Biomarkers of food intake and nutrient status are associated with glucose tolerance status and development of type 2 diabetes in older Swedish women

Biomarkers of food intake and nutrient status are associated with glucose tolerance status and development of type 2 diabetes in older Swedish women

Otto Savolainen,[1] *Mads Vendelbo Lind,*[1,2] *Göran Bergström,*[3] *Björn Fagerberg,*[3] *Ann-Sofie Sandberg,*[1] *and Alastair Ross*[1]

[1]Division of Food and Nutrition Science, Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden; [2]Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Frederiksberg, Denmark; and [3]Wallenberg Laboratory for Cardiovascular Research at the Center for Cardiovascular and Metabolic Research, Institute of Medicine, Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden

ABSTRACT

Background: Diet is frequently associated with both the development and prevention of type 2 diabetes (T2D), but there is a lack of objective tools for assessing the relation between diet and T2D. Biomarkers of dietary intake are unconfounded by recall and reporting bias, and using multiple dietary biomarkers could help strengthen the link between a healthy diet and the prevention of T2D.

Objective: The objective of this study was to explore how diet is related to glucose tolerance status (GTS) and to future development of T2D irrespective of common T2D and cardiovascular disease risk factors by using multiple dietary biomarkers.

Design: Dietary biomarkers were measured in plasma from 64-y-old Swedish women with different GTS [normal glucose tolerance (NGT; n = 190), impaired glucose tolerance (IGT; n = 209), and diabetes (n = 230)]. The same subjects were followed up after 5 y to determine changes in glucose tolerance (n = 167 for NGT, n = 174 for IGT, and n = 159 for diabetes). ANCOVA and logistic regression were used to explore baseline data for associations between dietary biomarkers, GTS, and new T2D cases at follow-up (n = 69).

Results: Of the 10 dietary biomarkers analyzed, β-alanine (beef) (P-raw $<$ 0.001), alkylresorcinols C17 and C19 (whole-grain wheat and rye) (P-raw = 0.003 and 0.011), eicosapentaenoic acid (fish) (P-raw = 0.041), 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) (fish) (P-raw = 0.002), linoleic acid (P-raw $<$ 0.001), oleic acid (P-raw = 0.003), and α-tocopherol (margarine and vegetable oil) (P-raw $<$ 0.001) were associated with GTS, and CMPF (fish) (OR: 0.72; 95% CI: 0.56, 0.93; P-raw = 0.013) and α-tocopherol (OR: 0.71; 95% CI: 0.51, 0.98; P-raw = 0.041) were inversely associated with future T2D development.

Conclusions: Several circulating dietary biomarkers were strongly associated with GTS after correction for known T2D risk factors, underlining the role of diet in the development and prevention of T2D. To our knowledge, this study is the first to use multiple dietary biomarkers to investigate the link between diet and disease risk. *Am J Clin Nutr* 2017;106:1302–10.

Keywords: prediabetes, diet, metabolome, biomarkers, nutrition, metabolomics

INTRODUCTION

Type 2 diabetes (T2D) is one of the fastest-growing health care problems worldwide. Globally 422 million adults had diabetes in 2014, 314 million more than in 1980, and the incidence of T2D is expected to rise (1). Prevention of T2D is the most effective strategy to reduce the cost and burden of this disease and the only way to arrest the increase in T2D incidence. Lifestyle factors are strongly associated with the development of T2D, and lifestyle changes, such as a healthier diet, reducing smoking, and increasing exercise are among the most effective means of preventing T2D (2–5).

Diets rich in whole grains, fruits, vegetables, legumes, and nuts, moderate in alcohol consumption, and lower in refined grains, red or processed meats, and sugar-sweetened beverages have been linked to a reduced risk of diabetes (6). However, the tools for assessing diet have long relied on dietary recall methods, such as food-frequency questionnaires and 24-h recalls. Although these methods provide a standardized tool for measuring dietary and nutrient intake, they have several well-known limitations, including reliance on self-reporting as well as multiple assumptions about food composition and serving size (7–9). To aid in the assessment of diet, biomarkers of dietary intake and nutrient status could serve as complimentary tools for the more accurate estimation of food and nutrient intake by combining both subjective (e.g., questionnaires) and objective (e.g., biomarkers) measures that have unrelated measurement errors (10).

The work of the Diabetes and Impaired glucose tolerance in Women and Atherosclerosis development study (DIWA) cohort was supported by grants from the Swedish Heart and Lung Foundation and grants from the Västra Götaland region.

Address correspondence to AR (e-mail: alastair.ross@chalmers.se).

Abbreviations used: CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; CVD, cardiovascular disease; GC-MS/MS, gas chromatography–tandem mass spectrometry; GTS, glucose tolerance status; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MRM, multiple-reaction monitoring; NGT, normal glucose tolerance; T2D, type 2 diabetes.

Received January 13, 2017. Accepted for publication August 18, 2017.

First published online September 13, 2017; doi: https://doi.org/10.3945/ajcn.117.152850.

