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Metabolomics identifies changes in fatty acid and amino acid profiles in serum of overweight older adults following a weight loss intervention

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Abstract

The application of metabolomics in nutritional research may be a useful tool to analyse and predict the response to a dietary intervention. The aim of this study was to examine metabolic changes in serum samples following exposure to an energy-restricted diet (-15% of daily energy requirements) over a period of 8 weeks in overweight and obese older adults (n=22) using a GC/MS metabolomic approach. After the 8 weeks, there were significant reductions in weight (7%) and metabolic improvement (glucose and lipid profile). Metabolomic analysis found that total saturated fatty acids (SFAs), including palmitic acid (C16:0) and stearic acid (C18:0) and monounsaturated fatty acids (MUFAs) were significantly decreased after the 8 week intervention. Furthermore, palmitoleic acid (C16:1) was found to be a negative predictor of change in body fat loss. Both the total ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) significantly decreased although the overall total amounts of PUFAs did not. The branched chain amino acid (BCAA) isoleucine significantly decreased in the serum samples after the intervention. In conclusion, this study demonstrated that the weight loss intervention based on a hypocaloric diet identified changes in the metabolic profiles of serum in overweight and obese older adults, with a reduction in anthropometric and biochemical parameters also found.

1. INTRODUCTION

The prevalence of overweight and obesity, established as excessive fat accumulation, has increased rapidly worldwide [10]. Fat excess is considered a major predisposing factor for a number of chronic diseases such as Type 2 diabetes mellitus, hypertension, dyslipidemias, cardiovascular disease (CVD) and cancer [53]. In turn, overweight and obesity prevalence is growing even among older adults (≥60 years) in developed countries [58]. Aging is associated with significant changes in body composition causing a decrease of muscle mass and an increase of total fat mass especially in the abdominal region. [33].

Essentially, many treatments for overweight and obesity include lifestyle modification through weight loss challenges and exercise with the purpose of balancing energy intake with energy expenditure. Nevertheless, most of the dietary interventions result not only in the desired body fat mass loss but also in a decrease in lean mass which is discouraged especially in older adults [40,48].

Metabolomics is a technique that aims to identify and quantify the metabolome [57]. It is the study of metabolites present in biological samples such as biofluids/cellular extracts and culture media. Its use in nutrition research is increasing and applications range from assessing novel biomarkers of dietary intake to utilization of metabolomics in intervention studies [4]. Application of this technique to analyse the response to a dietary intervention generates valuable information on the effect and predisposition of the prescribed diet on metabolic regulation. It also allows a connection between dietary intake and a particular metabolic phenotype

[15,46,50]. The two main approaches employed in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). These approaches both have their advantages and disadvantages and at present there is no unique analytical technique capable of measuring and identifying all metabolites in a single sample simultaneously. Therefore comprehensive metabolomic data needs to be assessed by bringing together data from different platforms [12,51,54]. A number of nutritional studies have used a gas chromatography/mass spectrometry (GC/MS) based metabolomic approach to analyse fatty acids (FAs) and amino acids (AAs) [34,41]

FAs play an important role in metabolic health taking part in many cellular processes, serving as energy reserves or regulating gene expression. Obese subjects report elevated concentrations of serum total FAs, which may have an impact on the development of metabolic syndrome and related disorders [22,50,52]. However, aside from the amount of total lipids, the type of fat has been suggested to be crucial in the development of obesity [36]. The FA composition in the human body mirrors not only the dietary fat composition but also the endogenous synthesis and metabolism of FAs, mainly by FA synthesis from carbohydrates (CHO), desaturation and elongation [2,55]. In this way desaturases have been suggested to play a role in the growth of metabolic disorders [1,23].

Due to the rising prevalence of overweight and obesity among older adults, the design of effective weight loss interventions in this age group is needed. Therefore, the aim of this study was to examine metabolic changes after the exposure to an energy-restricted diet over a 8-week period in overweight and obese older adults using a GC/MS metabolomic approach and also using anthropometric and biochemical data.

