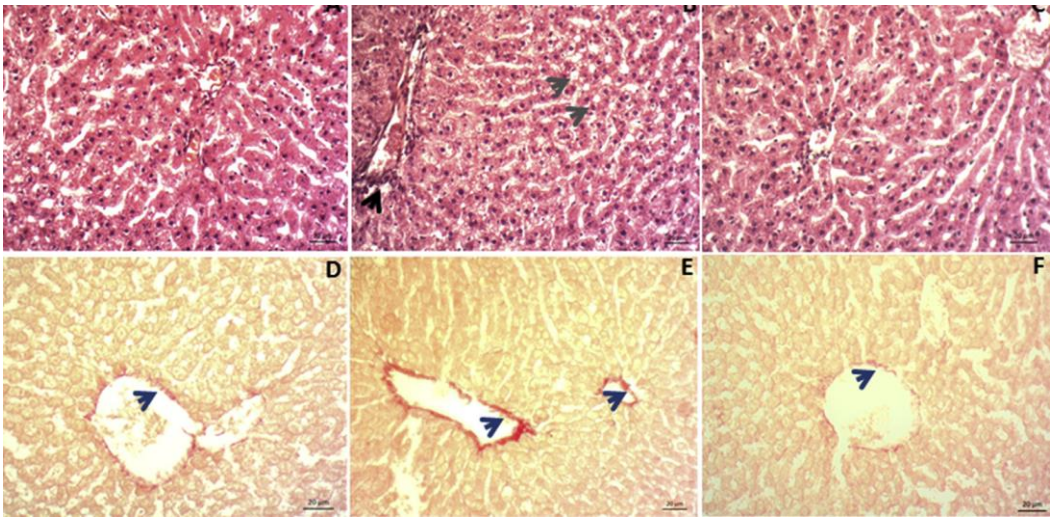

Figure 3 Inflammation (black arrows), fat deposition (gray arrows), and fibrosis (blue arrows) in the liver from ND (A, D), HFD (B, E), SD (C, F) rats. Hematoxylin and eosin staining (A, B, C) of the liver showed inflammatory cells and enlarged fat vacuoles in HFD rats. Picrosirius red staining (D, E, F) showed collagen deposition in the hepatic portal region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

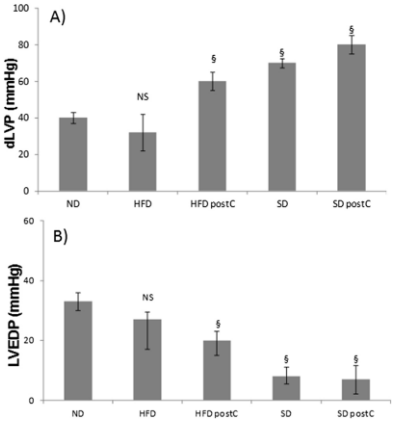

Figure 4 (A) Systolic function. Variation of developed LVP (dLVP) in ND (n Z 10), HFD and HFD post-C (n Z 10), SD, and SD post-C groups (n Z 10). Data are presented in mm Hg at the end of 120-min reperfusion with respect to baseline level of 10 experiments for each group. (B) Diastolic function. Left ventricular end-diastolic pressure (LVEDP): data are expressed in mm Hg at the end of 120-min reperfusion. Significance of difference (one-way ANOVA) from control values of each group versus ND: x Z $p < 0.05$.

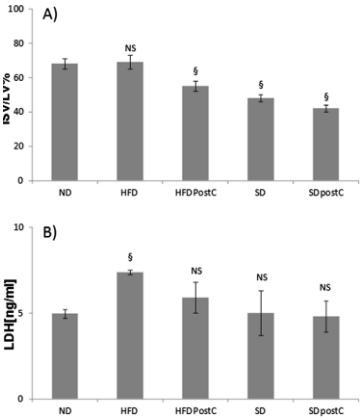

Figure 5 (A) Infarct size. The amount of necrotic tissue measured after 30-min global ischemia and 120-min reperfusion is expressed as percent of the left ventricle (% IS/LV) in ND (n Z 10), HFD, and HFD post-C (n Z 10), SD, and SD post-C groups (n Z 10). (B) LDH release. Values are expressed as means _ SEM of absolute data (ng/ml) in ND, HFD, HFD Post-C, SD, and SD post-C groups. Significance of difference (one-way ANOVA) from control values of each group versus ND: x Z $p < 0.05$.

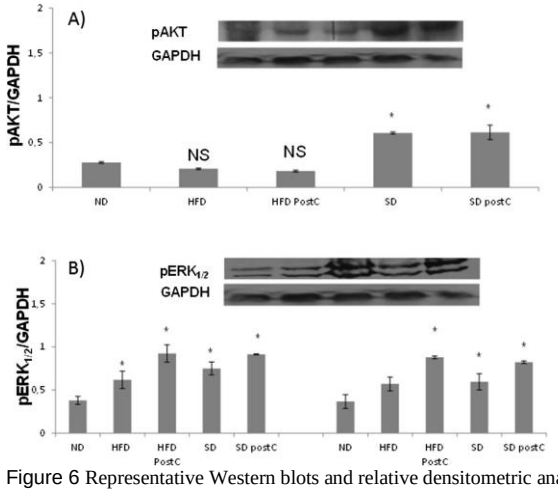

Figure 6 Representative Western blots and relative densitometric analysis of (A) AKT and (B) ERK1/2 phosphorylation in ND, HFD, HFD post-C, SD, and SD post-C groups. Percentage changes were evaluated as means _ SEM of three experiments for each group. Significance of difference (one-way ANOVA) from control values of each group versus ND:)p < 0.05.

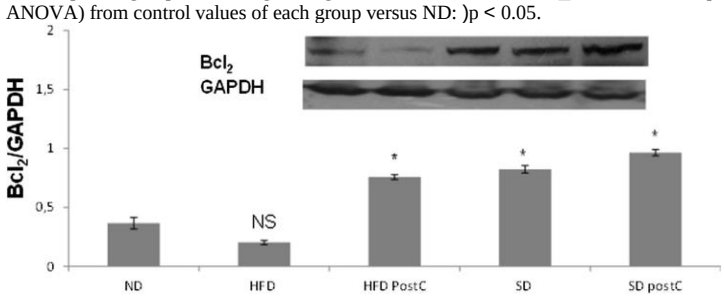

Figure 7 Representative Western blots and relative densitometric analysis of Bcl2 phosphorylation in the ND, HFD, HFD post-C, SD, and SDpostC groups. The percentage changes were evaluated as means _ SEM of three experiments for each group. Significance of difference (one-way ANOVA) from control values of each group versus ND:)p < 0.05.