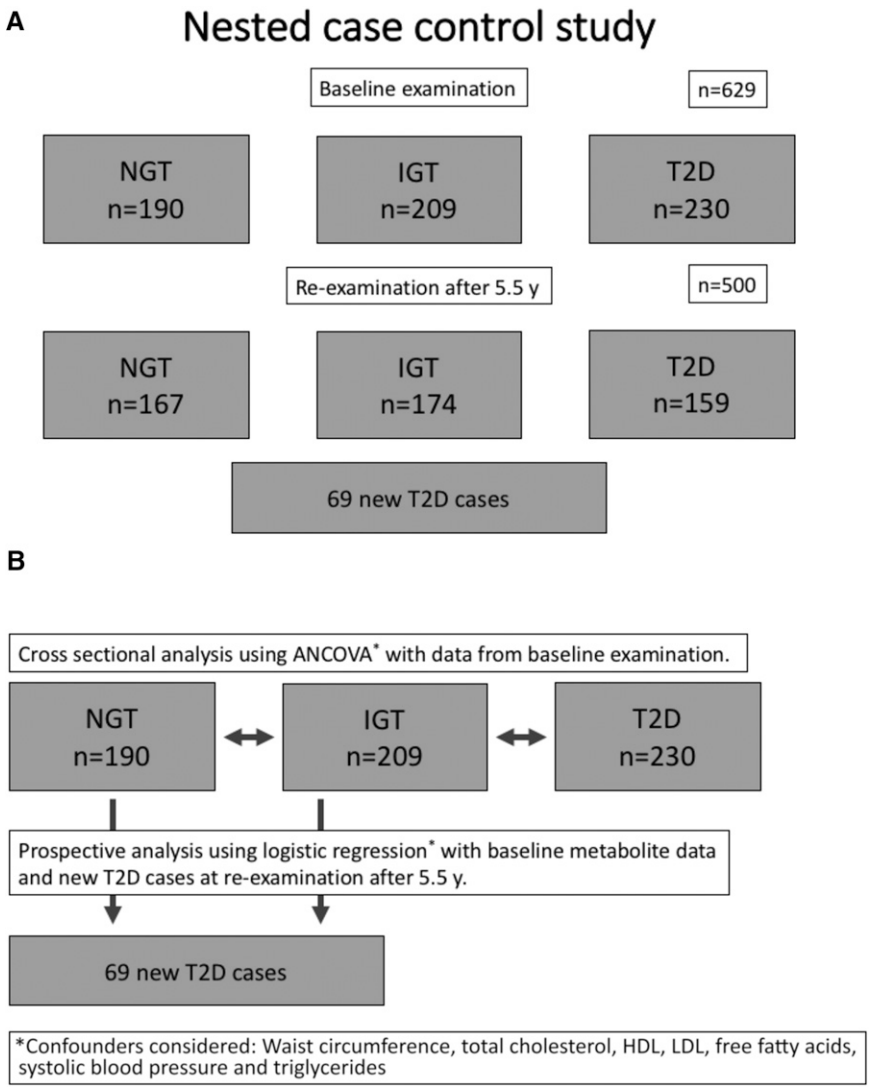

FIGURE 1 Structure of the nested case-control study (A) with the number of subjects in each glucose tolerance group and an illustration of the use of the cohort for statistical analysis (B). IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes.

Biomarkers also allow the exploration of associations between diet and disease in cohorts where dietary intake data are missing or inadequate. Recent studies have shown the usefulness of a single class of dietary intake biomarkers to find associations between diet and glucose tolerance as well as T2D (11, 12). These highlight the utility of biomarkers in research aiming to uncover relations between diet and T2D. To our knowledge, no work has been done with the use of multiple dietary and nutrient status biomarkers to reflect dietary intake to examine the association with both glycemic control and future development of T2D. The use of several markers of diet intake and nutrient status could give a wider perspective on the associations between diet and disease.

The aim of the present study was to investigate associations between a panel of biomarkers of diet and nutrient status detected by using a semitargeted metabolomics method and glucose tolerance status (GTS) and future T2D development in a prospective cohort of 64-y-old Swedish women.

METHODS

Study participants

A population-based cohort was started in 2001 by inviting all women turning 64 y old who were living in the Gothenburg region of Sweden to participate in a screening examination for T2D (13). At baseline, WHO criteria were used for the definitions of diabetes mellitus and impaired glucose tolerance (IGT) (14). The screening examination included fasting capillary whole blood glucose measurements in women with overt diabetes and repeated oral glucose tolerance tests (13, 15) in women without overt diabetes. Of the screened cohort of 2595 women, 9.5% had diabetes mellitus and 14.4% had IGT. Women with diabetes, IGT, and normal glucose tolerance (NGT) were randomly selected and invited to participate in a nested case-control study, which included a baseline and a follow-up examination after 5.5 y (**Figure 1**). In total, 629 women participated in the first examination, and samples from 614 subjects were analyzed by gas chromatography–tandem mass spectrometry

TABLE 1
Baseline characteristics of the 64-y-old women included in the study[1]

	NGT (n = 188)	IGT (n = 203)	T2D (n = 202)	P[2]	Subjects with new T2D at follow-up (n = 69)[3]
Waist circumference, cm	88.4 ± 9.0	92.4 ± 12.0	96.5 ± 11.1	<0.001	95.3 ± 12.6
BMI, kg/m^2	26.2 ± 3.4	27.8 ± 5.0	28.8 ± 4.3	<0.001	28.6 ± 5.3
Total cholesterol, mmol/L	6.1 ± 0.9	5.9 ± 1.0	5.7 ± 1.1	0.002	5.9 ± 1.0
HDL, mmol/L	1.8 ± 0.4	1.6 ± 0.4	1.6 ± 0.4	<0.001	1.5 ± 0.4
LDL, mmol/L	3.8 ± 0.9	3.7 ± 0.9	3.4 ± 1.0	0.004	3.7 ± 0.9
Free fatty acids, mmol/L	0.67 ± 0.26	0.80 ± 0.27	0.81 ± 0.29	<0.001	0.80 ± 0.26
Systolic blood pressure, mm Hg	136 ± 16	149 ± 17	151 ± 18	<0.001	148 ± 17
Serum triglycerides, mmol/L	1.3 ± 0.6	1.5 ± 0.7	1.5 ± 0.7	<0.001	1.7 ± 0.8

[1] Values are means ± SDs. IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes.
[2] ANCOVA P value for indicating the significance between NGT, IGT, and T2D.
[3] The 69 new cases of T2D are included twice in the table: once in their original glucose tolerance group and once in the group with new T2D at follow-up.

(GC-MS/MS)-based metabolomics (T2D: n = 202; IGT: n = 203; NGT: n = 188; type 1 diabetes: n = 21). A reexamination was performed after 5.5 y in 500 women with diabetes (n = 159), IGT (n = 174), or NGT (n = 167) according to the classification at baseline. The remaining 129 women from the original study did not participate in the reexamination because of death (n = 23) or severe disease (n = 3), they were no longer living in the area (n = 12), or they were unwilling to participate (n = 91) (15). Of those originally in the IGT and NGT groups, 69 were diagnosed as having T2D at follow-up. Dietary intake was not assessed in this cohort. Baseline characteristics for the women included in the analysis are shown in **Table 1**. The study was approved by the ethics committee at Sahlgrenska University Hospital in Gothenburg, Sweden.

