Exploring the links between diet and health in an Irish cohort: A lipidomic approach

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Abstract

Epidemiology and clinical studies provide clear evidence of the complex links between diet and health. To understand these links, reliable dietary assessment methods are pivotal. Biomarkers have emerged as more objective measures of intake compared to traditional dietary assessment methods. However, there are only a limited number of putative biomarkers of intake successfully identified and validated. The use of biomarkers that reflect food intake to examine diet related diseases represents the next step in biomarker research. Therefore, the aim of this study was to (1) identify and confirm biomarkers associated with dietary fat intake and (2) examine the relationship between those biomarkers with health parameters. Heatmap analysis identified a panel of 22 lipid biomarkers associated with total dietary fat intake in the Metabolic Challenge (MECHE) Study. Confirmation of four of these biomarkers demonstrated responsiveness to different levels of fat intake in a separate intervention study (NutriTech study). Linear regression identified a significant relationship between the panel of dietary fat biomarkers and HOMA-IR, with 3 lipid biomarkers (C16, PCaaC36:2 & PCaeC36:4) demonstrating significant associations. Identifying such links allows us to explore the relationship between diet and health, to determine whether these biomarkers can be modulated through diet to improve health outcomes.

Introduction

Epidemiology and clinical studies have shown clear evidence that a number of diseases with high morbidity and mortality are linked with diet; examples of such diseases include diabetes, cardiovascular disease (CVD) and a number of cancers.[1] Therefore, reliable dietary assessment methods are necessary when attempting to understand the complex links between diet and health. At present, the majority of epidemiologic studies rely on traditional self-reported dietary assessment methods, which are associated with errors such as under-reporting and recall bias/errors.[2, 3] To address some of these errors there is an increased interest in using dietary biomarkers to provide a more objective measure of intake.[4, 5]

Metabolomics has emerged as an important tool in dietary biomarker discovery. Applications of metabolomics to identify novel dietary biomarkers have in general taken three approaches (1) acute intervention studies with specific foods (2) cohort studies and (3) analysis of dietary patterns and metabolic profiles.[6, 7] In this first approach of acute intervention studies participants consume specific food items, biofluids are collected, and metabolomics techniques are applied with potential biomarkers identified. In cohort studies, low and high consumers of a specific food are usually selected and their metabolomic profiles compared, which can result in the discovery of potential dietary biomarkers.[6, 8] The third approach looks at the concept of using biomarkers to reflect dietary patterns, where profiles have been linked to habitual dietary patterns to identify nutritypes and biomarkers.[9-12]

These approaches have led to the identification of a number of putative biomarkers of exposure to certain foods and beverages such as red meat,[13, 14] citrus fruits,[15-17] cruciferous vegetables,[18, 19] coffee[20, 21] and sugar sweetened beverages.[8] Examples of such biomarkers include proline betaine which has been associated with citrus fruit intake in a range of different studies. [15-18] However, to date there is a dearth of studies demonstrating the potential application and/or sensitivity of such biomarkers.

One potential application includes the use of biomarkers that reflect food intake to study diet related diseases. Recently Wittenbecher and colleagues used a metabolomic and statistical approach to link red meat consumption to type 2 diabetes (T2D) in the European Prospective Investigation into Cancer and Nutrition-Potsdam cohort. Habitual diet was assessed with validated semi quantitative food-frequency questionnaires and serum samples were analysed by high-throughput flow injection tandem mass spectrometry. Statistical analysis identified six serum biomarkers (ferritin, glycine, diacyl phosphatidylcholines 36:4 and 38:4, lysophosphatidylcholine 17:0 and hydroxyl-sphingomyelin 14:1) to be associated with red meat consumption and diabetes risk in the study cohort. This is the first reported study to evaluate a large set of metabolites as potential mediators linking red meat intake and diabetes risk. Such results generate and underline experimentally testable hypotheses that can advise future dietary interventions in terms of design and biomarker assessment.[22] From a public health perspective it is important to investigate modifiable risk factors that contribute to alterations in metabolite concentrations impacting disease risk.[12] For the present study we focused on insulin resistance and the metabolic syndrome. Insulin resistance is the condition where the body does not respond correctly to insulin levels. It is commonly associated with obesity, hypertension and the MetS and often precedes the onset of T2D making it an important measure of metabolic health.

The objective of the present work was therefore to (1) identify and confirm biomarkers associated with total dietary fat intake and (2) examine the relationship between those biomarkers with health parameters.

Experimental Section

Ethics statements

Ethical approval for the MECHE study was obtained from the Research Ethics Committee in University College Dublin (LS-08-43-Gibney-Ryan). The NutriTech intervention study was approved

by the London Brent Ethics Committee (12/LO/0139). All procedures were conducted according to the principles expressed in the Declaration of Helsinki.

