Changes in plasma metabolite concentrations after a low-glycemic index diet intervention

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Abbreviations:

AHA, American Heart Association; **BCAA**, branched-chain amino acid; **BMI**, body mass index; **BP**, blood pressure; **BW**, body weight; **CRP**, C-reactive protein; **CVD**, cardiovascular disease; **DHA**, docosahexaenoic acid; **ERETIC**, electronic reference to access in vivo concentrations; **FA**, fatty acid; **FDR**, false discovery rate; **FFM**, fat-free mass; **FM**, fat mass; **GC**, gas chromatography; **HGI**, high-glycemic index; **HDL**, high-density lipoprotein; **HOMA-IR**, homeostatic model assessment of insulin resistance; **IL-6**, interleukin-6; **LC**, liquid chromatography; **LCAT**, lecitin:cholesterol acyltransferase; **LDL**, low-density lipoprotein; **LF**, low-fat; **LGI**, low-glycemic index; **LPC**,

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lysophosphatidylcholine; **MTBE**, methanol/methyl-tert-butyl ether; **mTORC1**, mammalian target of rapamycin complex 1; **NMR**, nuclear magnetic resonance; **PC**, phosphatidylcholine; **PE**, phosphatidylethanolamine; **PCA**, principal component analysis; **PLS-DA**, partial least squares discriminant analysis; **Q-TOF**, quadrupole time-of-fligth; **SM**, sphingomyelin; **T2D**, type 2 diabetes; **TG**, triglycerides; **TMAO**, trimethylamine-N-oxide; **TMS**, tetramethylsilane; **UHPLC**, ultra-high performance liquid chromatography; **VIP**, variable importance in projection; **VLC**, very-low carbohydrate; **WC**, waist circumference.

Keywords: glycemic index; lipidomics; metabolomics; nutritional intervention; targeted.

Clinical Trial Registry number and website:

This trial was registered at Current Controlled Trials (www.controlled-trials.com) as ISRCTN54971867.

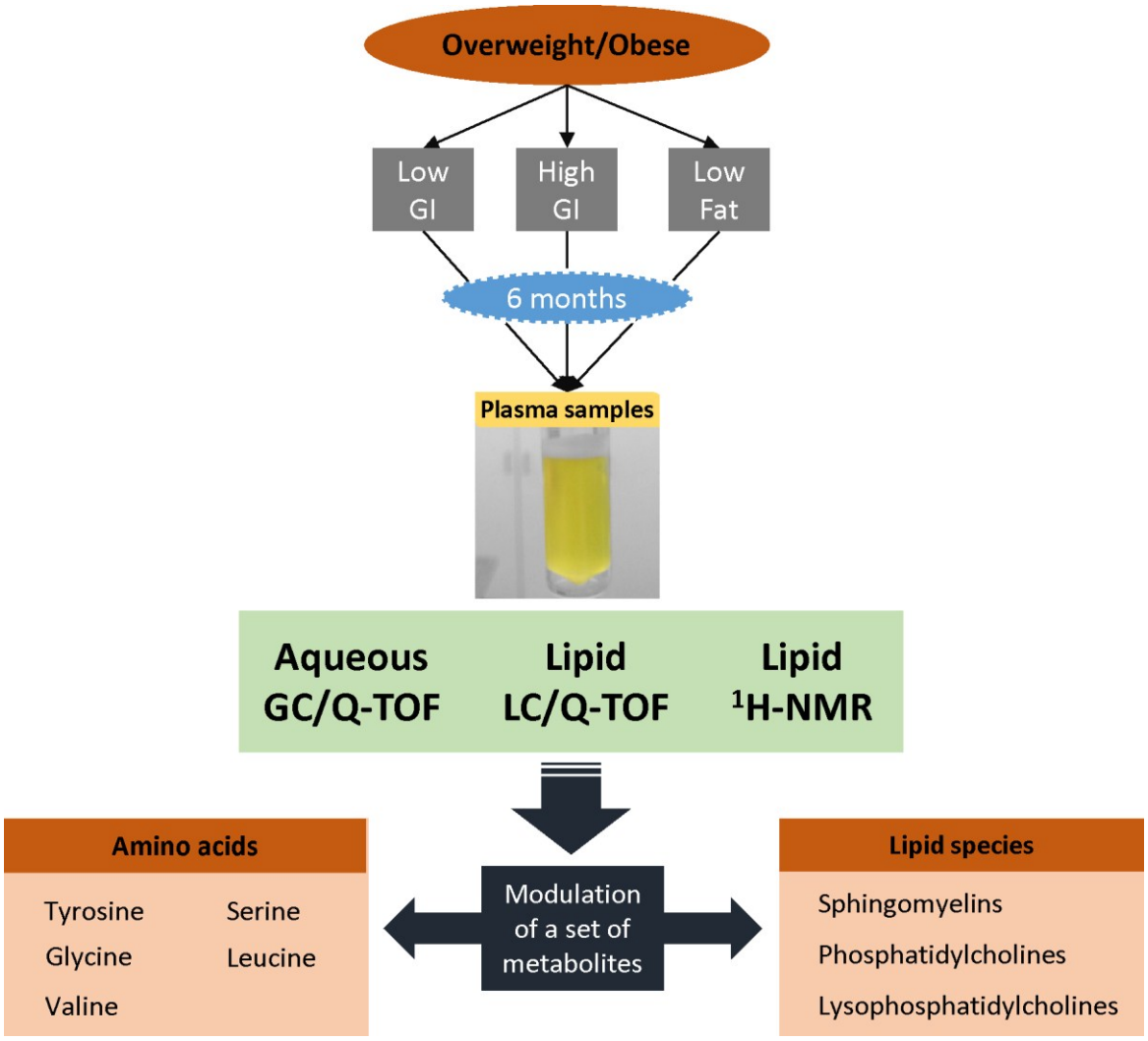
ABSTRACT

Scope*:* To examine whether a low-glycemic index (LGI) diet improves a set of plasma metabolites related to different metabolic diseases and compared to a high-glycemic index (HGI) diet and a low-fat (LF) diet.

Methods and Results*:* We conducted a parallel, randomized trial with three intervention diets: a LGI diet, a HGI diet and a LF diet. A total of 122 adult overweight and obese subjects were enrolled in the study for 6 months. Blood samples were collected at baseline and at the end of the intervention. We analyzed the plasma metabolomic profile of 102 subjects using three different approaches: GC/quadrupole-TOF, LC/quadrupole-TOF and NMR. Both univariate and multivariate analysis were performed. Serine levels were significantly higher following the LGI diet compared to both the HGI and LF diets (q=0.002), whereas leucine (q=0.015) and valine (q=0.024) were lower in the LGI diet compared to the LF diet. A set of two sphingomyelins, two lysophosphatidylcholines and six phosphatidylcholines were significantly modulated after the LGI diet compared to the HGI and LF diets (q<0.05). We found significant correlations between changes in plasma amino acids and lipid species with changes in body weight, glucose, insulin and some inflammatory markers.

Conclusion*:* Our results suggest that a LGI diet modulates certain circulating amino acids and lipid levels. These findings may explain the health benefits attributed to LGI diets in metabolic diseases such as type 2 diabetes.