Dietary biomarker measurement by using GC-MS/MS metabolomics

Dietary biomarkers were measured by using GC-MS/MS metabolomics as previously described (16). This method detects metabolites, including dietary biomarkers, by using both mass spectrometry–based scanning (untargeted) and more selective and sensitive single-ion monitoring and multiple-reaction monitoring (MRM) modes, which detect predefined metabolites. Plasma was extracted with methanol:water (9:1, vol:vol) derivatized by using methoxymation followed by silylation (16). Samples were injected onto a Shimadzu GCMS TQ-8030 GC-MS/MS (Shimadzu Europa GmbH), and both scan and MRM data were collected for further data analysis. Biomarkers previously associated with either dietary intake or nutrient status and that were detected in human plasma by this method are α- and δ-tocopherol (vitamin E), alkylresorcinols C17 and C19 (whole-grain wheat and rye), β-alanine (meat), 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) (fish), lauric acid (saturated fat), oleic acid (common dietary sources include olive and rapeseed oils, almonds, hazelnuts, and avocados), EPA (fish), and linoleic acid (common dietary sources include seeds, nuts, and vegetable oils) (**Table 2**).

Metabolomics data processing

A MATLAB (Mathworks) script and database developed at the Swedish Metabolomics Centre was used for targeted analysis of the full-scan data (23). The internal standard normalization of raw data was performed by using the method of Jonsson et al. (23). Internal standard variables from each sample were scaled to unit variance and modeled together by using principle component analysis (SIMCA+; Umetrics AB) to describe the overall variation in the multivariate space. Vectors from the first component were then used to correct for the analytic variation between samples. The MRM data were processed and normalized as described earlier (16). The 2 data sets, targeted measurements

TABLE 2
Dietary sources of the proposed biomarkers, based on a Swedish diet

Biomarker	Main nutritional sources in Sweden	Reference
β-Alanine	Circulating concentrations linked to the consumption of beef	17
Alkylresorcinol C17	Whole-grain wheat and rye	18, 19
Alkylresorcinol C19	Whole-grain wheat and rye	18, 19
CMPF[1]	Fish	20
EPA	Fish	21
Lauric acid	Saturated fat	21
Linoleic acid	Seeds, nuts, and vegetable oils	21
Oleic acid	Olive and rapeseed oils, almonds, hazelnuts, and avocados	22
α-Tocopherol	Pastry, margarine, eggs, bread, vegetables, and fruits	21
γ-Tocopherol	Corn and soybean oils and margarine	

[1] CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid.

TABLE 3
Identification of confounders for biomarker-outcome associations[1]

Outcome and biomarker	Stage 1[2]	Stage 2[3]
GTS[4]		
β-Alanine	HDL	HDL
Alkylresorcinol C17	FFA	FFA
Alkylresorcinol C19	—	—
CMPF	LDL	LDL
EPA	CHOL, HDL, LDL	CHOL, LDL
Lauric acid	FFA, HDL	FFA, HDL
Linoleic acid	FFA, HDL	FFA, HDL
Oleic acid	FFA, CHOL, HDL	FFA, CHOL, HDL
α-Tocopherol	CHOL, HDL, LDL, TG	CHOL, LDL
γ-Tocopherol	FFA, CHOL, HDL, LDL, TG, W	CHOL, HDL, LDL
New T2D[5]		
β-Alanine	W, HDL, TG, SBP	W, TG
Alkylresorcinol C17	W, HDL, TG	W
Alkylresorcinol C19	W, HDL, TG	W, TG
CMPF	W, HDL, TG, SBP	W, TG
EPA	W, HDL, TG, SBP	W, TG
Lauric acid	W, HDL, TG	TG, HDL
Linoleic acid	W, HDL, TG	TG
Oleic acid	FFA, W, HDL, TG, SBP	W, TG
α-Tocopherol	TG, HDL, W	TG
γ-Tocopherol	W, HDL, TG	W, TG

[1] CHOL, total cholesterol; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; FFA, free fatty acid; GTS, glucose tolerance status; SBP, systolic blood pressure; TG, triglyceride; T2D, type 2 diabetes; W, waist circumference.
[2] Stage 1 confounders tested individually for each biomarker-outcome combination.
[3] Stage 2 confounders after a step-wise model reduction by always removing the least significant confounder.
[4] Glucose tolerance class defined as normal glucose tolerance, impaired glucose tolerance, and T2D.
[5] New T2D at the follow-up examination of the study.

from full-scan data and MRM, were then merged for statistical analyses.

Biochemical analysis

Blood glucose, insulin, adiponectin, total cholesterol, HDL, LDL, free fatty acid, and triglyceride measurements were performed by using standard clinical chemistry techniques as previously described at the clinical chemistry laboratory of the Sahlgrenska University Hospital, Gothenburg (15).

Data analysis strategy

Baseline samples used for exploring the associations between the 10 biomarkers and GTS groups were defined as NGT, IGT, and T2D and new T2D cases at follow-up irrespective of common risk factors of T2D and cardiovascular disease (CVD). ANCOVA and Tukey's post hoc *t* test were used for comparing the GTS groups and logistic regression for testing if baseline biomarker values were associated with new T2D at follow-up (Figure 1). All data were checked for normal distribution, and skewed variables were log transformed. For logistic regression analysis, each biomarker was scaled to have a mean of 0 and an SD of 1 to have comparable units for all biomarkers. Because the results are not quantitative and are relative responses of mass spectrometer peak areas, differences between groups are reported as fold changes. As the focus of this work was the relation between dietary biomarkers and the development of T2D, subjects with previously diagnosed T2D or type 1 diabetes were excluded from the analyses. Waist circumference, total cholesterol, HDL, LDL, free fatty acids, systolic blood pressure, triglycerides, and medication for nondiabetic conditions were considered potential confounders and were first individually tested for each biomarker and outcome combination (stage 1). After this a step-wise model reduction was performed starting from the model including all potential confounders, followed by removal of the least significant variables one by one, and ending with a model including only significant ($P < 0.05$) confounders (stage 2) (**Table 3**). One type of diuretic medication (bendroflumethiazide) was found to confound the association of baseline values of the biomarkers with the development of diabetes at follow-up but was used by only 4 subjects. Removing these 4 subjects from the population did not change the results, which are reported based on all subjects. Results for the logistic regression are reported as ORs and 95% CIs for one SD change in concentration of a biomarker for significant biomarkers. Results from ANCOVA models comparing GTS were reported as fold differences compared with NGT and with IGT in the case of T2D. In line with the explorative nature of the work that does not allow for power calculations, we report both raw and Benjamini-Hochberg false discovery rate–corrected *P* values. We treated results with a raw *P* value of <0.05 as being of interest.