(1) Discovery Study: MECHE Study

Subjects

A total of 214 healthy adults aged 18-60 years were recruited between 2008 and 2010 and provided their written informed consent. Good health was defined as the absence of a known chronic or infectious disease and supported by a series of fasting blood tests. Detailed information on the study has been previously published.[23, 24] For the present study, participants (n=188) who had complete dietary data and lipidomic data were included (Table 1).

Sample Collection

Following a 12 hour overnight fast, serum and plasma samples were collected into serum tubes containing a clot activator coating, EDTA-coated and lithium heparin containing tubes for plasma isolation as previously described [23, 24]. Serum samples were allowed time to clot (30 minutes) at room temperature. EDTA and lithium tubes were placed directly on ice. All blood samples were centrifuged at 1800 g for 15 minutes at a temperature of 4 ºC and 500 µl aliquots were stored at -80 ºC until required for further analysis. For the lipidomic analysis lithium heparin samples were used.

Anthropometric and Biochemical Parameters

Height was measured using a wall-mounted Harpenden stadiometer and weight was measured on a calibrated beam balance platform digital body weight scale (SECA 888, Germany). Percentage body fat was measured using an air-displacement plethysmograph (BOD-POD GS system, UK) in accordance with the manufacturer's instructions. Percentage body fat measurements were taken in fasting state.

Clinical chemistry analysis was performed using an RxDaytona™ chemical autoanalyser (Randox Laboratories, UK) and Randox reagents. Details of the analytes and methods are as follows; total cholesterol (cholesterol oxidase); HDL-cholesterol (direct clearance); glucose (glucose oxidase) and triacylglycerol (lipase/glycerol kinase colorimetric) in plasma were measured by using a biochip array system (Evidence Investigator; Randox laboratories). Standard quality control procedures were followed on both analysers to ensure integrity of the data. The HOMA-IR was used as a proxy estimate of insulin sensitivity and was calculated as fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)/22.5.

Dietary Data

Dietary data was collected using the European Prospective Investigation into Cancer (EPIC) food frequency questionnaire (FFQ), which contains over 130 foods used to assess typical dietary intake over the previous year.[25] A total of 25 food groups were examined which included: alcohol, bread, butter/margarine, cakes, cereal, cereals other, cheese, dairy other, drinks, eggs, fish, fruit, legumes, meat, meat products, milk, nuts/seeds, offal, potatoes, sauces, soups, sugar, tea, vegetables and vegetable dishes. Prior to analysis food group data was normalised using a logarithmic transformation. Food groups are reported in grams per day (g/d) and macronutrient intakes are reported as percentage total energy (% TE).

Lipidomic Analysis

Plasma (lithium heparin) samples were sent to BIOCRATES Life Sciences AG (Innsbruck, Austria) for targeted lipidomic analysis, where the TargetIDQ™ P150 kit and in-house lipid assays were used to identify and quantify phospholipid, sphingolipid, ceramide and acylcarnitine metabolites. Metabolites were quantitatively analysed by a high-throughput flow injection electrospray ionization tandem mass spectrometry (ESI-MS/MS) screening method. For further information please refer to O'Gorman et al 2014.[9]

(2) Confirmation Study (NutriTech Study): Demonstration of dose response of biomarkers associated with dietary fat intake in an independent study

For the NutriTech study, participants were randomly assigned to one of five different treatment diets; red meat, fish, poultry, processed meat or a supplement and vegetarian option. Participants attended the National Institute for Health Research (NIHR)/Wellcome Trust Imperial Clinical Research Facility, Imperial College London for three days over three consecutive weeks. During this time participants consumed a standardised breakfast and their treatment meals at midday and evening. All meals were designed to provide similar intakes of dietary energy and fibre but macronutrient composition varied with carbohydrate decreasing from week 1 to week 3 and protein and fat intake increasing from week 1 to week 3: Week 1: 13% protein, 30% fat, 57% carbohydrate; Week 2: 20% protein, 35% fat, 45% carbohydrate and Week 3: 30% protein, 40% fat, 30% carbohydrate. For the purpose of this analysis we focused on the red meat group, with a particular interest in the increasing fat intake over the 3 weeks, to confirm our findings in the MECHE study. Ten participants were recruited to this red meat group. Eligibility criteria included males and females aged 18-65 years with a BMI of 18.5-35 kg/m^2 and free from any chronic medical condition. Information on the participants characteristics is provided in Supporting Information Table S-1. A fasting plasma sample was collected on day 4 of each intervention week: 4 ml of blood was collected in an EDTA tube and 10ml of blood was collected in a lithium heparin tube. All blood samples were inverted 8 times and processed immediately. If samples were not processed immediately, they were kept on ice and processed within 30 minutes. Blood samples were centrifuged at 1800 x g for 10 mins at 4°C and aliquoted in 0.5 ml x 4 aliquots and stored at -80°C for analysis. Plasma collected in EDTA tubes were used in this analysis.