Low glycemic index (LGI) diets have consistently been related to beneficial metabolic effects on plasma glucose concentrations. However, their potential role in the modulation of other metabolites has not been fully determined. Therefore, we examined whether a low glycemic index diet improves a set of plasma metabolites related to different metabolic diseases and compared to a high-glycemic index diet and a low-fat diet.



1. INTRODUCTION

A growing body of evidence from large epidemiological studies and clinical trials suggests that the glycemic index and glycemic load (GI/GL) play an important role in the prevention or management of several diseases, including obesity, type 2 diabetes (T2D), cardiovascular diseases (CVD) and some types of cancer [1].

Consumption of low GI/GL foods is expected to reduce blood glucose rise and insulin postprandial levels. Therefore, they could contribute to reduce hunger, increase satiety and decrease overeating and, in the long term, they may lead to a weight loss [2]. Results of clinical trials are also generally consistent, showing a decrease in plasma low-density lipoprotein (LDL) cholesterol and triglycerides (TG), and an increase in plasma concentrations of high-density lipoprotein (HDL) cholesterol after following low-GI (LGI) or low-GL diets [3]. Moreover, circulating levels of some inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6), are consistently reduced by a low-GI diet [4]. These findings contribute to explain some of the beneficial effects of LGI/low-GL diets in human health. However, the potential benefits of these type of diets for obesity and metabolic-related disorders could also be attributed to the modulation of other metabolites that have been studied to a lesser extent. This encourages the use of high-throughput technologies such as metabolomics to monitor different molecular species that may be modulated by health-promoting diets.

There is growing evidence that cardiometabolic disorders are characterized by a broad impact on the metabolic physiology, involving considerable changes in lipid species and aminoacids [5, 6]. Lipid metabolites with a high number of double bonds and a longer acyl chain [7], and increased tryptophan concentrations [8] have been inversely associated with the risk of CVD. Similarly, higher concentrations of acylcarnitines [9] and branched-chain aminoacids [10] have been associated with an increased risk of CVD. Specific metabolomic signatures, mainly based on lipid species and amino acids, have also been related to obesity [11] and T2D [12].

Metabolomic modulation after dietary interventions with differential GI content was first identified in 24h-urine samples from overweight subjects [13]. However, a few studies have used metabolomics to identify changes in circulating metabolites after subjects follow a LGI diet for a short length of time

[14, 15]. These studies found a significant modulation of plasma metabolites, including amino acids and specific lipid species, in the LGI diet intervention.

In previous studies we found that an LGI diet is more effective than a high-GI (HGI) or a low-fat (LF) diet for i) losing weight, ii) controlling glucose and insulin metabolism [16] and iii) enhancing the erythrocyte fatty acid membrane composition, in overweight and obese adults [17]. Here, we explore further whether this intervention will result in differences in plasma metabolites that could contribute to explain the health-protective benefits ascribed to LGI diets.

2. MATERIALS AND METHODS

2.1 Design and dietary intervention

The GLYNDIET study is a 6-month randomized, parallel, controlled, clinical trial conducted with 122 subjects between 30 and 60 years of age, with a body mass index (BMI) ranging from 27 to 35 kg/m^2. Details of the study design (i.e. inclusion and exclusion criteria and data collection methods) and the main results of the study are described elsewhere [18]. Briefly, participants were randomly assigned to a 500-kcal energy-restricted LGI, HGI or LF diet following the recommendations of the American Heart Association (AHA) [19], according to the energy requirements estimated using the WHO equations for resting energy expenditure and corrected with estimated physical activity in each subject. All diets provided similar amounts of fiber. Trained dietitians gave nutritional advice to the participants, with specific recommendations regarding the type of carbohydrates and cooking methods to use. To help participants adhere to the intervention, they were provided with biweekly menus and seasonal recipes. The study protocol, which complied with the Helsinki Declaration, was approved by the institutional review board and registered in the International Standard Randomized Controlled Trial Number (ISRCTN54971867). All eligible candidates provided written informed consent.

2.2 Data collection and anthropometric, nutritional and biochemical measurements

Individual examinations were scheduled at baseline, after 15 days of the intervention, and then monthly until the end of the study. Body weight, height, BMI and waist circumference (WC) were recorded at each visit. Body composition was measured with a bioelectrical impedance analysis (TANITA TBF-300; Tanita). Blood pressure was measured in the non-dominant arm with a validated

semiautomatic oscillometer (Omron HEM-705CP; OMRON Corp) in duplicate. At baseline and at the end of the intervention, dietary data were assessed with 3-day dietary records (including two workdays and a weekend day), and energy and nutrient intake were estimated with a Spanish food-composition table [20] and the International GI database [21].

Overnight-fasting blood samples were collected at baseline and at the last study visit. Plasma fasting glucose was determined with standard enzymatic automated methods (COBAS; Roche Diagnostics Ltd) and fasting insulin was determined with a MILLIPLEX® MAP Plex Kit (Merck Millipore, MA, USA). Interleukin-6 (IL-6) (R&D Systems, MN, USA) and C-reactive protein (CRP) (IBL, Hamburg, Germany) were determined in plasma using commercial ELISAs.

2.3 Metabolomic procedures: multiplatform targeted metabolomics

Plasma metabolite profiling included an automated metabolite extraction and a multiplatform analysis using three different infrastructures: Proton nuclear magnetic resonance (^{1}H NMR), liquid chromatography coupled to mass spectrometry (LC-MS) and gas chromatography coupled to mass spectrometry (GC-MS). To normalize the signaling from different samples throughout the entire analysis internal standards were added to the extractions that were analyzed by MS.

2.3.1 Automation of multiple plasma sample extraction

Aqueous extractions of 250 µL of plasma were performed with a methanol/water solution in a Bravo automated liquid handling platform (Agilent Technologies, CA). Lipid extractions of 100 µL of plasma were performed by a biphasic extraction with methanol/methyl-tert-butyl ether (MTBE). Solvents were added automatically to the samples and after the appropriate shaking and centrifugation steps the supernatants were dispensed in 96-well plates and stored until analysis with GC-MS, LC-MS/MS and NMR. Internal standards for GC and LC were previously dispensed by the robot to the same plates where supernatants were collected. Quality controls (i.e. pool of samples) were used in both GC and LC to discard drift in the instrumental response.

2.3.2 Lipid ^{1}H-NMR profiling

Samples were prepared and extracted and the regions identified following the procedure in Vinaixa *et al.* [22] then compared directly with lipid standards. ^{1}H-NMR spectra were recorded at 300K on an

Avance III 600 spectrometer (Bruker, Germany) operating at a proton frequency of 600.20 MHz using a 5 mm PBBO gradient probe. Lipid samples were measured and recorded in PROCNO 11 using a simple pre-saturation sequence (recycle delay (RD)–90°–ACQ pre-saturation pulse (zgpr) program) to eliminate the residual water moisture of deuterated methanol. After pre-processing and visual checking of the NMR dataset, specific ^{1}H regions of diacylgycerols, triglycerides and total lipids based on terminal methyl and methylene signals were identified in the spectra using a comparison in the AMIX 3.9 software (Bruker, Germany). Curated identified regions across the spectra were integrated using the same AMIX 3.9 software package and exported to excel spreadsheets to obtain relative concentrations.