Predictive models were used to test if the baseline values of the biomarkers can predict the subjects who developed T2D during the 5.5-y follow-up and how the prediction with biomarkers compares with other prediction models for the development of T2D. Four different prediction models based on the biomarkers in this study and earlier established predictors in this cohort (15)

TABLE 4
Comparison of biomarker concentration across glucose tolerance groups by using ANCOVA, false discovery rate–corrected ANCOVA, and Tukey's post hoc *t* test[1]

			P			Fold change		
Biomarker	*P*	*P*-corrected	IGT-NGT	T2D-NGT	T2D-IGT	IGT/NGT	T2D/NGT	T2D/IGT
β-Alanine	<0.001	<0.001	0.003	<0.001	0.092	1.14	1.22	1.07
Alkylresorcinol C17	0.003	0.005	0.001	0.073	0.288	0.87	0.93	1.06
Alkylresorcinol C19	0.011	0.016	0.003	0.178	0.237	0.90	0.94	1.05
CMPF	0.002	0.004	0.232	<0.001	0.008	1.05	1.20	1.14
EPA	0.041	0.051	0.013	0.145	0.920	0.93	0.93	1.00
Lauric acid	0.050	0.056	0.033	0.861	0.093	1.05	1.00	0.95
Linoleic acid	<0.001	<0.001	<0.001	0.004	0.021	0.90	1.02	1.14
Oleic acid	0.003	0.005	0.003	0.776	0.121	0.96	0.99	1.04
α-Tocopherol	<0.001	<0.001	<0.001	<0.001	0.983	0.84	0.84	1.00
γ-Tocopherol	0.170	0.170	0.089	0.137	0.852	1.07	1.08	1.01

[1] The *P* values are from the models adjusted for stage 2 confounders and fold changes for comparing the baseline levels of biomarkers between glucose tolerance groups defined as NGT (n = 188), IGT (n = 203), and T2D (n = 202). CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes.

were tested: *1*) diet and nutrition biomarkers model based on the biomarkers measured in this study, *2*) anthropometry model based on smoking, alcohol consumption, waist circumference, systolic blood pressure, and family history, *3*) adiponectin model based on serum adiponectin concentration, HOMA-IR, smoking, and IGT and impaired fasting glucose (IFG) at baseline, and *4*) combination model based on a combined model from predictors in models 1 and 3. All dietary biomarkers were used for prediction in models 1 and 4. The area under the receiver operating characteristics curve was used to assess the discriminating power of the prediction models and was calculated with the trapezoidal rule by using the R-package pROC (version 1.8). All statistical analyses were performed by using the R statistical environment (version 3.0.2).

RESULTS

Of the subjects with blood plasma samples available from the baseline measurement, 188 subjects had NGT, 203 had IGT, and 202 had T2D. As expected, subjects with NGT had lower waist circumference, systolic blood pressure, and free fatty acid and triglyceride concentrations compared with subjects with IGT and T2D and had slightly higher or similar HDL, LDL, and total cholesterol concentrations compared with subjects with IGT and T2D (Table 1).

Associations between dietary biomarkers and glucose tolerance

Of the 10 diet and nutrient biomarkers measured by using the GC-MS/MS metabolomics method, at baseline β-alanine, alkylresorcinols C17 and C19, EPA, lauric, linoleic and oleic acids, and α-tocopherol differed between the NGT and IGT groups, and β-alanine, CMPF, linoleic acid, and α-tocopherol differed between the NGT and T2D groups (**Table 4**). CMPF and linoleic acid differed between the IGT and T2D groups. γ-Tocopherol did not differ between any of the GTSs. Among the significant biomarkers, β-alanine and CMPF were lower in subjects with NGT compared with IGT and T2D. The 2 alkylresorcinols were higher in subjects with NGT compared with IGT, α-tocopherol was higher in subjects with NGT compared with IGT and T2D, oleic acid and EPA were higher in subjects with NGT compared with IGT, linoleic acid was higher in subjects with NGT compared with IGT and higher in subjects with T2D compared with NGT and IGT (see Table 4 for fold changes).

Baseline concentrations of biomarkers and future development of T2D

α-Tocopherol and CMPF were inversely associated with new T2D at the follow-up examination (P = 0.01; OR: 0.72; 95% CI: 0.56, 0.93 and P = 0.04; OR: 0.71; 95% CI: 0.51, 0.98, respectively) (**Table 5**). The *P* values were no longer <0.05 after correction for multiple comparisons. None of the other biomarkers measured was associated with future T2D risk. Prediction of new T2D at the follow-up examination based on a model that included all the diet and nutrient biomarkers had nominally higher sensitivity and selectivity (AUC: 0.724;

TABLE 5
ORs (95% CIs) and *P* values from logistic regression models for the biomarkers associated with the development of T2D at the follow-up examination (n = 69)[1]

Biomarker	*P*	Corrected *P*[2]	OR (95% CI)
β-Alanine	0.185	0.271	0.82 (0.61, 1.10)
Alkylresorcinol C17	0.303	0.378	0.86 (0.63, 1.14)
Alkylresorcinol C19	0.190	0.271	0.81 (0.59, 1.10)
CMPF	0.041	0.137	0.71 (0.51, 0.98)
EPA	0.673	0.747	1.06 (0.80, 1.40)
Lauric acid	0.038	0.137	1.35 (1.02, 1.81)
Linoleic acid	0.135	0.271	0.79 (0.58, 1.08)
Oleic acid	0.186	0.271	0.83 (0.62, 1.09)
α-Tocopherol	0.013	0.127	0.72 (0.56, 0.93)
γ-Tocopherol	0.992	0.992	1.00 (0.77, 1.29)

[1] CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; T2D, type 2 diabetes.
[2] *P* value corrected for multiple testing by the using false discovery rate.