Plasma samples were sent to BIOCRATES Life Sciences AG (Innsbruck, Austria) for targeted lipidomic analysis where the TargetIDQ™ P180 kit was used to identify and quantify phospholipid, sphingolipid

and acylcarnitine metabolites. Metabolites were quantitatively analysed by a high-throughput flow injection electrospray ionization tandem mass spectrometry (ESI-MS/MS) screening method. A total of 145 lipids were identified and quantified by ESI-MS/MS; 40 acylcarnitines, 14 lysophosphatidylcholines (LPCs), 76 phosphatidylcholines (PCs) and 15 sphingomyelins (SM).

Statistical Analyses

Firstly, heatmap analysis was carried out using regularised canonical correlation analysis (rCCA)[26] in the mixOmics package[27] in r (version 3.1.3). The statistical package is used to assess correlations between two multivariate datasets, in this instance it was employed to visualise the relationship between participant's lipid profiles and their nutrient intake with a particular focus on total dietary fat and saturated fatty acid (SFA) intakes (% TE). Multiple linear regression analysis was employed to (1) examine associations between the lipids with total dietary fat (% TE) and SFA intake (% TE) and (2) to identify relationships between food groups and the identified panel of lipids. P-values of ≤ 0.05 were considered statistically significant. Beta coefficients were reported and used to determine the direction of the relationships between lipids and nutrient intake/food groups. Our power calculations revealed that 188 participants had power to detect a 0.2 standard deviation change in the dependent variable for each standard deviation change in a predictor variable

A linear regression model was used to evaluate the relationship between biomarkers associated with dietary fat intake and HOMA-IR. Participants were then grouped according to their metabolic risk. The groups were classified based on the National Adult Cholesterol Education Program Adult Population III (ATPIII) guidelines. The ATPIII definition does not give one risk factor greater precedence over another, but by having three or more risk factors present, an individual is deemed to have the metabolic syndrome (MetS). Under the definition three of the following five conditions must be present: central adiposity, high blood pressure, low high lipoprotein cholesterol (HDL-C), high fasting glucose levels and elevated triacylglyceride (TAG) levels.[28]

Group 1 (n=129) were those defined as having no risk, group 2 (n=53) were those that had some risk, which was defined as having 1-2 risk factors present. Group 3 were defined as those who have the MetS (>3 risk factors) (n=6). The panel of 22 biomarkers associated with dietary fat intake was then evaluated across the groups using an ANOVA and post hoc Bonferroni's test to determine if any of these lipids were significantly different between groups. Further to this, an ordinal logistic regression model was used to determine which variables were associated with an increased risk of the MetS. The variables included in the model were the panel of 22 biomarkers associated with dietary fat, sex, age, BMI and total dietary fat intake (% TE).

A repeated measures ANOVA was used to evaluate plasma metabolites from week 1 to week 3 in the NutriTech study controlling for gender and BMI. Multiple comparisons were adjusted for by using Bonferrroni's correction.

Results

Identifying biomarkers associated with total dietary fat intake (Meche Study)

A total of 229 lipids were identified and quantified by ESI-MS/MS (Biocrates p150 and lipidassay) ; 14 acylcarnitines, 12 lysophosphatidylcholines (LPCs), 71 phosphatidylcholines (PCs), 9 lysophosphatidylethanolamines (LPEs), 54 phosphatidylethanolamines (PEs), 3 phosphatidylglycerols (PGs), 14 phosphatidylserines (PS), 38 ceramides (Cer) and 14 sphingomyelins (SM).

Heatmap analysis was performed with the use of rCCA to visualise correlations between participant's lipid profiles with their nutrient intakes. A panel of 22 lipids were identified as having strong positive correlations with either total dietary fat intake (% TE) or SFA intake (% TE) (Figure 1). The lipid panel was made up of the following lipid species; 10 PCs, 8 acylcarnitines, 2 LPCs, 1 PS and 1 Cer.

A multiple regression model was used to determine if this panel of 22 lipids had the ability to predict total dietary fat intake (% TE) and SFA intake (% TE). The panel of lipid biomarkers had a significant

relationship with both total dietary fat intake (P 7.0 x 10^{-3}) and SFA intake (P 1.0 x 10^{-3}) (Supporting Information Table S-2), indicating that this panel of lipid metabolites are associated with dietary fat intake and are potential biomarkers.