2.3.3 Lipid LC-MS profiling

The lipid species in plasma samples were determined by ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole-time of flight (qTOF) high resolution mass spectrometry (MS) (6550 iFunnel series, Agilent Technologies, Spain). The ionization was performed in positive electrospray, and the mass calibration reference was used in all the analyses to keep the mass accuracy below 5 ppm. Lipids were separated in a C18 reversed phase column (Kinetex C18-EVO from Phenomenex) and a ternary mobile phase (water/methanol/2-propanol) was used. Each lipid was quantified with an internal standard calibration method using one analytical standard and one deuterated internal standard for each lipid family.

2.3.4 Aqueous GC-MS profiling

Samples were analysed in a 7890A Series GC coupled to a triple quadrupole (QqQ) (7000 series; Agilent Technologies, Barcelona, Spain). The chromatographic column was a J&W Scientific HP5-MS (30 m x 0.25 mm i.d., 0.25 μm film; Agilent Technologies, Barcelona, Spain), and helium (99.999% purity) was used as a carrier gas. Ionization was carried out with electronic impact recording data in “Full Scan” mode.

Quantification was performed by internal standard calibration, using the corresponding analytical standard for each determined metabolite and a deuterated internal standard depending on the family of

metabolite. The internal standards used were succinic d_4 acid, glycerol $^{13}C_3$, norvaline, L-methionine-(carboxy-^{13}C,methyl-d_3), D-glucose $^{13}C_6$, myristic-d_{27} acid and alpha-tocopherol d_6.

2.4 Statistical analysis

Descriptive data of participants at baseline and changes during the intervention periods are shown as means ± SEM for continuous variables, and number (%) for categorical variables. Normality was assessed with the Kolmogorov-Smirnov test and variables without a normal distribution were log2-transformed before the analysis. Changes in clinical, demographic and anthropometric variables according to the intervention groups were assessed with ANOVA with Bonferroni correction for multiple comparisons. Within-group changes in metabolic variables and nutrient intake were assessed with a paired *t* test. Changes are expressed as a percentage in the multivariate analysis (to account for baseline values). Between-group differences were assessed with ANCOVA adjusting for baseline values and for changes in body weight (BW) [23]. Spearman's correlation coefficients were used to evaluate whether the changes (final-basal of each intervention period) in different clinical parameters correlated with changes in metabolites in the different interventions.

To conduct the multivariate analysis, data was first centered and scaled using the standard deviation as the scaling factor [24]. Principal component analysis (PCA) was applied and the first two principal components were selected - based on eigenvalues higher than 1 - in order to identify outliers and detect grouping trends. A partial least squares discriminant analysis (PLS-DA) approach was used to determine whether the intervention diets led to holistic differences in metabolic responses. We used leave one out cross-validation method to test the predictive performance of the model and Q^2 (i.e. estimate of predictive ability of the model) values were calculated [25]. The results from the univariate analysis were further adjusted for the FDR (false discovery rate) [26]. The *q-values* derived from the ANCOVA for the three groups are specified in the tables. All analyses were performed using the R software v. 3.4.0, including the package mixOmics for multivariate analysis. All tests were 2-sided, and the significance was set at $P<0.05$ or $q<0.05$.

3. RESULTS

3.1 Study participants' characteristics

A total of 543 participants were screened by phone to identify 215 eligible participants. Of these, 122 met all inclusion criteria and were randomly assigned to one of the three intervention groups (**Supplementary Figure 1**). During the intervention, 17 of the 122 randomized participants (14%) dropped out of the study. Three participants were finally excluded from the analysis due to a withdrawal of informed consent (n=1), lack of plasma sample (n=1) or high outlying values in the PCA of plasma baseline metabolites (n=1; **Supplementary Figure 2.A**). A total of 102 participants were finally included in the analyses (**Supplementary Figure 1**).

The characteristics of the participants according to the intervention groups are given in **Table 1**. No significant differences were observed in sex, age, anthropometric measurements, blood pressure and the prevalence of comorbidities across the three groups.

3.2 Differences in metabolites according to the dietary intervention

A total of 126 metabolites were analysed and quantified by targeted GC/Q-TOF, LC/Q-TOF and NMR (**Supplementary Table 1).** At baseline, no significant differences in the metabolite profiles were observed among the three dietary intervention groups (data not shown). Individual metabolites with significant changes among the intervention groups, the direction of their change and the *q*-value (*P*-value after FDR adjustment) are reported in **Table 2**. We additionally included as **Supplementary Table 2** raw baseline and 6-month changes in the concentration of significant metabolites according to intervention period. Five amino acids were significantly modulated across the groups. Plasma serine levels were found to be significantly increased following the LGI diet compared to both the HGI and LF diets (q=0.002). Moreover, tyrosine was reduced and glycine was increased in the LGI diet compared to the HGI diet. We also found a significant decrease in leucine and valine in the LGI diet compared to the LF diet.

Significant differences in the modulation of lipid species were identified among the intervention diets. C32:1 SM was significantly reduced after the LGI diet compared to the HGI and LF diets, whereas C42:3 SM was reduced in the LGI diet compared to the HGI diet. C20:3 LPC was significantly

increased in the LF diet versus the HGI and LGI diets (q=0.016), whereas C18:2 LPC was significantly increased in the LGI diet versus the HGI and LF diets (q=0.006). The highest impact of the LGI diet was in the modulation of certain PCs. A total of 13 PCs were found to be significantly modulated by the intervention diets, but only 6 PCs remained significant after adjustments. C32:1, C34:2e, C36:2e, C36:5e, C38:5 and C40:6 PC were significantly reduced after the LGI diet compared to the HGI and/or LF diets (**Table 2**). C32:1, C34:2e, C36:2e and C38:5 PC were reduced in the LGI diet compared to the HGI and LF diets, whereas C36:5e PC was reduced in the LGI diet compared to the HGI diet. Moreover, C40:6 PC was increased in the HGI diet compared to the LGI and LF diets (q=0.019).

A significant reduction in total LPC in the LGI diet compared to the HGI diet was observed in the non-adjusted analysis (q=0.042), but statistical significance was lost when we adjusted for baseline values and changes in body weight (q=0.072). When changes in metabolites were adjusted by changes in HOMA-IR (data not shown), the results remained in the same reported direction except for total LPC, which was statistically significant (q=0.021).

The intra-group analysis showed a significant increase in total PCs after a LF diet (q=0.031), together with a significant reduction in plasma linoleic levels in both the HGI and LF diets (q=0.012 and 0.041 respectively). Subjects following either a LGI or HGI diet significantly reduced their plasma levels of free cholesterol, esterified cholesterol, total cholesterol, triglycerides and LPC, and increased their PC and docosahexaenoic acid (DHA) levels. Plasma total PCs and citric acid levels were reduced in all the intervention diets (q<0.05).

PCA of the changes in plasma metabolites across the dietary interventions showed no grouping trends. The total amount of variance explained by the first two principal components was 34% (23% and 11% respectively). PLS-DA score plot of the first two components neither revealed a clear separation among the intervention diets. Moreover, cross-validation reported low predictive performance of the model as reflected in Q^2 values: 0.12 for the first component and 0.17 for the second component. The primary and secondary components accounted for a total of 25% variability. The metabolites with the greatest contribution based on variable importance in projection (VIP) $\geq$ 2 were: glutamic acid, glycine and C18:2 LPC, in the first component; and valine and C32:1 SM, in the second component

(**Supplementary Figure 2.B**). In **Figure 1**, we show the radar plot with the mean centered and scaled change values in each intervention period for those metabolites found to be significantly modulated (q<0.05). There is a differential pattern for the set of significantly modulated metabolites according to the intervention group.