2. MATERIAL AND METHODS

Study population

Twenty-two of the twenty-six enrolled Caucasian healthy older adults with overweight or obesity (BMI between 27-34.9 kg/m^2) finished the study. All participants were non-smokers, followed a diet free of antioxidants or vitamin supplements and presented a stable weight (± 3 kg) for the previous 3 months. Diabetes mellitus, history of previous psychiatric disorders or chronic diseases related with the metabolism of nutrients was considered as exclusion criteria. The volunteers were recruited through a local newspaper and the Department database. Prior to beginning the study, subjects attended the Metabolic Unit of the University of Navarra, where the physician informed them in detail about the study conditions and they signed the written informed consent.

Study protocol

The present study was designed as a prospective intervention study in which subjects followed a personalised and hypocaloric diet (-15% of daily energy requirements) over 8 weeks. The macronutrient distribution was as follows: 45% of calories from CHO, less than 30% from lipids and 25% from proteins. The diet was designed by trained dieticians using a food exchange system and a menu indicating what the volunteers should choose each day of the week in order to follow a healthy diet. In addition, the volunteers were instructed to weigh all the food they consumed and were advised to eat 5 meals per day. Similarly, they were asked to continue with their usual physical activity which was controlled with pedometers (Omron, HJ-152K-E, Japan).

This study was approved by the Ethics Committee of the University of Navarra (033/2011) and conforms to the principles outlined in the Declaration of Helsinki.

Anthropometric and biochemical measurements

Anthropometric and body composition measurements were taken at the beginning and at the end of the study. Body weight was assessed to the nearest 0.1 kg using a Tanita bioelectrical impedance (SC-330, Tanita, Tokyo, Japan) and height was measured using a wall-mounted stadiometer (Seca 220, Vogel & Halke, Germany) to the nearest 1 mm. Body Mass Index (BMI) was determined as the body weight divided by the squared height (kg/m^2). All measurements were carried out after an overnight fast and with the subjects in their underwear. Waist circumference was measured at the narrowest point between the rib cage and the iliac crest and the hip circumference at the widest point over the buttocks. Body composition was measured by a dual-energy X-ray absorptiometry (DEXA Lunar Prodigy, GE Medical Systems, Madison, WI, USA).

Serum samples were collected at baseline and at the end of the study, after a 12-h overnight fast from each volunteer. Serum glucose, total cholesterol, HDL-c, triglycerides and non-esterified fatty acids (NEFA) were measured in an autoanalyser Pentra C-200 (HORIBA ABX, Madrid, Spain) with commercially available kits. LDL-c levels were calculated following the Friedewald formula: LDL-c = Total cholesterol − HDL-c − TG/5 [14].

Metabolite extraction & GC/MS analysis

For analysis of FAs, 300 μl of serum was combined with 50 μl of nonadecanoic acid (C19:0) (2 mg/ml methanol) as an internal standard and extracted using a 1:2 mixture of chloroform:methanol based on the method of Bligh & Dyer [3]. Briefly, extracts were derivatised by methylation using methanolic BF_3. Derivatives were re-suspended in 200 μl of hexane and 1 μl was injected into the GC/MS. The GC/MS system comprised of an Agilent 7890A GC coupled with a 5975C MS. The GC temperature was initially 70 °C for 2 min, increased at 15 °C/min to 190 °C and held for 9 min, then increased at 5 °C/min to 230 °C and held for 13 min and finally raised to 320 °C at 20 °C/min and held for 10 min.

Aqueous compounds were isolated using a methanolic extraction [21] following deproteinisation with acetonitrile. An aliquot of 100 μl of serum were combined with 20 μl of ^{13}C myristic acid (2 mg/ml methanol) as an internal standard prior to extraction with 800 μl methanol. Following drying, samples were methoximised using 60 μl of methoxyamine hydrochloride (20 mg/ml pyridine) for 17 hours at room temperature prior to silylation with 60 μl of *N*-methyl-*N*-(trimethylsilyl)fluoroacetamide for 1 hour. Samples were diluted with 210 μl of hexane and analysed by GC/MS. The GC temperature was initially 70 °C for 2 min, increased at 5 °C/min to 260 °C, held for 41 min and finally raised to 320 °C at 30 °C/min and held for 3 min. After a solvent delay of 1 min full scan, mass spectra were recorded within a scan range of 45-650 amu (atomic mass units).