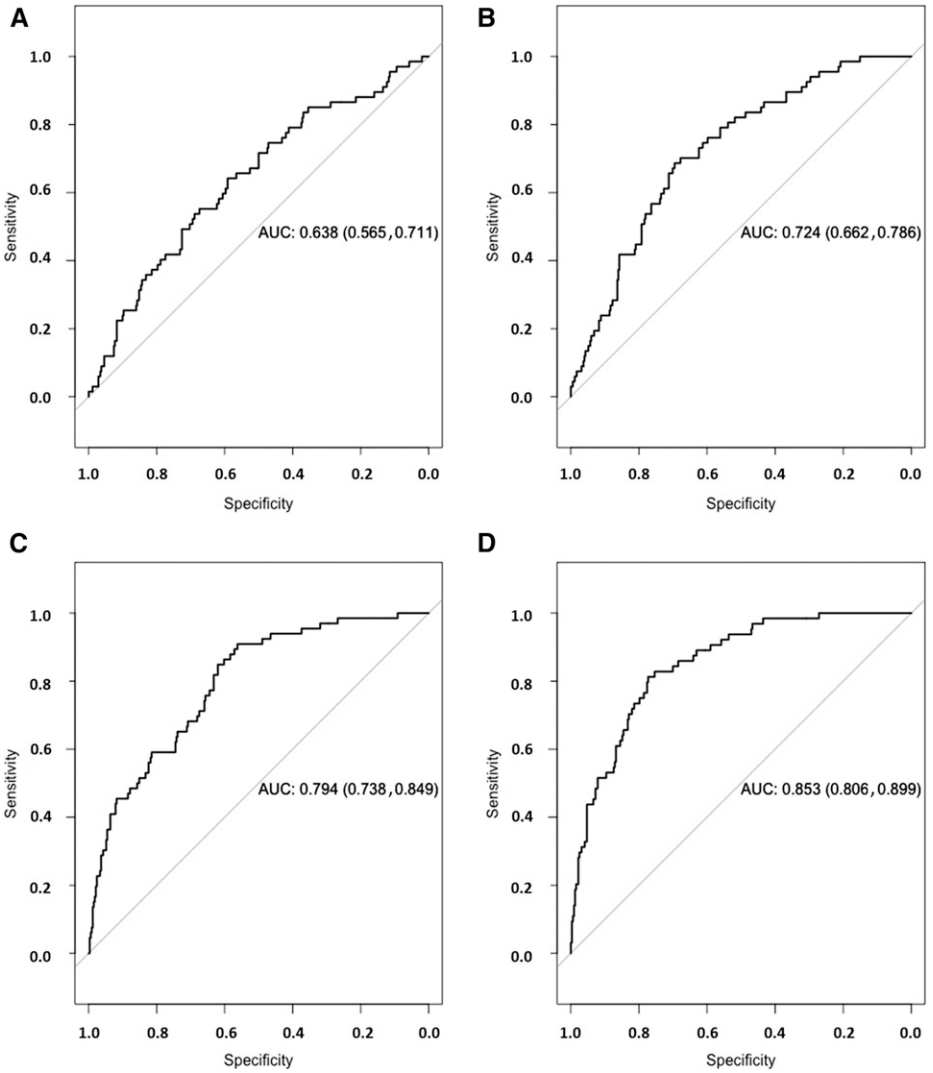

FIGURE 2 Receiver operating characteristics curve with the AUC (95% CI) for predicting incident type 2 diabetes during the 5.5-y (n = 69 new cases) follow-up including (A) smoking, alcohol consumption, waist circumference, systolic blood pressure, and heredity; (B) dietary and nutritional biomarkers; (C) serum adiponectin concentration, HOMA-IR, smoking, impaired glucose tolerance, and impaired fasting glucose at baseline; and (D) a combination of the data in panels B and C as variables in the model.

95% CI: 0662, 0.786) than prediction based on anthropometry (AUC: 0.638; 95% CI: 0.565, 0.711). Inclusion of diet and nutrient status biomarkers improved prediction based on serum adiponectin, smoking, IGT, IFG alone from an AUC of 0.794 (95% CI: 0.738, 0.849) to an AUC of 0.853 (95% CI: 0.806, 0.899) (**Figure 2**).

DISCUSSION

We have explored the associations between multiple dietary biomarkers and glucose tolerance accounting for common risk factors of T2D and CVD and have prospectively assessed whether these were associated with new cases of T2D at a 5-y follow-up. In addition, we tested if these biomarkers could be used for the prediction of T2D and compared how the prediction based on dietary and nutritional biomarkers compares with established predictive markers of T2D. We found that 8 of the 10 diet-related biomarkers detected were associated with GTS, supporting the notion that diet is an important factor for preventing T2D. The results based on the dietary biomarkers suggest that an overall higher consumption of whole-grain products, fatty fish, and vegetable oils is associated with better GTS, whereas a higher consumption of meat was associated with a greater risk of IGT. This pattern is in line with many healthy diet recommendations, including the American Diabetes Association (24) and the Nordic Nutrition Recommendations (25). Furthermore, by using the combined dietary biomarkers it was possible to predict new cases of T2D during the 5.5-y follow up with specificity and sensitivity similar to using serum adiponectin concentration, HOMA-IR, smoking, and IGT and IFG at baseline, which were the best predictors of T2D in this cohort (15).

Diet is commonly associated with many risk factors for T2D (4), and positive dietary changes are among the recommendations for people at risk of developing T2D. On finding the association between dietary biomarkers and GTS and T2D development, we hypothesized that correcting for common risk factors of T2D and CVD, including systolic blood pressure, total cholesterol, LDL, HDL, triglycerides, free fatty acids, and obesity, would attenuate

the effects of the biomarkers, based on a healthy diet leading to an overall healthier lifestyle. Surprisingly, the associations of the dietary biomarkers with GTS remained, irrespective of the risk factors, suggesting that whole grains, fish, saturated fat, margarine and vegetable oil, vitamin E, and meat may be related to and mediate T2D development beyond their known or suggested effects on obesity and metabolism (26–28).

Of the biomarkers used, several may have a direct link to the development of T2D. Plasma alkylresorcinols are well-established markers of whole-grain wheat and rye intake (18, 29, 30) and were found to improve insulin sensitivity in rats (31). Although they have not been directly studied for effects on glucose metabolism in humans, this is the first study to our knowledge to find a relation between alkylresorcinol concentrations and GTS and it supports earlier work that has found a relation between the ratios of specific alkylresorcinol homologs and T2D incidence (12). CMPF has been suggested to be a biomarker for fish intake (20), although increased circulating concentrations have been found to lead to a loss of glucose control via β cell dysfunction (32). Furthermore, CMPF was associated with T2D development in a prospective study (33). Our results show divergent associations, with CMPF being associated with both IGT and a reduced risk of developing T2D, suggesting that plasma CMPF concentrations may reflect 2 different outcomes: as a modulator of β cell dysfunction leading to T2D, and in populations without T2D and as a possible biomarker of fish intake, suggesting healthier dietary choices. α-Tocopherol reflects vitamin E status, and its relations with complications of T2D are not yet fully elucidated (34–36), although a recent study described vitamin E metabolism as associated with glucose control in both lean and obese subjects (37). Tocopherol metabolism can also be influenced by other dietary components (38), further confounding the direct relation between α-tocopherol and disease. EPA is one of the n–3 fatty acids commonly associated with marine foods (39) and recommended for improved cardiometabolic health (40). Also, EPA supplementation has been shown to improve glycemic control (41, 42). Recently we found that β-alanine was increased after beef intake compared with fish because of a high amount of β-alanine in beef mince compared with fish (17), suggesting that β-alanine may reflect beef intake. Other studies have linked β-alanine and carnosine (β-alanine conjugated with the amino acid histidine) with T2D, which may also explain this result (43–45).