3.3 Correlations between changes in plasma metabolites and changes in clinical variables

Spearman correlation coefficients between changes in metabolites and changes in clinical parameters are shown in **Table 3.** BW, waist circumference (WC), fat mass (FM), glucose, insulin and HOMA-IR were positively correlated to LPC. Body weight was directly correlated with tyrosine, C32:1 SM, C20:3 LPC and C34:2e PC. Fat mass was directly correlated with valine, whereas fat-free mass (FFM) was inversely correlated with C20:3 LPC and directly correlated with C32:1 SM. Fasting glucose was positively correlated with C36:2e PC whereas HOMA-IR was positively correlated with valine, leucine, tyrosine, C20:3 LPC, C36:5e PC, C38:5 PC and LPC. Both CRP and IL-6 were inversely correlated with plasma serine levels but IL-6 levels were further positively correlated with both leucine and tyrosine levels.

4. DISCUSSION

In the present clinical trial, we found differential regulation of certain amino acids and phospholipids after a LGI diet compared to a high-glycemic index diet and a low-fat diet, which could contribute to explaining the health benefits attributed to LGI diets.

Plasma targeted metabolomics in the nutrition field is a promising tool for understanding the effects of diets or food items on metabolic pathways [27]. However, to the best of our knowledge, the potential modulatory effect of the glycemic index or glycemic load on circulating metabolome has only been previously assessed in two different trials with a short follow-up period and using exclusively a LC-MS platform. Barton *et al.* found significant differences in 14 plasma metabolites out of a total of 155 analyzed in 19 healthy adults after a crossover trial of two 28-day diet periods of high and low-GI diets [14]. Similarly, the second study was conducted with 21 obese adults following a crossover design trial for 4-weeks in each period. The authors identified a cluster of 152 metabolites with significantly different concentrations between at least two of the diets (including a LF, LGI, and very-low carbohydrate diet) [15]. Some metabolites, such as cytosine, certain TGs, hydroxyproline,

hippurate, 5-aminolevulinic acid and pipecolic acid, differentiated the LGI diet from the other two interventions.

In our medium-term, randomized, parallel, clinical trial, we found increased levels of serine and glycine, together with reductions in tyrosine, leucine and valine in the LGI diet versus the HGI and/or LF diets. Higher concentrations of serine and glycine were recently associated with a lower risk of developing hypertension within 10 years of follow-up [28]. In general, serine and glycine are known to act as neurotransmitters, to be important for the catalytic function of many enzymes and to represent essential elements of many lipids (e.g., sphingolipids and glycerophospholipids) [29]. Therefore, the increased levels that we found after the LGI diet not only suggest that this diet plays a protective role against the onset of T2D, but also against the risk of hypertension. This is also supported by recent research that indicates that supplementation with serine as well as glycine may have anti-inflammatory and antioxidant properties as well as blood pressure (BP)-lowering effects [30, 31]. The link between GI and hypertension was evaluated in a recent systematic review and meta-analysis of 14 trials comprising 1,097 subjects, and it was found that a lower GI diet could lead to relevant reductions in blood pressure [32]. We did not find any blood pressure modulation in the GLYNDIET study [16] or any significant correlations between these amino acids and HOMA-IR or BP as indirect markers of T2D and hypertension risk. Nevertheless, this may be partly explained by the fact that none of the study subjects had pre-diabetes or T2D and the prevalence of hypertension was low. Moreover, as visceral obesity has been previously associated with lower circulating levels of amino acids, such as glycine and serine [33], the study gives further support to the beneficial effect of the LGI diet on visceral obesity even after adjusting for changes in BW.

Our results also showed a significant reduction in leucine and valine following a LGI diet compared to the LF diet. Circulating levels of branched-chain amino acids (BCAA) tend to be increased in obese individuals and are associated with worse metabolic health and future IR or T2D (reviewed in [34]). One of the hypothesized mechanisms between BCAAs and T2D involves BCAAs and insulin synergistically activating the mammalian target of rapamycin complex 1 (mTORC1), resulting in the uncoupling of insulin signaling at an early stage [35]. However, it is still unclear whether BCAAs are a causative factor in IR and T2D or just a biomarker of impaired insulin action. Interestingly, BCAAs

may serve as substrates for the glucose-alanine cycle in skeletal muscle and, through alanine aminotransferase–catalyzed transamination reactions, this may result in increased substrate availability for hepatic gluconeogenesis, thereby increasing hepatic glucose production [36]. Conversely, glycine is a gluconeogenic amino acid, thus reduced levels may also reflect increased gluconeogenesis. Our findings regarding the modulation of leucine are in line with those from a previous weight-loss intervention study that found that a mild-caloric restriction diet for three years achieved weight loss, HOMA-IR and TG reduction, and also improved a set of plasma metabolites, such as reducing leucine [37]. There are emerging evidences supporting a role of BCAA in adipocyte differentiation and adipose tissue expansion since differences in leucine and valine metabolism have been shown between 3T3-L1 preadipocytes and 3T3-L1 adipocytes [38]. Even though little is known about the potential mechanisms that could regulate this process, the lack of specific amino acids in the culture medium of 3T3-L1 fibroblasts inhibits their differentiation to adipocytes [39]. Overall, this suggests that amino acids play a putative role in glucose, insulin and fat homeostasis and evidences the beneficial role of LGI diets.

Importantly, we found a significant positive correlation between changes in leucine levels and IL-6, together with a negative correlation between serine and CRP levels. This establish a further link between levels of certain circulating amino acids and important inflammatory markers.

In addition to the amino acid profile, changes in the plasma lipidome were observed in the three dietary interventions. Differences in PCs, SM and LPCs have been reported in obesity and other metabolic disorders [40]. In fact, these lipid species are considered the main constituents of cellular membranes that may be involved in cellular signal transduction, and represent a major fraction of the human plasma lipidome because they are most abundant in all lipoproteins [41]. In our study, we found a reduction in the levels of six PCs after subjects followed the LGI diet. In this regard, Floegel *et al.* found that increased values of C32:1 PC are positively correlated with T2D [42], thus the direction of our findings regarding lipid species also supports a beneficial role of LGI against T2D. Moreover, we found a significant positive correlation between changes in some of these PCs and changes in BW, WC and/or FM, linking PC modulation with anthropometric changes. LPC species

are products of PC hydrolysis by secretory phospholipases and lecitin:cholesterol acyltransferase (LCAT) and are carried on high-density lipoprotein, low-density lipoprotein and albumin [43]. LPCs are ligands for G-protein coupled receptors that may exert either pro-infammatory or anti-infammatory responses depending on the FA chain esterified [43]. LPC levels have been found to be increased in serum from obese [44] and T2D patients [45]. Importantly, in monozygotic twins, obesity was primarily related to increases in LPC and to decreases in ether phospholipids [46]. However, this is not consistently reported as some studies show decreased serum LPC species in individuals with impaired glucose tolerance [47] and obesity [48]. Moreover, in a study of diet-induced weight loss in obese subjects, a reduction in PC and predominantly short chain fatty acid triglycerides in serum was observed, while other lipid classes such as sphingolipids and LPC were unaffected by weight loss [49]. The fact that we observed a significant decrease in total LPCs in the LGI versus the HGI diets, suggest that levels of total LPCs may be a composite of the individual effects of BW modulation and dietary factors on each member of this lipid class. In fact, we found a positive correlation between BW and other anthropometric parameters, with plasma LPC levels. Remarkably, as total LPC levels significantly decreased in the LGI diet compared to the HGI diet when we adjusted for changes in HOMA-IR, this could be further evidence for the link between LPC modulation and glucose/insulin homeostasis. Considering specific LPCs, overweight and obese individuals had lower C18:1 and C18:2 LPCs [50], whereas waist circumference was inversely associated with these two LPCs [51]. Moreover, higher levels of C18:2 LPC were independently associated with a decreased risk of T2D in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study [42]. Therefore, the increased plasma levels that we found after a LGI diet in C18:2 LPC compared to after HGI and LF diets, together with a negative correlation between changes in this metabolite and changes in BW, provides evidence of the protective role played by the LGI diet in relation to T2D, and suggests an improvement in the anthropometric parameters in overweight/obese subjects.