Metabolite identification & quantification

Calibration was achieved by comparison of peak areas for amino and FAs with reference to known standards (Amino acid standard A9906 and Supelco 37 component FAME mix, Sigma Aldrich, Ireland) using Agilent Chemstation (MSD E.02.00.493) and by comparison of their mass spectra with those in the National Institute of Standards and Technology (NIST) library 2.0. Automatic peak detection was carried out with Agilent Chemstation. Mass spectra deconvolution was performed with the Automated Mass Spectral Deconvolution and

Identification System (AMDIS, version 2.65). Peaks with a signal to noise (S/N) ratio lower than 30 were rejected, which is an acceptable level to avoid false positives as reported by Norli and colleagues [42]. To obtain accurate peak areas for internal standard and specific peaks/compounds, one quant mass for each peak was specified as the target ion and three masses were selected as qualifier ions. Each data file was then manually analysed for false positives/negatives in Agilent Chemstation.

Enzyme activity determination

The desaturase activity was calculated using the ratio of individual FAs according to the following criteria: C16 $\Delta 9$-desaturase = (C16:1/C16:0), C18 $\Delta 9$-desaturase = (C18:1n-9 /C18:0), $\Delta 6$-desaturase = (C18:3n-6/C18:2n-6) and $\Delta 5$-desaturase = (C20:4n-6/C20:3n-6) [2]. The elongase activity index of FAs was assessed from the ratio C18:0/C16:0 [45].

Statistical analysis

Data are expressed as mean ± standard deviation (SD), unless otherwise specified. The Shapiro Wilk test was used to analyse the normality of the measured variables. The differences between baseline measurements and those taken after the 8 week intervention were assessed using a paired t-test or by using the nonparametric Wilcoxon test when variables followed a non-normal distribution. Correlation analyses were applied to assess the potential relationships between specific metabolites with biochemical and anthropometrical parameters. Linear regression analysis was performed to predict changes in anthropometric variables according to FA levels at baseline. Average weight loss between groups (more weight loss vs less weight loss) was assessed using an independent measure t-test. All statistical procedures were conducted using SPSS version 15 for Windows (SPSS Ibérica, Madrid, Spain). $P < 0.05$ was considered statistically significant.

3. RESULT

After the 8-week weight loss intervention, there were significant reductions in body weight, BMI, waist circumference, total fat mass, lean mass and diastolic blood pressure (Table 1). Physical activity did not change during the weight loss intervention (Table 1). In addition, the dietary program was effective in reducing total cholesterol, LDL-c and transaminases, however it also decreased HDL-c concentrations (Table 2).

The FA concentrations of serum samples at baseline and after the 8-week intervention are reported in Table 3. Analysis of the FAs revealed a significant decrease in total SFAs ($p < 0.05$), including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and lignoceric acid (C24:0) after the 8-week intervention. Final serum levels of total MUFAs including oleic acid (C18:1) and cis-11–eicosenoic acid (C20:1) were significantly decreased after the 8-week intervention. Although total PUFA levels did not significantly decrease, linoleic acid (C18:2n-6), arachidonic acid (C20:4n-6), cis-8, 11, 14, 17–eicosatrienoic acid (C20:3n-6), cis-11, 14–eicosadienoic acid (C20:2) and cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6n-3) were significantly reduced following the 8 week diet. Total ω-6 and ω-3 PUFAs also significantly decreased, although the ratio of ω-6/ ω-3 did not change following the intervention.

The activity of $\Delta 5$-desaturase significantly increased after weight loss, whereas the activity of the remaining investigated enzymes did not significantly change from the beginning of the study to the end of the study (Table

3). Analysis of correlation showed significant positive association between the change in elongase activity and the variation of total cholesterol (r=0.648, p=0.003), HDL-c (r=0.457, p=0.049) and LDL-c (r=0.562, p=0.012). Furthermore, the resulting change in percentage of body fat was positively predicted by the baseline circulating concentrations of palmitoleic acid (C16:1) (Figure 1).

In this study weight loss was categorised into two groups (greater weight loss (7 .4 kg) vs less weight loss (3.4 kg)) in order to identify whether there were differences in the metabolomic profiles between the groups. In this context, we found that individuals who achieved greater weight loss also reduced their total MUFA levels (p=0.021), particularly oleic acid (C18:1) (p=0.042) and stearic acid (C18:0) (p=0.024).