Although these biomarkers are by no means comprehensive in their coverage of diet, they do reflect several food groups that are of interest for T2D development. These biomarker results support previous findings on whole grains (12, 46–48), α-tocopherol (49, 50), fish (51), and a reduction of T2D risk, although the literature in relation to fish intake and T2D is not conclusive (52). Also, the present potential association of meat intake supports earlier findings of meat intake and increased T2D risk (53–55). There were slightly higher linoleic acid concentrations in subjects with T2D than in subjects with NGT, supporting the earlier work suggesting that excess circulating linoleic acid could have adverse health effects (56).

One of the strengths of the current study is the application of a multiple-nutritional biomarker approach in a free-living population, which gives a holistic picture of normal within population dietary variation and its association with GTS and the development of T2D. Biomarkers give an unbiased view of food intake but do have their own set of limitations based on variations in food composition, bioavailability, and metabolism (39, 57, 58). In this context, the results should be interpreted with some caution. α-Tocopherol and EPA are used in several biological processes, and although they are of dietary origin (although EPA can be synthesized from α-linolenic acid), plasma concentrations are influenced by several factors other than diet. For example, EPA is metabolized during elevated inflammation (59), which can confound its relation with fish intake and further genetic factors might also affect synthesis (60). Additionally, there may be some bias introduced by the use of a broad extraction-and-analysis method that covers a wide range of compound classes. A general extraction method was used that has been optimized to reproducibly extract many different compounds for metabolomics analysis by GC-MS (61) and may not be optimal for all compounds, especially those that are highly lipophilic, including the alkylresorcinols and tocopherols. An advantage of this approach is that the method could be expanded to cover a wider range of biomarkers as they continue to be discovered, thus improving our ability to cover a wide range of foods and nutrients. Furthermore, there are many gaps in our knowledge about how well a broad range of dietary biomarkers perform in observational settings, in part because of a lack of validation studies for many biomarkers. In the present study, the relatively large, well-defined, and homogeneous cohort provides a good starting point for testing the use of a broad panel of dietary biomarkers, and the results suggest that the use of dietary and nutrient status biomarkers could be a useful way to study the relation between diet and disease. Further studies with the use of this methodology in cohorts with diet intake information should be carried out to confirm that this approach of using one method to cover multiple biomarkers is valid.

Despite the known limitations, the wider application of dietary biomarkers to improve dietary intake estimates in research is encouraged, and several studies have demonstrated the potential of using several markers in the context of intervention studies (29, 30, 62). The biomarker panel measured in the current study covers some of the important food groups and represents the first multi-dietary biomarker analysis in the study of T2D. More work is needed to improve biomarker coverage for future studies, and further study in cohorts with existing dietary recall data are needed to further validate the approach.

In conclusion, our results suggest that a diet high in whole grains, vegetable oil, and fish, as well as α-tocopherol as measured by a multiple-biomarker approach can be a factor in controlling GTS and T2D development in free-living, 64-y-old Swedish women irrespective of common T2D and CVD risk factors. The multiple dietary and nutrient-status biomarker approach can capture different dietary components with one method and represents a step toward a universal methodology for the unbiased assessment of diet for use alone or in conjunction with self-reported dietary assessment.

We thank Nils-Gunnar Carlsson for technical advice, Pipsa Pajarinen and Annette Almgren for their assistance with the sample analysis, and Marie Louise Ekholm, Birgitta Jannemark, and Josefin Kjelldahl for collection and sample delivery.

The authors' responsibilities were as follows—OS: conducted the metabolomics analyses, performed statistical analyses, interpreted the data, and wrote the manuscript; MVL: interpreted the data and provided intellectual input; AR: conceived and designed the metabolomics study, interpreted the