We would like to comment on certain strengths and limitations of the study. First, because the study was conducted with non-diabetic, obese subjects, our findings should not be generalized to subjects with T2D and who are not overweight or obese. Second, because not all the lipid classes or the members of each specific class were reported, we cannot discard that there was alternative modulation

by other means. Third, we cannot discriminate between specific isomers in some lipid species, nor between the fatty acid content of some lipids such as PCs and LPCs. Finally, despite the amino acid intake not necessarily correlates with their plasma concentrations due to their complex metabolism [52], we cannot completely discard a residual confounding effect due to different content of dietary amino acids. In addition, even though the intervention was conducted during two years covering all seasons, a potential residual effect could not be completely dismiss. Among the strengths are the study's medium-term duration, the sample size and the randomized design that was balanced for gender and age in the three interventions. Unlike previous studies, we also included LF and HGI diets. Moreover, the use of a targeted and multi-platform approach allowed a wide plasma metabolomic range to be covered, and the use of standards allowed their plasma concentration to be determined. Importantly, we have adjusted our analysis to changes in body weight to isolate the independent effect of the intervention diets on plasma metabolites.

In conclusion, following a LGI diet may help to improve the blood levels of certain amino acids and lipids related to metabolic diseases such as T2D, beyond their proven beneficial effect on insulin resistance and weight-loss. Further research is needed to explore alternative pathways by which LGI and low GL diets could contribute globally to human health and specifically to the prevention and/or treatment of different cardiovascular and metabolic diseases.

Author contributions:

MB had full access to all the data in the study and takes full responsibility for the integrity and accuracy of the data analysis. Study concept and design: MB, JS-S and PH-A. Acquisition of data: PH-A. Analysis and interpretation of data: PH-A, SG, DC, NC and MB. Drafting of the manuscript: PH-A, MB and SG. Critical revision of the manuscript for important intellectual content: MB and JS-S. Statistical analysis: PH-A and MB. Obtained funding: MB. Administrative, technical, and material support. All the authors read and approved the final manuscript.

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Conflict of interest statement:

I certify that neither I nor my co-authors have a conflict of interest as described above that is relevant to the subject matter or materials included in this Work.

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5. REFERENCES

[1] WHO, World Health Organization, Obesity and overweight. *Glob. Strateg Diet, Phys. Act. Heal.* 2016, 1–2.

[2] Radulian, G., Rusu, E., Dragomir, A., Posea, M., Metabolic effects of low glycaemic index diets. *Nutr. J.* 2009, *8*, 5.

[3] Bouche, C., Rizkalla, S.W., Luo, J., Vidal, H., Veronese, A., Pacher, N., Fouquet, C., Lang, V., Slama, G., Five-week, low-glycemic index diet decreases total fat mass and improves plasma lipid profile in moderately overweight nondiabetic men . *Diabetes Care* 2002, *25*, 822–828.

[4] Qi, L., Hu, F.B., Dietary glycemic load, whole grains, and systemic inflammation in diabetes: the epidemiological evidence. *Curr. Opin. Lipidol.* 2007, *18*, 3–8.

[5] Park, S., Sadanala, K.C., Kim, E.-K., A Metabolomic Approach to Understanding the Metabolic Link between Obesity and Diabetes. *Mol. Cells* 2015, *38*, 587–596.

[6] Newgard, C.B., Metabolomics and Metabolic Diseases: Where Do We Stand? *Cell Metab.* 2017, *25*, 43–56.

[7] Toledo, E., Wang, D.D., Ruiz-Canela, M., Clish, C.B., Razquin, C., Zheng, Y., Guasch-Ferré, M., Hruby, A., Corella, D., Gómez-Gracia, E., Fiol, M., Estruch, R., Ros, E., Lapetra, J., Fito, M., Aros, F., Serra-Majem, L., Liang, L., Salas-Salvadó, J., Hu, F.B., Martínez-González, M.A., Plasma lipidomic profiles and cardiovascular events in a randomized intervention trial with the Mediterranean diet. *Am. J. Clin. Nutr.* 2017, *106*, 973–983.

[8] Yu, E., Ruiz-Canela, M., Guasch-Ferré, M., Zheng, Y., Toledo, E., Clish, C.B., Salas-Salvadó, J., Liang, L., Wang, D.D., Corella, D., Fitó, M., Gómez-Gracia, E., Lapetra, J., Estruch, R., Ros, E., Cofán, M., Arós, F., Romaguera, D., Serra-Majem, L., Sorlí, J. V, Hu, F.B., Martinez-Gonzalez, M.A., Increases in Plasma Tryptophan Are Inversely Associated with Incident Cardiovascular Disease in the Prevención con Dieta Mediterránea (PREDIMED) Study. *J. Nutr.* 2017, *147*, 314–322.

[9] Guasch-Ferré, M., Zheng, Y., Ruiz-Canela, M., Hruby, A., Martínez-González, M.A., Clish, C.B., Corella, D., Estruch, R., Ros, E., Fitó, M., Dennis, C., Morales-Gil, I.M., Arós, F., Fiol, M., Lapetra, J., Serra-Majem, L., Hu, F.B., Salas-Salvadó, J., Plasma acylcarnitines and risk of cardiovascular disease: effect of Mediterranean diet interventions. *Am. J. Clin. Nutr.* 2016, *103*, 1408–16.

[10] Ruiz-Canela, M., Toledo, E., Clish, C.B., Hruby, A., Liang, L., Salas-Salvadó, J., Razquin, C., Corella, D., Estruch, R., Ros, E., Fitó, M., Gómez-Gracia, E., Arós, F., Fiol, M., Lapetra, J., Serra-Majem, L., Martínez-González, M.A., Hu, F.B., Plasma Branched-Chain Amino Acids and Incident Cardiovascular Disease in the PREDIMED Trial. *Clin. Chem.* 2016, *62*, 582–92.