A positive association between triglycerides and total SFAs (r=0.517, p=0.023), including myristic acid (C14:0) (r=0.486, p=0.035), pentadecanoic acid (C15:0) (r=0.702, p=0.001), palmitic acid (C16:0) (r=0.456, p=0.050), heptadecanoic acid (C17:0) (r=0.474, p=0.040), behenic acid (C22:0) (r=0.546, p=0.016) and tricosanoic acid (C23:0) (r=0.507, p=0.027) were found at baseline. Likewise, the change in triglyceride levels were positively associated with the variation in pentadecanoic acid (C15:0) (r=0.748, p=<0.001), heptadecanoic acid (C17:0) (r=0.489, p=0.033), behenic acid (C22:0) (r=0.481, p=0.037) and lignoceric acid (C24:0) (r=0.732, p=<0.001).

A total of 4 AAs were identified and semi-quantified in the serum (Table 4), of these 3 were BCAAs. Of the BCAAs it was found that isoleucine significantly decreased in the serum following the intervention (p=0.02).

4. DISCUSION

The effectiveness of the dietary intervention was reflected in the decrease in body weight, BMI, waist circumference, total fat mass and the diastolic blood pressure. However, subjects also showed a decline in lean mass. In general, lean mass reduces after following a hypocaloric diet, with this being more notable with aging [27,33]. In order to avoid losing lean mass the prescribed hypocaloric diet presented a higher percentage of protein (25%). Despite our attempt to prevent lean mass loss, it was significantly decreased in subjects after the dietary intervention.

Subjects were asked to continue with their usual physical activity, which was controlled throughout the weight loss treatment with pedometers so as to control the effect of physical activity on weight loss. Consequently, the variations in anthropometric, biochemical parameters and metabolite concentrations cannot be associated to changes in physical activity, but to the dietary weight loss intervention.

Levels of NEFA in serum decreased, although not significantly after the weight loss intervention. In this sense, high levels of total FAs in blood have been positively related with CVD, particularly with obesity and diabetes [59]. However, evidence suggests that the dietary fat quality rather than quantity might have a greater influence on disease risk [20,22,36]. In this context the analysis of the contribution of each FA has emerged indicating that SFAs are positively associated with the development of obesity and diabetes, increasing comorbidities related to metabolic disease [20,22].

In this study, total SFAs in serum significantly decreased with the hypocaloric diet, among them palmitic acid (C16:0) and stearic acid (C18:0), which have been previously related with the incidence of Type 2 diabetes [19,24]. Also, total SFAs may increase CVD risk by raising levels of LDL-c and total cholesterol [13]. Total

MUFAs and in particular oleic acid serum levels decreased in this study. The health benefits of (C18:1), which represents the most abundant MUFA provided in the diet have been described previously [22,47]. Both total ω-6 and ω-3 PUFAs decreased significantly in this study. The ω-6 PUFAs are thought to promote adipogenesis and increase expression of lipogenic genes, while the ω-3 PUFAs have been suggested to do the opposite [30,37]. Nevertheless, the association between weight loss and the ω-3 PUFAs remains controversial [38]. Linoleic acid (C18:2n-6) as well as cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid (C22:6n-3), which have been suggested to decrease obesity features [7,8], decreased significantly in this study. Mice under calorie restriction have shown increased expression of genes responsible of FA β-oxidation compared with ad libitum-fed controls [5], what may be implicated in the reduction of body fat after weight loss. Therefore, it can be proposed that in this study the energy restriction has increased FA β-oxidation [25], decreasing body fat and for that reason these metabolites are presented in lower amounts in the bloodstream.