data, and provided intellectual input; BF and GB: were responsible for the cohort study and interpreted the data; and A-SS: conceived the metabolomics study and provided important intellectual input. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. WHO. Global report on diabetes. Geneva: WHO; 2016.
2. Diabetes Prevention Program Research Group; Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, et al. 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. Lancet 2009;374:1677–86.
3. Perreault L, Pan Q, Mather KJ, Watson KE, Hamman RF, Kahn SE. Effect of regression from prediabetes to normal glucose regulation on long-term reduction in diabetes risk: results from the Diabetes Prevention Program Outcomes Study. Lancet 2012;379:2243–51.
4. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343–50.
5. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, Li H, Li H, Jiang Y, An Y, et al. The long-term effect of lifestyle interventions to prevent diabetes in the China Da Qing Diabetes Prevention Study: a 20-year follow-up study. Lancet 2008;371:1783–9.
6. Ley SH, Hamdy O, Mohan V, Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet 2014;383:1999–2007.
7. Illner AK, Freisling H, Boeing H, Huybrechts I, Crispim SP, Slimani N. Review and evaluation of innovative technologies for measuring diet in nutritional epidemiology. Int J Epidemiol 2012;41:1187–203.
8. Byers T. Food frequency dietary assessment: how bad is good enough? Am J Epidemiol 2001;154:1087–8.
9. Kristal AR, Peters U, Potter JD. Is it time to abandon the food frequency questionnaire? Cancer Epidemiol Biomarkers Prev 2005;14:2826–8.
10. Kaaks RJ. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. Am J Clin Nutr 1997;65:1232S–9S.
11. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Åkesson B, Landin-Olsson M, Rosqvist F, Iggman D, Schwab U, Herzig K-H, et al. Plasma alkylresorcinols C17:0 C21:0 ratio, a biomarker of relative whole-grain rye intake, is associated to insulin sensitivity: a randomized study. Eur J Clin Nutr 2014;68:453–8.
12. Biskup I, Kyrø C, Marklund M, Olsen A, van Dam RM, Tjønneland A, Overvad K, Lindahl B, Johansson I, Landberg R. Plasma alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and risk of type 2 diabetes in Scandinavian men and women. Am J Clin Nutr 2016;104:88–96.
13. Brohall G, Behre CJ, Hulthe J, Wikstrand J, Fagerberg B. Prevalence of diabetes and impaired glucose tolerance in 64-year-old Swedish women: experiences of using repeated oral glucose tolerance tests. Diabetes Care 2006;29:363–7.
14. Alberti KGMM, Zimmet PZ, Consultation WHO. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Diabet Med 1998;15:539–53.
15. Fagerberg B, Kellis D, Bergstrom G, Behre CJ. Adiponectin in relation to insulin sensitivity and insulin secretion in the development of type 2 diabetes: a prospective study in 64-year-old women. J Intern Med 2011;269:636–43.
16. Savolainen OI, Sandberg A-S, Ross AB. A simultaneous metabolic profiling and quantitative multimetabolite metabolomic method for human plasma using gas-chromatography tandem mass spectrometry. J Proteome Res 2016;15:259–65.
17. Ross AB, Svelander C, Undeland I, Pinto R, Sandberg A-S. Herring and beef meals lead to differences in plasma 2-aminoadipic acid, β-alanine, 4-hydroxyproline, cetoleic acid, and docosahexaenoic acid concentrations in overweight men. J Nutr 2015;145:2456–63.
18. Ross AB. Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. J Nutr Metab 2012;2012:462967.
19. Ross AB, Shepherd MJ, Schüpphaus M, Sinclair V, Alfaro B, Kamal-Eldin A, Åman P. Alkylresorcinols in cereals and cereal products. J Agric Food Chem 2003;51:4111–8.
20. Hanhineva K, Lankinen MA, Pedret A, Schwab U, Kolehmainen M, Paananen J, de Mello V, Sola R, Lehtonen M, Poutanen K, et al. Nontargeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish, and bilberries in a randomized controlled trial. J Nutr 2015;145:7–17.
21. Livsmedelsverket [Swedish Food Agency] [Internet]. Livsmedels- och näringsintag bland vuxna i Sverige. [Food and nutrient intake among adults in Sweden, 2010-2011.] [cited 2017 Mar 13]. Available from: https://www.livsmedelsverket.se/globalassets/matvanor-halsa-miljo/kostrad-matvanor/matvaneundersokningar/riksmaten_2010_20111.pdf (in Swedish).
22. Gunnerud U, Jansson N. Hälsomässiga effekter av palmolja - från vetenskap till folkhälsa. Swedish nutrition foundation [Internet]. c2014 [cited 2017 Mar 13]. Available from: http://snf.ideon.se/wp-content/uploads/2015/01/palmoljerapport_FINALversion.pdf.
23. Jonsson P, Johansson AI, Gullberg J, Trygg JAJ, Grung B, Marklund S, Sjostrom M, Antti H, Moritz T. High-throughput data analysis for detecting and identifying differences between samples in GC/MS-based metabolomic analyses. Anal Chem 2005;77:5635–42.
24. Evert AB, Boucher JL, Cypress M, Dunbar SA, Franz MJ, Mayer-Davis EJ, Neumiller JJ, Nwankwo R, Verdi CL, Urbanski P, et al. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care 2013;36:3821–42.
25. Bere E, Brug J. Towards health-promoting and environmentally friendly regional diets – a Nordic example. Public Health Nutr 2009;12:91–6.
26. Who J, Consultation F. Diet, nutrition and the prevention of chronic diseases. Geneva: WHO; 2003.
27. James PT, Rigby N, Leach R; International Obesity Task Force. The obesity epidemic, metabolic syndrome and future prevention strategies. Eur J Cardiovasc Prev Rehabil 2004;11:3–8.
28. Roberts CK, Barnard RJ. Effects of exercise and diet on chronic disease. J Appl Physiol 2005;98:3–30.
29. McKeown NM, Marklund M, Ma J, Ross AB, Lichtenstein AH, Livingston KA, Jacques PF, Rasmussen HM, Blumberg JB, Chen C-YO. Comparison of plasma alkylresorcinols (AR) and urinary AR metabolites as biomarkers of compliance in a short-term, whole-grain intervention study. Eur J Nutr 2016;55:1235–44.
30. Andersson A, Marklund M, Diana M, Landberg R. Plasma alkylresorcinol concentrations correlate with whole grain wheat and rye intake and show moderate reproducibility over a 2- to 3-month period in free-living Swedish adults. J Nutr 2011;141:1712–8.
31. Oishi K, Yamamoto S, Itoh N, Nakao R, Yasumoto Y, Tanaka K, Kikuchi Y, Fukudome S-I, Okita K, Takano-Ishikawa Y. Wheat alkylresorcinols suppress high-fat, high-sucrose diet-induced obesity and glucose intolerance by increasing insulin sensitivity and cholesterol excretion in male mice. J Nutr 2015;145:199–206.
32. Prentice KJ, Luu L, Allister EM, Liu Y, Jun LS, Sloop KW, Hardy AB, Wei L, Jia W, Fantus IG, et al. The furan fatty acid metabolite CMPF is elevated in diabetes and induces beta cell dysfunction. Cell Metab 2014;19:653–66.
33. Liu Y, Prentice KJ, Eversley JA, Hu C, Batchuluun B, Leavey K, Hansen JB, Wei DW, Cox B, Dai FF, et al. Rapid elevation in CMPF may act as a tipping point in diabetes development. Cell Reports 2016;14:2889–900.
34. Xu R, Zhang S, Tao A, Chen G, Zhang M. Influence of vitamin E supplementation on glycaemic control: a meta-analysis of randomised controlled trials. PLoS One 2014;9:e95008.
35. Farvid MS, Jalali M, Siassi F, Hosseini M. Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. Diabetes Care 2005;28:2458–64.
36. Gómez-Pérez FJ, Valles-Sanchez VE, López-Alvarenga JC, Choza-Romero R, Ibarra Pascuali JJ, González Orellana R, Pérez Ortiz OB, Rodríguez Padilla EG, Aguilar Salinas CA, Rull JA. Vitamin E modifies neither fructosamine nor HbA1c levels in poorly controlled diabetes. Rev Invest Clin 1996;48:421–4.
37. Lee S, Zhang C, Kilicarslan M, Piening BD, Bjornson E, Hallström BM, Groen AK, Ferrannini E, Laakso M, Snyder M, et al. Integrated network analysis reveals an association between plasma mannose levels and insulin resistance. Cell Metab 2016;24:172–84.
38. Hamre K. Metabolism, interactions, requirements and functions of vitamin E in fish. Aquacult Nutr 2011;17:98–115.
39. Arab L. Biomarkers of fat and fatty acid intake. J Nutr 2003;133 Suppl 3:925S–32S.