Examination of the relationship between this panel of biomarkers associated with dietary fat and the food group intakes revealed a number of significant relationships (Table 2). For example, meat and meat products had significant relationships with a phosphatidylcholine (PCaeC36:4), an acylcarnitine (C16) and a ceramide (NC-C19:0-OH.Cer2H). Milk and dairy products had significant relationships with a phosphatidylcholine (PCaeC38:4) and two acylcarnitines (C5 and C3). Interestingly vegetables had significant negative relationships with two potential dietary fat biomarkers (C18:0 and NC-C19:0-OH.Cer2H).

The response of the biomarkers associated with dietary fat intake to differing amounts of fat intake: Biomarker confirmation (NutriTech Study)

Of the panel of 22 biomarkers associated with dietary fat intake identified in the MECHE study, 19 were also measured in the NutriTech study. Examination of these revealed that 8 were found to be increasing as dietary fat intake increased from week 1 to week 3 in the intervention study (C3, C4, C5, PCaaC36:1, PCaaC40:4, PCaeC36:4, PCaeC36:5 and PCaeC38:5). Further analysis revealed that four metabolites were significantly increased (PCaeC36:4, PCaeC36:5, PCaeC36:3 and PCaeC38:5), demonstrating that these biomarkers were responsive to differential dietary fat intake (Figure 2).

Exploration of the relationship between biomarkers associated with dietary fat intake and insulin resistance (HOMA-IR) and MetS risk score.

The panel of 22 biomarkers associated with dietary fat intake had a significant association with HOMA-IR (P 1.0 x 10^{-6}), with 3 lipid metabolites (C16, PCaaC36:2 and PCaeC36:4) having strong positive associations (Table 3). Participants in the Meche Study were then grouped according to their metabolic risk.[28] The panel of dietary fat biomarkers were then evaluated across the groups. Of the

panel of 22 dietary fat biomarkers, 5 were significantly altered between the groups (Table 4), which included 3 PCs (PCaaC38:4, PCaaC40:4 and PCaeC36:4) and 2 acylcarnitines (C3 and C5). These lipid metabolites were all found to be significantly elevated in participants with a higher risk score for the MetS.

An ordinal logistic regression model identified increasing age, BMI and PCaeC36:4 (Table 5) as contributors to an increasing risk of developing the MetS. Total dietary fat (% TE) was also included in the model; however it did not have a significant effect on the risk of developing the MetS, highlighting the importance of obtaining more sensitive measurements of dietary intake.

Discussion

The present study identified a panel of 22 lipid biomarkers which were related to total dietary fat intake. Importantly, four of these biomarkers demonstrated responsiveness to different levels of fat intake in an intervention study. Furthermore, it was demonstrated that this panel of biomarkers had a significant relationship with HOMA-IR and MetS scores.

Dietary fat is an important component of the diet for a number of physiological functions.[29, 30] However an imbalance in lipid and fatty acid intake has been linked to adverse health effects such as increased risk of diseases for example CVD, diabetes and MetS.[31, 32] For such reasons there is a need to identify robust validated biomarkers of total dietary fat intake, which at present is lacking.[33] In our study we focused on a panel of 22 lipid metabolites that had strong positive correlations with both total dietary fat intake and SFA intake. Previous work in our research group also identified a number of PCs as potential biomarkers of dietary fat intake.[9] The study identified a lipid pattern (LP1) that could discriminate between low and high consumers of dietary fat with good sensitivity and specificity. This lipid pattern included four long chain PCs among other lipid species. Indeed the long chain PCs were also implicated in a recent study, which together with a number of SM were found to

be increased in the serum of mice following a high fat diet (HFD) for 14 weeks compared to those on a standard chow diet.[34] The present study makes a significant contribution by identifying a panel of biomarkers related to dietary fat intake. Importantly, it was demonstrated that the biomarkers respond to differing amounts of dietary fat intake in a controlled intervention. To the best of our knowledge, this is the first demonstration of a dose response to metabolomics derived dietary biomarkers in an independent study.