This research also found that for individuals who lost more body weight they also had reduced levels of stearic acid (C18:0), total MUFAs and oleic acid (C18:1) levels. This might be explained by a lower FA production or a higher oxidation of these compounds, thus decreasing serum levels leading to a greater body weight reduction. Furthermore, it was observed that subjects with higher circulating values of palmitoleic acid (C16:1) experienced lower reduction in percentage body fat. The role of palmitoleic acid in human metabolism has not been fully clarified. Animal models have shown that adipose-derived palmitoleic acid may contribute to resistance to diet-induced obesity by inhibiting stearoylcoenzyme A desaturase 1 activity in the liver [6]. However, studies carried out in humans have not observed this effect [16], and others have observed a detrimental influence of this MUFA on health [44,56]. High levels of this particular FA have been associated with increased risk of suffering cardiovascular diseases, since it has been positively associated with metabolic syndrome (MetS) [56], including hypertriglyceridemia [44] and abdominal adiposity [16]. Mice supplemented with palmitoleate presented higher fat deposition, hepatic steatosis and also increased hepatic expression of sterol regulatory element-binding protein 1c and FA synthase, demonstrating the pro-lipogenic effect of this MUFA [17]. Moreover, in a Chinese population, high erythrocyte palmitoleic acid concentrations were related with lower plasma adiponectin and higher inflammatory markers [60]. Palmitoleic acid (C16:1) serum concentrations mostly show *de novo* hepatic FA synthesis from palmitic acid (16:0) by the C16 Δ9-desaturase enzyme [43,60]. Therefore, it can be speculated that subjects with higher palmitoleic acid (C16:1) at baseline could be predisposed to present a lower response to the dietary treatment by decreasing less amount of body fat.

The present finding of a positive association between SFAs and triglycerides is consistent with previous data in which serum SFAs have been suggested to increase triglyceride levels [29], whereas PUFAs are thought to reduce triglycerides levels but we did not find this association.

It is known that exist an inverse balance between CHO and FA β-oxidation [25]. The role of CHO in controlling the balance between fat intake and fat oxidation is well-established, since CHO consumption reduces the use of fat for fuel [32]. De novo lipogenesis (DNL) reflects the conversion of excess CHO to new FA and triacylglycerol, which are key substrates for the formation of TG and cholesterol [18]. FA desaturases are enzymes that create MUFAs from SFAs [9]. The use of desaturase indices is particularly useful when liver tissue samples are not available [23]. The activity of the enzymes elongase, Δ6-desaturase, C16 Δ9-desaturase and C18 Δ9-desaturase did not change during the weight loss intervention. Increased levels of the previous

enzymes have been detected in subjects with obesity and MetS [2,23]. Nevertheless, Δ5-desaturase significantly increased after the 8 week intervention, indicating that activity appears to be decreased in obese individuals [55]. Therefore, the increase of this desaturase during the study suggests a potential benefit to the participants.

Higher levels of circulating BCAAs have been reported in obese individuals compared to lean individuals [35,39], with a number of studies reporting a reduction in BCAAs levels after weight loss [26,28]. BCAAs predicted improvements in insulin resistance in patients participating in a weight loss intervention [31,49], and a positive association between BCAAs and insulin resistance has been also reported [34,49]. In the current study, insulin levels were not determined. Regarding BCAAs values, only isoleucine serum levels significantly decreased. Existing evidence suggests that the reduction in isoleucine levels promote lipolysis via induction of lipolytic genes and by the suppression of lipogenesis in liver [11].

Although these results are interesting, the study has a number of limitations such as the small number of participants. Future studies in larger cohorts would be required in order to validate these findings. Additionally, dietary intake data was not available: such data might be helpful in further understanding the alterations in the diet following the 8-week intervention.

In conclusion, this study demonstrates that the weight loss intervention based on a hypocaloric diet not only improved anthropometric and biochemical parameters but also metabolite serum levels in overweight and obese older adults. Metabolomic analysis identified a significant decrease in FAs and isoleucine levels and an increase in Δ5-desaturase activity. Moreover, the MUFA palmitoleic acid (C16:1) predicted the change in the percentage of body fat and an association between SFAs and triglycerides levels was observed.