[11] Carayol, M., Leitzmann, M.F., Ferrari, P., Zamora-Ros, R., Achaintre, D., Stepien, M., Schmidt, J.A., Travis, R.C., Overvad, K., Tjønneland, A., Hansen, L., Kaaks, R., Kühn, T., Boeing, H., Bachlechner, U., Trichopoulou, A., Bamia, C., Palli, D., Agnoli, C., Tumino, R., Vineis, P., Panico, S., Quirós, J.R., Sánchez-Cantalejo, E., Huerta, J.M., Ardanaz, E., Arriola, L., Agudo, A., Nilsson, J., Melander, O., Bueno-de-Mesquita, B., Peeters, P.H., Wareham, N., Khaw, K.-T., Jenab, M., Key, T.J., Scalbert, A., Rinaldi, S., Blood Metabolic Signatures of Body Mass Index: A Targeted Metabolomics Study in the EPIC Cohort. *J. Proteome Res.* 2017, *16*, 3137–3146.

[12] Liu, J., Semiz, S., van der Lee, S.J., van der Spek, A., Verhoeven, A., van Klinken, J.B., Sijbrands, E., Harms, A.C., Hankemeier, T., van Dijk, K.W., van Duijn, C.M., Demirkan, A.,

Metabolomics based markers predict type 2 diabetes in a 14-year follow-up study. *Metabolomics* 2017, *13*, 104.

[13] Rasmussen, L.G., Winning, H., Savorani, F., Ritz, C., Engelsen, S.B., Astrup, A., Larsen, T.M., Dragsted, L.O., Assessment of dietary exposure related to dietary GI and fibre intake in a nutritional metabolomic study of human urine. *Genes Nutr.* 2012, *7*, 281–93.

[14] Barton, S., Navarro, S.L., Buas, M.F., Schwarz, Y., Gu, H., Djukovic, D., Raftery, D., Kratz, M., Neuhouser, M.L., Lampe, J.W., Targeted plasma metabolome response to variations in dietary glycemic load in a randomized, controlled, crossover feeding trial in healthy adults. *Food Funct.* 2015, *6*, 2949–2956.

[15] Esko, T., Hirschhorn, J.N., Feldman, H.A., Hsu, Y.H.H., Deik, A.A., Clish, C.B., Ebbeling, C.B., Ludwig, D.S., Metabolomic profiles as reliable biomarkers of dietary composition. *Am. J. Clin. Nutr.* 2017, *105*, 547–554.

[16] Juanola-Falgarona, M., Salas-Salvadó, J., Ibarrola-Jurado, N., Rabassa-Soler, A., Díaz-López, A., Guasch-Ferré, M., Hernández-Alonso, P., Balanza, R., Bulló, M., Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. *Am. J. Clin. Nutr.* 2014, *100*, 27–35.

[17] Giardina, S., Sala-Vila, A., Hernández-Alonso, P., Calvo, C., Salas-Salvadó, J., Bulló, M., Carbohydrate quality and quantity affects the composition of the red blood cell fatty acid membrane in overweight and obese individuals. *Clin. Nutr.* 2017.

[18] Juanola-Falgarona, M., Ibarrola-Jurado, N., Salas-Salvado, J., Rabassa-Soler, A., Bullo, M., Design and Methods of the Glyndiet Study; Assessing the Role of Glycemic Index on Weight Loss and Metabolic Risk Markers . *Nutr. Hosp.* 2013, *28*, 382–390.

[19] Sacks, F.M., Lichtenstein, A.H., Wu, J.H.Y., Appel, L.J., Creager, M.A., Kris-Etherton, P.M., Miller, M., Rimm, E.B., Rudel, L.L., Robinson, J.G., Stone, N.J., Van Horn, L. V, American Heart Association, O. behalf of the A.H., Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. *Circulation* 2017, *136*, e1–e23.

[20] Feinberg, M., Favier, J.C., Trque, C., Ireland-Ripert, J., Répertoire général des aliments (REGAL). Table de composition, Lavoisier, Paris 1995.

[21] Atkinson, F.S., Foster-Powell, K., Brand-Miller, J.C., International tables of glycemic index and glycemic load values: 2008 . *Diabetes Care* 2008, *31*, 2281–2283.

[22] Vinaixa, M., Ángel Rodríguez, M., Rull, A., Beltrán, R., Bladé, C., Brezmes, J., Cañellas, N., Joven, J., Correig, X., Metabolomic Assessment of the Effect of Dietary Cholesterol in the Progressive Development of Fatty Liver Disease. *J. Proteome Res.* 2010, *9*, 2527–2538.

[23] Harris, J.E., Sheean, P.M., Gleason, P.M., Bruemmer, B., Boushey, C., Publishing Nutrition Research: A Review of Multivariate Techniques—Part 2: Analysis of Variance. *J. Acad. Nutr. Diet.* 2012, *112*, 90–98.

[24] van den Berg, R.A., Hoefsloot, H.C.J., Westerhuis, J.A., Smilde, A.K., van der Werf, M.J., Centering, scaling, and transformations: Improving the biological information content of metabolomics data. *BMC Genomics* 2006, *7*.

[25] Westerhuis, J.A., Hoefsloot, H.C.J., Smit, S., Vis, D.J., Smilde, A.K., Velzen, E.J.J., Duijnhoven, J.P.M., Dorsten, F.A., Assessment of PLSDA cross validation. *Metabolomics* 2008, *4*, 81–89.

[26] Glickman, M.E., Rao, S.R., Schultz, M.R., False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. *J. Clin. Epidemiol.* 2014, *67*, 850–

857.

[27] Brennan, L., Metabolomics in nutrition research: current status and perspectives. *Biochem. Soc. Trans.* 2013, *41*, 670–3.

[28] Dietrich, S., Floegel, A., Weikert, C., Pischon, T., Boeing, H., Drogan, D., Identification of Serum Metabolites Associated with Incident Hypertension in the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. *Hypertension* 2016, *68*, 471–477.

[29] Wu, G., Amino acids: Metabolism, functions, and nutrition. *Amino Acids* 2009, *37*, 1–17.

[30] Mishra, R.C., Tripathy, S., Desai, K.M., Quest, D., Lu, Y., Akhtar, J., Gopalakrishnan, V., Nitric oxide synthase inhibition promotes endothelium-dependent vasodilatation and the antihypertensive effect of L-serine. *Hypertension* 2008, *51*, 791–796.

[31] Diaz-Flores, M., Cruz, M., Duran-Reyes, G., Munguia-Miranda, C., Loza-Rodriguez, H., Pulido-Casas, E., Torres-Ramirez, N., Gaja-Rodriguez, O., Kumate, J., Baiza-Gutman, L.A., Hernandez-Saavedra, D., Oral supplementation with glycine reduces oxidative stress in patients with metabolic syndrome, improving their systolic blood pressure. *Can. J. Physiol. Pharmacol.* 2013, *91*, 855–860.

[32] Evans, C. EL, Greenwood, D.C., Threapleton, D.E., Gale, C.P., Cleghorn, C.L., Burley, V.J., Glycemic index, glycemic load, and blood pressure: a systematic review and meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* 2017, *105*, 1176–1190.

[33] Takashina, C., Tsujino, I., Watanabe, T., Sakaue, S., Ikeda, D., Yamada, A., Sato, T., Ohira, H., Otsuka, Y., Oyama-Manabe, N., Ito, Y.M., Nishimura, M., Associations among the plasma amino acid profile, obesity, and glucose metabolism in Japanese adults with normal glucose tolerance. *Nutr. Metab. (Lond).* 2016, *13*, 5.

[34] Lynch, C.J., Adams, S.H., Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat. Rev. Endocrinol.* 2014, *10*, 723–36.