The main contributors to total dietary fat intake in the Irish diet are fresh meat, meat products and dairy consumption.[35, 36] Importantly, significant relationships between the panel of dietary fat biomarkers and key contributing foods were identified. In particular PCaeC36:4 had a significant positive association with meat (P 1.2×10^{-5}) and meat products (P 4.73×10^{-7}). Furthermore, others have also identified PCaeC36:4 as having an association with meat intake.[22] A long chain PC (PCaeC38:4) and acylcarnitines (C3 & C5) were found to have significant positive associations with dairy in our study. Previous work has also demonstrated a relationship between PCaeC38:4 and high fat dairy products and subsequently identified a dietary pattern characterised with red meat, poultry and butter to be associated with a higher risk of T2D.[12]

An important aspect of this research was the use of dietary biomarkers to link intake to health/disease parameters. This concept has been successfully explored in the field of epidemiology with a particular focus on environmental exposures; the model that has emerged is coined a “meet in the middle” approach which aims to identify intermediate biomarkers linking exposure and disease.[37] Although, the approach used here was not identical, the fundamental concept of using the exposure biomarkers (in this case dietary exposure) to link to health/disease is similar. The identification of a link between dietary fat intake and HOMA-IR is significant as it provides an insight into which lipid metabolites and pathways are potential disease mediators and therefore could be modulated using dietary interventions to improve health outcomes. In terms of HOMA-IR and the MetS, PCs and acylcarnitines had significant positive relationships, with PCaeC36:4 linking dietary fat

intake with both HOMA-IR and the MetS. PCs together with the LPCs and SMs are the main components of cellular membranes and part of blood lipo-proteins and in recent times have been associated with a number of diseases including T2D.[38] Mechanisms underlying the relationship between PCs and incidence of diabetes are still unclear. Acylcarnitines have traditionally been used to measure inborn errors of metabolism, but in recent years have been shown to be markers of insulin resistance.[39, 40] The identification of these biomarkers that link diet and health is an important next step in biomarker research. Identifying such links allows us to investigate modifiable risk factors that are related to disease risk.

The identification of these links between diet and health also confirms metabolomics as an invaluable tool for identifying biomarkers of metabolic diseases, which are required in order to understand the relationship between nutrition and such chronic disorders. The importance of the approach taken here is highlighted by the fact that no association between dietary fat intake estimated from an FFQ and HOMA-IR was observed. The use of such intermediate biomarkers will have an important role in future studies. Support for this also comes from previous studies where biomarkers of certain foods demonstrated clear relationships with health outcomes not found when using traditional methods. For example in one such study, urinary sugars and plasma vitamin C, but not FFQ based estimates of intake were found to be associated with obesity.[41] Subsequent work by these authors identified that the risk of ischaemic heart disease was associated with plasma vitamin C ($P < 0.001$) and intake of vitamin C and fruit and vegetables assessed by a food diary (P quintile trends <0.001, 0.001), however this association was not captured by the FFQ (P quintile trends 0.923, 0.186).[42]

The strengths of this study include the fact that a confirmation step was included for the biomarkers in an independent study. The demonstration of a dose response adds significant credibility that the biomarker is associated with intake. Furthermore the wide range of lipids profiled is also a strength of the study. The participants in the discovery study and the confirmation study were predominately of Caucasian origin and future work is needed to validate these markers in other populations.

Conclusions

In conclusion, this study has identified a panel of potentially important total dietary fat biomarkers, a number of which were then confirmed in a dose response intervention study. An important aspect of this work was the demonstration of a link between certain biomarkers and health parameters. Such an approach proves very useful in Identifying links between diet and health, thus allowing us to explore the relationship between the two and to determine whether lipid biomarkers can be modulated through diet to improve health outcomes.

Associated Content Available: Supporting Information

Supporting Table S-1: NutriTech food intake study participant's characteristics

Supporting Table S-2: Multiple regression models to evaluate the overall relationship of the lipid panel with total dietary fat intake and SFA intake.

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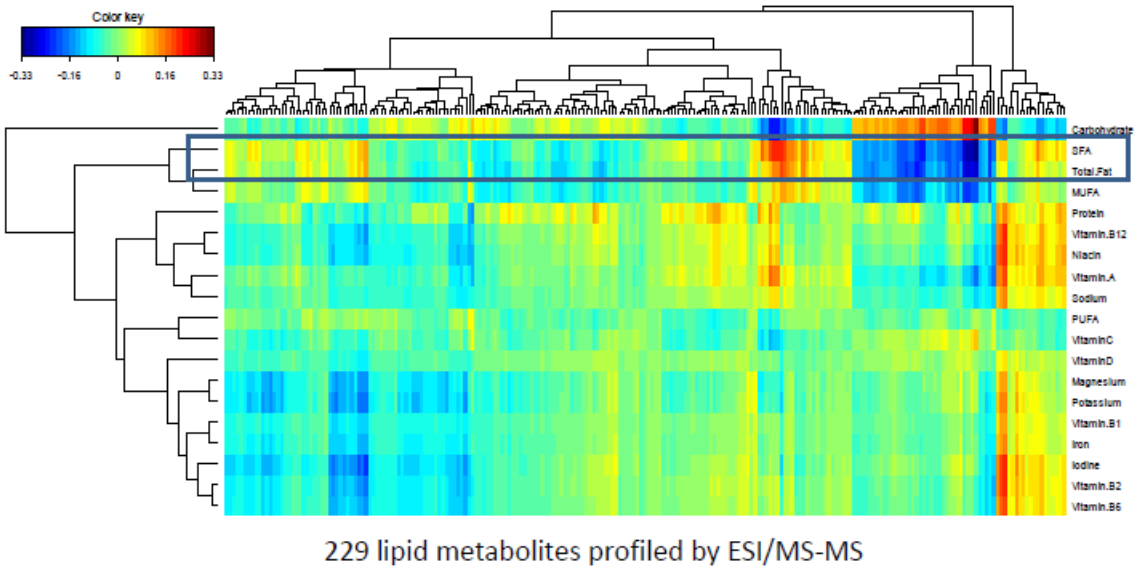
Figure Legends