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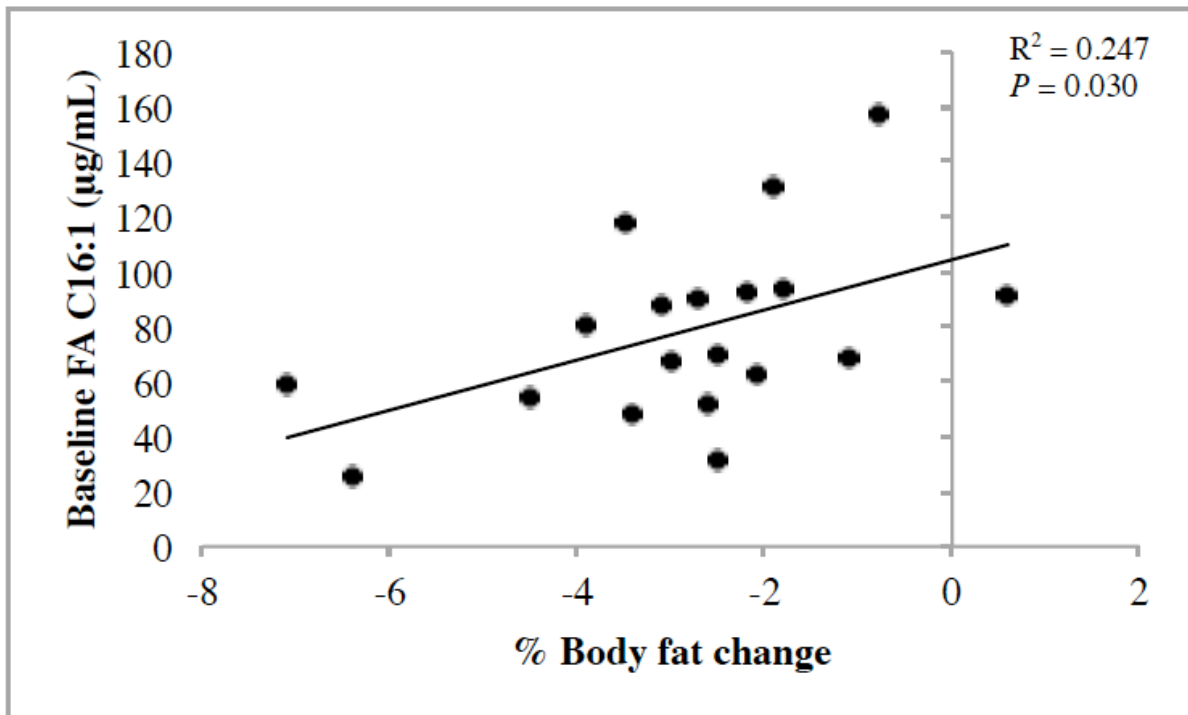
Figure 1. Association between baseline FA C16:1 serum values and % of body fat change in response to the 8-week energy restriction intervention (*n*=19).

FA: fatty acid.

Table 1. General characteristics of the study population (*n*=22) at baseline and at the end of the dietary intervention (8 weeks).

	Baseline	8 weeks	*P-value*
Age (years)	60 ± 5		
Female sex. % (n)	68.2% (15)		
Anthropometric variables			
Weight (kg)	76.8 ± 10.3	71.4 ± 8.6	<0.001
BMI (kg/m^2)	29.7 ± 2.0	27.6 ± 1.9	<0.001
Waist circumference (cm)	92.7 ± 7.5	87.7 ± 6.9	<0.001
Total fat mass (kg)	30.6 ± 5.1	26.4 ± 5.3	<0.001
Lean mass (kg)	43.4 ± 9.7	42.4 ± 9.4	<0.001
Other variables			
Systolic Pressure (mmHg)	126.9 ± 22.3	118.1 ± 13.8	ns
Diastolic Pressure (mmHg)	79.3 ± 8.8	72.6 ± 9.2	0.004
Pedometer (steps/day)	12447 ± 6835	11562 ± 5558	ns

Data are expressed as means ± SD. P-values were based on paired t-test.
BMI: body mass index; SD: standard deviation.

Table 2. Biochemical parameters of the study population (*n*=22) at baseline and at the end of the dietary intervention (8 weeks).

	Baseline	**8 weeks**	*P-value*
Glucose (mg/dL)	95.2 ± 7.2	91.9 ± 8.2	0.046
Total Cholesterol (mg/dL)	240 ± 37	212 ± 23	<0.001
HDL-col (mg/dL)[a]	55.3 ± 10.6	47.3 ± 8.0	<0.001
LDL-col (mg/dL)	165.6 ± 32.7	147.8 ± 21.7	0.006
Triglycerides (mg/dL)	95.2 ± 33.8	86.5 ± 39.5	ns
NEFA (µg/ml)	138.7 ± 52.6	115.5 ± 38.5	ns
Total proteins (g/L)	68.8 ± 3.3	66.9 ± 4.2	0.034
Alanine aminotransferase (U/L)	22.8 ± 7.0	19.8 ± 5.3	0.017
Aspartate aminotransferase (U/L)	22.7 ± 6.5	19.1 ± 4.7	0.002

Data are expressed as means ± SD. P-values were based on paired t-test or Wilcoxon test. HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; NEFA: non-esterified fatty acids; SD: standard deviation.
[a] P-value based on non-parametric Wilcoxon test compared the two time points of the study.