[35] Yoon, M.-S., Mee-Sup, The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism. *Nutrients* 2016, *8*, 405.

[36] Ruderman, N.B., Muscle amino acid metabolism and gluconeogenesis. *Annu. Rev. Med.* 1975, *26*, 245–258.

[37] Kim, M., Lee, S.-H., Lee, J.H., Global Metabolic Profiling of Plasma Shows that Three-Year Mild-Caloric Restriction Lessens an Age-Related Increase in Sphingomyelin and Reduces L-leucine and L-phenylalanine in Overweight and Obese Subjects. *Aging Dis.* 2016, *7*, 721–733.

[38] Salinas-Rubio, D., Tovar, A.R., Noriega, L.G., Emerging perspectives on branched-chain amino acid metabolism during adipocyte differentiation. *Curr. Opin. Clin. Nutr. Metab. Care* 2018, *21*, 49–57.

[39] Estrada-Alcalde, I., Tenorio-Guzman, M.R., Tovar, A.R., Salinas-Rubio, D., Torre-Villalvazo, I., Torres, N., Noriega, L.G., Metabolic Fate of Branched-Chain Amino Acids During Adipogenesis, in Adipocytes From Obese Mice and C2C12 Myotubes. *J. Cell. Biochem.* 2017, *118*, 808–818.

[40] Graessler, J., Schwudke, D., Schwarz, P.E.H., Herzog, R., Shevchenko, A., Bornstein, S.R., Top-down lipidomics reveals ether lipid deficiency in blood plasma of hypertensive patients. *PLoS One* 2009, *4*.

[41] Cole, L.K., Vance, J.E., Vance, D.E., Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* 2012, *1821*, 754–761.

[42] Floegel, A., Stefan, N., Yu, Z., M??hlenbruch, K., Drogan, D., Joost, H.G., Fritsche, A., H??ring, H.U., De Angelis, M.H., Peters, A., Roden, M., Prehn, C., Wang-Sattler, R., Illig, T., Schulze, M.B., Adamski, J., Boeing, H., Pischon, T., Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes* 2013, *62*, 639–648.

[43] Wiesner, P., Leidl, K., Boettcher, A., Schmitz, G., Liebisch, G., Lipid profiling of FPLC separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J. Lipid Res.* 2009, *50*, 574–585.

[44] Pietil??inen, K.H., Naukkarinen, J., Rissanen, A., Saharinen, J., Ellonen, P., Ker??nen, H., Suomalainen, A., G??tz, A., Suortti, T., Yki-J??rvinen, H., Ore??i??, M., Kaprio, J., Peltonen, L., Global transcript profiles of fat in monozygotic twins discordant for BMI: Pathways behind acquired obesity. *PLoS Med.* 2008, *5*, 0472–0483.

[45] Rabini, R.A., Galassi, R., Fumelli, P., Dousset, N., Solera, M.L., Valdiguie, P., Curatola, G., Ferretti, G., Taus, M., Mazzanti, L., Reduced Na(+)-K(+)-ATPase activity and plasma lysophosphatidylcholine concentrations in diabetic patients. *Diabetes* 1994, *43*, 915–9.

[46] Pietiläinen, K.H., Sysi-Aho, M., Rissanen, A., Seppänen-Laakso, T., Yki-Järvinen, H., Kaprio, J., Orešič, M., Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects - A monozygotic twin study. *PLoS One* 2007, *2*.

[47] Zhao, X., Fritsche, J., Wang, J., Chen, J., Rittig, K., Schmitt-Kopplin, P., Fritsche, A., Häring, H.U., Schleicher, E.D., Xu, G., Lehmann, R., Metabonomic fingerprints of fasting plasma and spot urine reveal human pre-diabetic metabolic traits. *Metabolomics* 2010, *6*, 362–374.

[48] Barber, M.N., Risis, S., Yang, C., Meikle, P.J., Staples, M., Febbraio, M.A., Bruce, C.R., Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. *PLoS One* 2012, *7*.

[49] Schwab, U., Seppänen-Laakso, T., Yetukuri, L., Ågren, J., Kolehmainen, M., Laaksonen, D.E., Ruskeepää, A.L., Gylling, H., Uusitupa, M., Orešcić, M., Triacylglycerol fatty acid composition in diet-induced weight loss in subjects with abnormal glucose metabolism - The GENOBIN study. *PLoS One* 2008, *3*.

[50] Kim, J.Y., Park, J.Y., Kim, O.Y., Ham, B.M., Kim, H.-J.J., Kwon, D.Y., Jang, Y., Lee, J.H., Metabolic profiling of plasma in overweight/obese and lean men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). *J. Proteome Res.* 2010, *9*, 4368–4375.

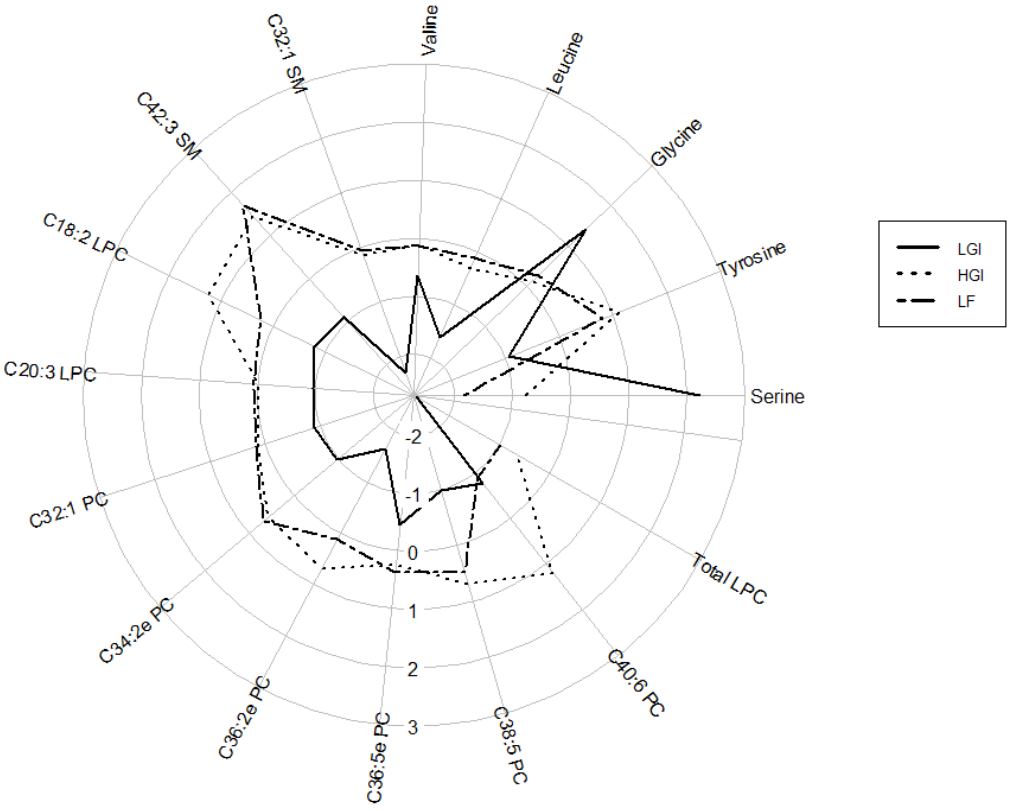
[51] Rauschert, S., Uhl, O., Koletzko, B., Kirchberg, F., Mori, T.A., Huang, R.-C., Beilin, L.J., Hellmuth, C., Oddy, W.H., Lipidomics Reveals Associations of Phospholipids With Obesity and Insulin Resistance in Young Adults. *J. Clin. Endocrinol. Metab.* 2016, *101*, 871–879.