Figure 1: Heatmap analysis performed using regularised canonical analysis showing the association between plasma lipid profiles and nutrient profiles (% TE). The x-axis represents the measured lipids and the y-axis the nutrient profiles. Correlation strengths are indicated by the colour key. The panel of dietary fat biomarkers are highlighted by the box. SFA: saturated fatty acid; TotalFat: Total dietary fat; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. ESI/MS-MS: Electrospray ionisation tandem mass spectrometry.

Figure 2: Dose response of lipid metabolites significantly increasing from week 1 to week 3 in the NutriTech study following an increase in dietary fat. Data are averages (μM) ± SEM (n=10). * indicates a P-value <0.05 for repeated measures anova controlling for gender and BMI.

Figures



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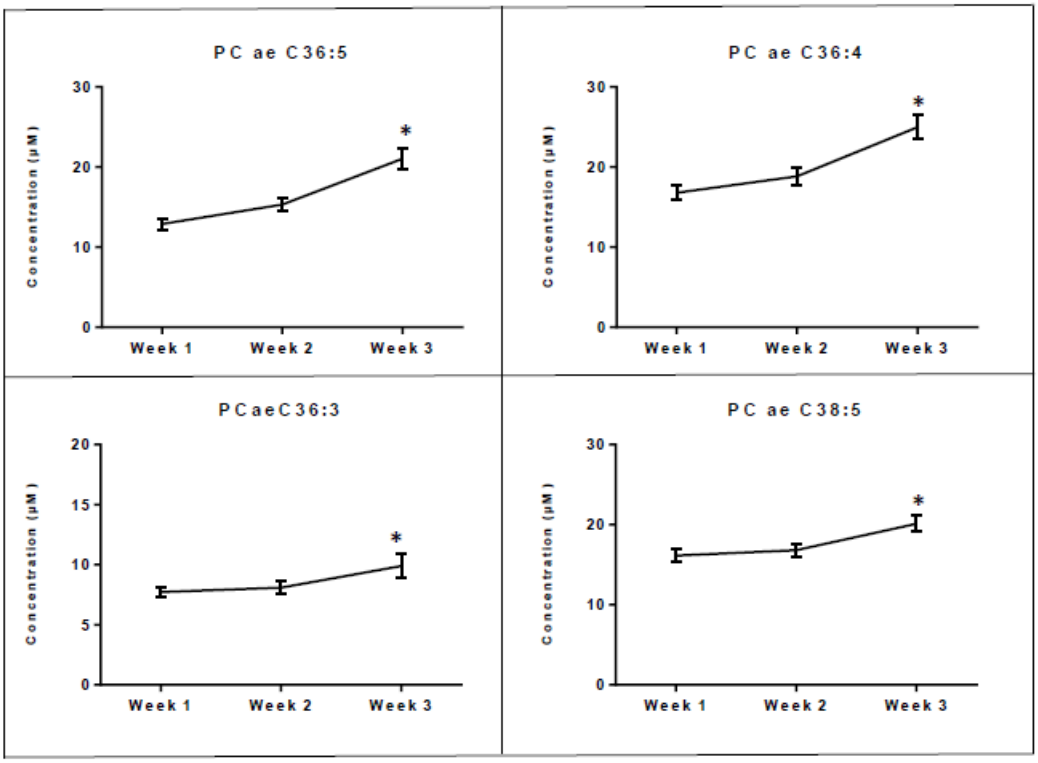

Figure 2: Dose response of lipid metabolites significantly increasing from week 1 to week 3 in the NutriTech study following an increase in dietary fat. Data are averages (µM) ± SEM (n=10). * indicates a P-value <0.05 for repeated measures anova controlling for gender and BMI.