Table 3. Fatty acid composition of serum samples (*n*=19) taken at baseline and at the end of the dietary intervention (8 weeks).

Fatty acid (µg/ml)	Baseline	8 weeks	*P-value*
SFAs	1783.8 ± 588.7	1449.8 ± 520.8	0.002
Myristic acid (C14:0)[a]	68.16 ± 40.72	49.51 ± 26.46	<0.001
Pentadecanoic acid (C15:0)	7.07 ± 2.41	6.95 ± 2.73	ns
Palmitic acid (C16:0)[a]	1097.44 ± 479.92	901.69 ± 381.39	<0.001
Heptadecanoic acid (C17:0)	8.80 ± 2.77	7.69 ± 3.55	ns
Stearic acid (C18:0)	566.26 ± 220.57	452.94 ± 166.59	0.003
Arachidic acid (C20:0)	8.45 ± 4.39	8.31 ± 3.26	ns
Behenic acid (C22:0)	12.65 ± 4.36	11.30 ± 4.37	ns
Tricosanoic acid (C23:0)	5.67 ± 2.03	4.96 ± 2.06	ns
Lignoceric acid (C24:0)	9.30 ± 3.88	6.45 ± 2.24	0.003
MUFAs	2011.6 ± 497.1	1765.6 ± 528.5	0.001
Palmitoleic acid (C16:1)	78.17 ± 32.88	63.47 ± 25.53	ns
Oleic acid (C18:1n9c)[a]	1902.75 ± 488.09	1676.62 ± 532.75	<0.001
Cis-11 Eicosenoic acid (C20:1)	8.69 ± 6.22	6.43 ± 2.77	0.046
Nervonic acid (C24:1)	21.98 ± 11.16	19.08 ± 8.10	ns
PUFAs	2367.5 ± 383.0	1901.2 ± 391.9	ns
Polyunsaturated ω-6	2204.6 ± 383.9	1775.6 ± 393.3	<0.001
γ-Linolenic acid (C18:3n6)	8.67 ± 4.12	8.16 ± 5.19	ns
Linoleic acid (C18:2n6c)[a]	1548.39 ±	1264.42 ± 361.06	<0.001
Arachidonic acid (C20:4n6)[a]	551.88 ± 172.93	446.62 ± 128.99	0.004
Cis-8,11,14-Eicosatrienoic acid (C20:3n6)	90.27 ± 39.12	52.23 ± 25.69	<0.001
Cis-11,14-Eicosadienoic acid (C20:2n6)	5.36 ± 2.90	4.15 ± 2.67	0.017
Polyunsaturated ω-3	162.9 ± 61.0	125.6 ± 27.0	0.007
Cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	78.93 ± 51.24	58.83 ± 26.27	ns
Cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)[1]	84.01 ± 49.22	66.81 ± 34.26	0.004
Ratio ω-6/ ω-3	15.3 ± 6.2	14.9 ± 5.1	ns
C16 Δ9- desaturase[b]	0.088 ± 0.060	0.079 ± 0.036	ns
C18 Δ9- desaturase[c]	3.97 ± 2.45	4.22 ± 2.20	ns
Δ6- desaturase[d]	0.006 ± 0.002	0.007 ± 0.005	ns
Δ5- desaturase[e]	7.48 ± 4.08	10.00 ± 4.35	0.023
Elongase[f]	0.60 ± 0.29	0.54 ± 0.19	ns

Data expressed as mean ± SD. P-values were based on paired t-test or Wilcoxon test.
MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; SD: standard deviation.
[a] P-value based on non-parametric Wilcoxon test compared the two time points of the study. [b] C16 Δ9-desaturase = (C16:1/C16:0); [c] C18 Δ9-desaturase (C18:1n-9/C18:0); [d] Δ6-desaturase = (C18:3n-6/C18:2n-6); [e] Δ5-desaturase = (C20:4n-6/C20:3n-6); [f] elongase activity C18:0/C16:0; PUFAs ω-6 = C18:3n6 + C18:2n6c + C20:4n6 + C20:3n6 + C20:2n6; PUFAs ω-3 = C20:5n3 + C22:6n3.