[52] Jennings, A., MacGregor, A., Pallister, T., Spector, T., Cassidy, A., Associations between branched chain amino acid intake and biomarkers of adiposity and cardiometabolic health independent of genetic factors: A twin study. *Int. J. Cardiol.* 2016, *223*, 992–998.

FIGURES CAPTION

Figure 1. Radar plot of significantly modulated metabolites.

Data show the mean centered and scaled change value for each metabolite in each intervention period. Bold lines connect each metabolite. Acronyms: HGI, high-glycemic index; LF, low fat; LGI, low-glycemic index; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin.



TABLES

Table 1. Baseline characteristics of study participants

Variable	LGI diet (n=36)	HGI diet (n=36)	LF diet (n=30)	*P*-value
Female sex	28 (78)	30 (83)	24 (77)	0.789
Age (y)	42.14 ± 1.21	44.86 ± 1.32	44.87 ± 1.45	0.238
Weight (kg)	83.59 ± 1.61	82.62 ± 1.72	83.95 ± 1.92	0.856
BMI (kg/m^2)	31.31 ± 0.36	30.77 ± 0.38	30.74 ± 0.38	0.477
Waist circumference (cm)	101.4 ± 1.3	100.6 ± 1.4	103.9 ± 1.2	0.202
Free fat mass (kg)	50.28 ± 1.34	49.74 ± 1.58	50.76 ± 1.64	0.893
Systolic blood pressure (mmHg)	127.8 ± 2.4	129.0 ± 2.5	133.3 ± 2.4	0.270
Diastolic blood pressure (mmHg)	79.5 ± 1.4	82.1 ± 1.66	83.8 ± 1.7	0.154
Hypercholesterolemia, n (%)	2 (6)	2 (6)	5 (16)	0.219
Hypertension, n (%)	5 (14)	5 (14)	5 (16)	0.957
Smoking status, n (%)	7 (19)	5 (14)	4 (13)	0.720
Leisure-time physical activity (MET-min/day)	1012.9 ± 236.0	1270.8 ± 211.7	828.5 ± 155.5	0.332

N=102 subjects. Data are expressed as mean ± SEM except for qualitative variables, expressed as number (%). HGI, high-glycemic index; LF, low fat; LGI, low-glycemic index. *P*-values of the difference between intervention groups (ANOVA for the continuous variables and a $\chi 2$ test for categorical variables).

Table 2. Metabolites significantly modulated by intervention diets.

Metabolites	*q*-value	LGI (n=36)	HGI (n=36)	LF (n=30)
GC/Q-TOF				
Serine	0.002 [a,b]	↑	↓	↓
Tyrosine	0.012 [a]	↓	↑	↑
Glycine	0.002 [a]	↑	↑	↑
Leucine	0.015 [b]	↓	↓	↓
Valine	0.024 [b]	↓	↓	↑
LC/Q-TOF				
C32:1 SM	0.019 [a,b]	↓↓	↓	↓
C42:3 SM	0.034 [a]	↓	↑	↑
C18:2 LPC	0.006 [a,b]	↑	↓	↓
C20:3 LPC	0.016 [b,c]	↓	↓	↑
C32:1 PC[+]	0.006 [a,b]	↓	↑	↑
C34:2e PC[+]	0.002 [a,b]	↓	↑	↑
C36:2e PC[+]	0.016 [a,b]	↓	↑	↑
C36:5e PC[+]	0.004 [a]	↓	↑	↑
C38:5 PC	0.048 [a,b]	↓	↑	↑
C40:6 PC	0.019 [a,c]	↓	↑	↓
NMR				
Total LPC	0.072 [a*]	↓↓	↓	↓

N=102 subjects. ANCOVA results adjusting for baseline values and changes in body weight during the intervention. We conducted a false discovery rate adjustment within each metabolomic platform, thus *q*-values are reported instead of *P*-values. Acronyms: ↓, reduction; ↑, increase; GC, gas chromatography; HGI, high glycemic index; LC, liquid chromatography; LF, low fat; LGI, low glycemic index; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; Q-TOF, quadrupole time-of-flight; SM, sphingomyelin. Legend: [a], $q<0.05$ LGI vs HGI; [b], $q<0.05$ LGI vs LF; [c], $q<0.05$ HGI vs LF. [*], $q=0.042$ in the ANOVA without adjusting for covariates; [+], ether-linked isobaric species of plasmanyl (e) analogue of glycerophospholipids.

Table 3. Correlations between changes in metabolites and changes in anthropometric and biochemical parameters.

Metabolites	BW	WC	FM	FFM	Glucose	Insulin	HOMA-IR	CRP	IL-6
Glycine	0.149	-0.028	0.095	0.185	0.068	0.089	0.008	-0.144	0.022
Valine	0.189	0.182	0.233 *	-0.035	0.045	0.267 *	0.278 *	-0.005	-0.056
Leucine	0.104	0.054	0.06	0.095	0.216 *	0.288 *	0.272 *	0.023	0.2 *
Serine	0.079	0.042	0.044	0.078	0.032	0.048	0.056	-0.243 *	-0.322 **
Tyrosine	0.227 *	0.165	0.227 *	0.105	0.226 *	0.288 *	0.298 *	0.012	0.229 *
C32:1 SM	0.276 *	0.185	0.321 *	0.205 *	0.147	0.193	0.185	-0.134	0.031
C42:3 SM	-0.137	-0.074	-0.084	-0.131	0.036	0.014	-0.005	-0.048	-0.039
C18:2 LPC	-0.379 *	0.041	-0.127	0.049	0.259	0.168	0.118	-0.022	-0.14
C20:3 LPC	0.283 *	0.189	0.287 *	-0.228 *	0.207 *	0.293 *	0.295 *	0.019	-0.04
C32:1 PC†	-0.076	0.09	-0.011	-0.099	0.069	0.147	0.156	0.01	0.022
C34:2e PC†	0.294 *	-0.078	0.267 *	-0.099	0.035	0.052	0.053	-0.108	0.07
C36:2e PC†	-0.091	0.041	-0.063	-0.097	0.256 *	0.112	0.166	0.039	-0.021
C36:5e PC †	0.042	0.15	0.054	-0.024	0.241 *	0.191	0.195 *	-0.041	-0.152
C38:5 PC	0.082	0.211 *	0.122	0.043	0.179 *	0.102 *	0.233 *	-0.018	-0.067
C40:6 PC	0.008	0.05	0.009	0.058	-0.046	0.133	0.121	-0.052	0.036
LPC	0.305 *	0.294 *	0.317 *	0.064	0.288 *	0.248 *	0.249 *	-0.053	-0.052

N=102 subjects. Spearman correlation coefficients between metabolites and clinical variables. Acronyms: BW, body weight; CRP, C-reactive protein; FFM, fat-free mass; FM, fat mass; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin-6; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin; WC, waist circumference. Legend: *, *P*-value<0.05; **, *P*-value<0.001; †, ether-linked isobaric species of plasmanyl (e) analogue of glycerophospholipids.