Tables

Table 1: Anthropometric and biochemical parameters of the study population

	Male (n= 90)	Female (n= 98)	P-value
Age (years)	32 ± 10	32 ± 12	ns
Weight (kg)	82.1 ± 14	67.06 ± 14.5	1.18×10^{-11}
BMI (Kg m^{-2})	26.3 ± 4	24.4 ± 5.2	6.6×10^{-3}
Body fat (%)	20.5 ± 9.1	31 ± 9.4	4.42×10^{-12}
glucose (mM)	4.86 ± 0.45	4.77 ± 0.39	ns
Insulin (µIU ml^{-1})	7.43 ± 6.74	7 ± 4.14	ns
Triacylglycerol (mM)	1.19 ± 0.83	0.89 ± 0.4	3.0×10^{-3}
Total cholesterol (mM)	4.59 ± 1.02	4.55 ± 0.88	ns
HDL-c (mM)	1.35 ± 0.31	1.72 ± 0.37	4.14×10^{-12}
LDL-c (mM)	2.69 ± 0.93	2.43 ± 0.78	4.42×10^{-2}
HOMA-IR	2.01 ± 1.76	1.8 ± 1.32	ns

Data are presented as means ± standard deviation (SD); BMI: body mass index; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HOMA: homeostasis model assessment; ns: not significant

Table 2: The relationship between biomarkers associated with dietary fat and food group intakes

Food Group (g/d)	Lipid Metabolites	B-coefficient	P-value
Alcohol	C8:1	0.178	1.4×10^{-3}
	LPCaC20:3	0.146	4.3×10^{-2}
Butter & Margarine	C8:1	0.189	9.0×10^{-3}
Cereal Other	C3	0.286	1.35×10^{-4}
Dairy Other	PCaeC38:4	0.199	2.8×10^{-2}
	C3	0.211	8.0×10^{-3}
Drinks	NC-C19:0-OH.Cer2H	0.156	2.9×10^{-2}
	PCaeC36:3	0.283	1.0×10^{-3}
Eggs	C5	0.243	1.0×10^{-3}
Fish	PCaeC36:1	0.529	7.0×10^{-6}
	PCaeC36:5	0.284	2.0×10^{-3}
Meat	PCaeC36:4	0.695	1.2×10^{-5}
	C16	0.189	6.0×10^{-3}
Meat Products	PCaeC36:4	0.806	4.73×10^{-7}
	NC-C19:0-OH.Cer2H	0.261	9.9×10^{-5}
Milk	PCaeC38:4	0.254	5.0×10^{-3}
	C5	0.178	1.3×10^{-2}
Offal	C3	0.36	2.0×10^{-6}
Potatoes	C8:1	0.210	3.0×10^{-3}
	PSaeC36:2	0.206	4.0×10^{-3}
Sauces	C8:1	0.231	1.0×10^{-3}
Vegetables	C18:0	-0.192	7.0×10^{-3}
	NC-C19:0-OH.Cer2H	-0.187	9.0×10^{-3}

g/d: gram per day; ns: not significant

Table 3: The relationship between biomarkers associated with dietary fat intake and HOMA-IR

Dietary fat Biomarker	β-coefficient	P-value
C16	0.311	1.0×10^{-3}
PCaaC36:2	0.378	2.62×10^{-5}
PCaeC36:4	0.794	1.75×10^{-6}

Overall model using the panel of 22 dietary fat biomarkers: P 1.0×10^{-6}

Table 4: The relationship between biomarkers associated with dietary fat intake and metabolic risk

Dietary fat biomarker	Group 1 (n=129)	Group 2(n=53)	Group 3 (n=6)	P-value
C3	0.29 ± 0.1[3]	0.31 ± 0.09[3]	0.46 ±0.1[1,2]	5.8 x 10^{-5}
C4	0.2 ± 0.12	0.18 ± 0.05	0.22 ± 0.09	ns
C5	0.1 ± 0.03[3]	0.11 ± 0.03[3]	0.15 ± 0.04[1,2]	1.7 x 10^{-3}
C16	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	ns
C18	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	ns
C8:1	0.11 ± 0.06	0.13 ± 0.06	0.15 ± 0.07	ns
C14:1	0.18 ± 0.05	0.18 ± 0.05	0.17 ± 0.03	ns
C18:1	0.12 ± 0.03	0.12 ± 0.03	0.13 ± 0.01	ns
PCaaC36:1	40.61 ± 10.79	39.9 ± 12.2	41.94 ± 6.84	ns
PCaaC36:2	227.85 ± 50.21	229.08 ± 51.82	265.67 ± 37.33	ns
PCaaC38:4	87.79 ± 21.79[3]	93.16 ±25.07[3]	118.42 ± 25.66[1,2]	3.85 x 10^{-3}
PCaaC40:4	2.8 ± 0.72[3]	2.83 ± 0.7[3]	3.78 ± 1.26[1,2]	6.38 x 10^{-3}
PCaeC36:1	6.69 ± 1.42	6.46 ± 1.69	5.44 ± 0.62	ns
PCaeC36:3	8.29 ± 1.84	7.92 ± 1.87	7.81 ± 0.81	ns
PCaeC36:4	15.99 ± 3.66[3]	16.33 ± 3.91	20.12 ± 2.56[3]	3.0 x 10^{-2}
PCaeC36:5	10.94 ± 3.04	11.21 ± 3.5	12.42 ± 1.67	ns
PCaeC38:4	12.55 ± 2.66	12.34 ± 2.77	12.56 ± 1.5	ns
PCaeC38:5	17.123 ± 3.63	17.07 ± 3.92	19.3 ± 1.65	ns
LPCaC20:3	2.04 ± 0.75	1.83 ± 0.64	1.93 ± 0.4	ns
LPCaC20:4	5.48 ± 2.01	4.89 ± 1.53	4.85 ± 1.28	ns
NC-C19:0-OH.Cer 2H	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	ns
PSaeC36:2	0.004 ± 0.003	0.004 ± 0.003	0.004 ± 0.004	ns