40. Eyre H, Kahn R, Robertson RM, Clark NG, Doyle C, Gansler T, Glynn T, Hong Y, Smith RA, Taubert K, et al. Preventing cancer, cardiovascular disease, and diabetes: a common agenda for the American Cancer Society, the American Diabetes Association, and the American Heart Association. CA Cancer J Clin 2004;54: 190–207.
41. Sarbolouki S, Javanbakht MH, Derakhshanian H, Hosseinzadeh P, Zareei M, Hashemi SB, Dorosty AR, Eshraghian MR, Djalali M. Eicosapentaenoic acid improves insulin sensitivity and blood sugar in overweight type 2 diabetes mellitus patients: a double-blind randomised clinical trial. Singapore Med J 2013;54:387–90.
42. Sawada T, Tsubata H, Hashimoto N, Takabe M, Miyata T, Aoki K, Yamashita S, Oishi S, Osue T, Yokoi K, et al. Effects of 6-month eicosapentaenoic acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. An open label, single blinded, prospective randomized controlled trial. Cardiovasc Diabetol 2016;15: 121.
43. de Oliveira FA, Shahin MH, Gong Y, McDonough CW, Beitelshees AL, Gums JG, Chapman AB, Boerwinkle E, Turner ST, Frye RF, et al. Novel plasma biomarker of atenolol-induced hyperglycemia identified through a metabolomics-genomics integrative approach. Metabolomics 2016;12.
44. Zhou Y, Qiu L, Xiao Q, Wang Y, Meng X, Xu R, Wang S, Na R. Obesity and diabetes related plasma amino acid alterations. Clin Biochem 2013;46:1447–52.
45. Gualano B, Everaert I, Stegen S, Artioli GG, Taes Y, Roschel H, Achten E, Otaduy MC, Junior AHL, Harris R, et al. Reduced muscle carnosine content in type 2, but not in type 1 diabetic patients. Amino Acids 2012;43:21–4.
46. de Munter JS, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med 2007;4:e261.
47. Liu S, Manson JE, Stamper MJ, Hu FB, Giovannucci E, Colditz GA, Hennekens CH, Willet WC. A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. Am J Public Health 2000;90:1409–15.
48. Fung TT, Hu FB, Pereira MA, Liu S, Stamper MJ, Colditz GA, Willet WC. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. Am J Clin Nutr 2002;76:535–40.
49. Mayer-Davis EJ, Costacou T, King I, Zaccaro DJ, Bell RA; Insulin Resistance and Atherosclerosis Study (IRAS). Plasma and dietary vitamin E in relation to incidence of type 2 diabetes: the Insulin Resistance and Atherosclerosis Study (IRAS). Diabetes Care 2002;25: 2172–7.
50. Arnlöv J, Zethelius B, Riserus U, Basu S, Berne C, Vessby B, Alfthan G, Helmersson J; Uppsala Longitudinal Study of Adult Men Study. Serum and dietary beta-carotene and alpha-tocopherol and incidence of type 2 diabetes mellitus in a community-based study of Swedish men: report from the Uppsala Longitudinal Study of Adult Men (ULSAM) study. Diabetologia 2009;52:97–105.
51. Patel PS, Sharp SJ, Luben RN, Khaw KT, Bingham SA, Wareham NJ, Forouhi NG. Association between type of dietary fish and seafood intake and the risk of incident type 2 diabetes: the European prospective investigation of cancer (EPIC)-Norfolk cohort study. Diabetes Care 2009;32:1857–63.
52. Xun P, He K. Fish consumption and incidence of diabetes: meta-analysis of data from 438,000 individuals in 12 independent prospective cohorts with an average 11-year follow-up. Diabetes Care 2012;35:930–8.
53. InterAct Consortium; Bendinelli B, Palli D, Masala G, Sharp SJ, Schulze MB, Guevara M, van der ADL, Sera F, Amiano P, et al. Association between dietary meat consumption and incident type 2 diabetes: the EPIC-InterAct study. Diabetologia 2013;56:47–59.
54. Männistö S, Kontto J, Kataja-Tuomola M, Albanes D, Virtamo J. High processed meat consumption is a risk factor of type 2 diabetes in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study. Br J Nutr 2010;103:1817–22.
55. Fung TT, Schulze M, Manson JE. Dietary patterns, meat intake, and the risk of type 2 diabetes in women. Arch Intern Med 2004;164:2235–40.
56. Choque B, Catheline D, Rioux V, Legrand P. Linoleic acid: between doubts and certainties. Biochimie 2014;96:14–21.
57. Landberg R, Linko A-M, Kamal-Eldin A, Vessby B, Adlercreutz H, Åman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. J Nutr 2006;136:2760–5.
58. Lind MV, Savolainen OI, Ross AB. The use of mass spectrometry for analysing metabolite biomarkers in epidemiology: methodological and statistical considerations for application to large numbers of biological samples. Eur J Epidemiol 2016;31:717–33.
59. Johansson S, Lönnqvist A, Ostman S, Sandberg A-S, Wold AE. Long-chain polyunsaturated fatty acids are consumed during allergic inflammation and affect T helper type 1 (Th1)- and Th2-mediated hypersensitivity differently. Clin Exp Immunol 2010;160:411–9.
60. Minihane AM. Impact of genotype on EPA and DHA status and responsiveness to increased intakes. Nutrients 2016;8:123.
61. A J, Trygg J, Gullberg J, Johansson AI, Jonsson P, Antti H, Marklund SL, Moritz T. Extraction and GC/MS analysis of the human blood plasma metabolome. Anal Chem 2005;77:8086–94.
62. Marklund M, Landberg R, Andersson A, Åman P, Kamal-Eldin A. Alkylresorcinol metabolites in urine correlate with the intake of whole grains and cereal fibre in free-living Swedish adults. Br J Nutr 2013; 109:129–36.

The American Journal of Clinical Nutrition