Group 1: No risk of metabolic syndrome; Group 2: Some risk factors present (1-2); Group 3: Metabolic syndrome present (> 3 factors). ns: not significant

[1] Significance to Group 1; [2] Significance to Group 2, [3] Significance to Group 3

Table 5: An ordinal logistic regression model using the panel of biomarkers associated with dietary fat intake, sex, age, BMI and total dietary fat intake (% TE) to determine which variables are associated with an increased risk of the MetS

Variable	B-coefficient	Standard error (SE)	P-value
Age	0.64	0.02	6.9×10^{-3}
BMI	0.14	0.05	8.3×10^{-3}
Total dietary fat intake (% TE)	-0.02	0.04	ns
C3	5.57	3.33	ns
C4	-8.31	3.34	1.3×10^{-2}
C5	2.34	7.72	ns
C16	7.4	18.81	ns
C18	8.02	27	ns
C8:1	2.64	3.59	ns
C14:1	-3.35	6.75	ns
C18:1	1.66	13.83	ns
PCaaC36:1	0.02	0.04	ns
PCaaC36:2	0.01	0.01	ns
PCaaC38:4	0.03	0.03	ns
PCaaC40:4	-0.32	0.66	ns
PCaeC36:1	-0.78	0.32	1.6×10^{-2}
PCaeC36:3	-0.14	0.3	ns
PCaeC36:4	0.41	0.19	3.3×10^{-2}
PCaeC36:5	-0.08	0.16	ns
PCaeC38:4	-0.04	0.25	ns
PCaeC38:5	-0.21	0.19	ns
LPCaC20:3	0.11	0.57	ns
LPCaC20:4	-0.64	0.26	1.5×10^{-2}
NC-C19:0-OH.Cer 2H	-42.24	165.63	ns
PSaeC36:2	48.29	75.73	ns

BMI: body mass index; ns: not significant

Supporting information

Supporting Table S-1: NutriTech food intake study participant's characteristics

Characteristic	
Gender	5 (M) 5 (F)
Age (y)	58 ± 4[1]
Weight (kg)	90.9 ± 17.7
Height (m)	1.7 ± 0.1
BMI (kg/m^2)	30.9 ± 3.2

[1]Data are Mean ± SD.

Supporting Table S-2: Multiple regression models to evaluate the overall relationship of the lipid panel with total dietary fat intake and SFA intake.

Dependent Variable	**Independent Variables**	**P-Value**
Total dietary fat intake (% TE)	C3, C4, C5, C8:1, C16, C18, C14:1, C18:1, PCaaC36:1, PCaaC36:2, PCaaC38:4, PCaaC40:4, PCaeC36:1, PCaeC36:3, PCaeC36:4, PCaeC36:5, PCaeC38:4, PCaeC38:5, LPC20:3, LPC20:4, N-C19:0(OH)-Cer(2H), PSaeC36:2	7.0×10^{-3}
SFA intake (% TE)	C3, C4, C5, C8:1, C16, C18, C14:1, C18:1, PCaaC36:1, PCaaC36:2, PCaaC38:4, PCaaC40:4, PCaeC36:1, PCaeC36:3, PCaeC36:4, PCaeC36:5, PCaeC38:4, PCaeC38:5, LPC20:3, LPC20:4, N-C19:0(OH)-Cer(2H), PSaeC36:2	1.0×10^{-3}

SFA: Saturated fatty acids
Regression method used: ENTER
% TE: Percentage of total energy