

A Health-Conscious Food Pattern Is Associated with Prediabetes and Gut Microbiota in the Malmö Offspring Study

Ulrika Ericson,¹ Louise Brunkwall,¹ Sophie Hellstrand,¹ Peter M Nilsson,² and Marju Orho-Melander¹

¹Department of Clinical Sciences, Diabetes and Cardiovascular Disease, Genetic Epidemiology, Lund University, Malmö, Sweden; and

²Department of Internal Medicine, Skåne University Hospital, Lund University, Malmö, Sweden

ABSTRACT

Background: Diet is a determinant of gut microbiota. Both diet and gut microbiota have been linked to metabolic diseases.

Objective: We aimed to examine data-driven food patterns in relation to the prevalence of prediabetes and gut microbiota composition and food pattern-associated bacteria in relation to prediabetes.

Methods: Food patterns were extracted using principal component analysis in 1726 individuals (aged 18–71 y, 55% women, mean BMI = 25.5 kg/m²) without diabetes from the population-based Malmö Offspring Study. The gut (fecal) microbiota was analyzed by sequencing the 16S ribosomal RNA gene (V1–V3 region). Prediabetes classification was based on fasting glucose ≥ 6.0 mmol/L and/or glycosylated hemoglobin ≥ 42 mmol/L at baseline and/or type 2 diabetes diagnosis during follow-up (0–3.8 y). Logistic regression was used to investigate cross-sectional associations with prediabetes, and the general linear model to examine associations between food patterns and bacterial genera.

Results: Two food patterns, the Health-conscious and the Sugar and High-Fat Dairy patterns, were identified. Adherence to the Health-conscious pattern was associated with a lower prevalence of prediabetes (OR comparing highest quintile with lowest: 0.54; 95% CI: 0.32, 0.92; *P*-trend = 0.03) and with the abundance of several gut bacterial genera, of which the most robust findings were with a higher abundance of *Roseburia* and *Lachnospira* and with a lower abundance of *Eubacterium*. *Roseburia* was also associated with a lower prevalence of prediabetes (OR comparing highest quintile with lowest: 0.56; 95% CI: 0.35, 0.92; *P*-trend = 0.01) and the association between the Health-conscious pattern and prediabetes was attenuated after adjustment for abundance of *Roseburia* and BMI. Adherence to the Sugar and High-Fat Dairy pattern was associated with a higher prevalence of prediabetes in women (*P*-trend across food pattern quintiles = 0.03).

Conclusions: In this Swedish population-based study, a Health-conscious food pattern showed an inverse association with the prevalence of prediabetes. Potential underlying explanations may involve links between healthy diet and BMI, as well as gut microbiota, especially a higher abundance of *Roseburia*. *J Nutr* 2020;150:861–872.

Keywords: food intake, food patterns, epidemiology, type 2 diabetes, gut microbiota

Introduction

Type 2 diabetes (T2D) affects the quality of life of many individuals worldwide and the high and increasing prevalence is an enormous burden to society. Identification of modifiable lifestyle factors including diet, that have beneficial effects on glucose metabolism and counteract the development of T2D is consequently of great value to public health. However, diet is a complex exposure of interacting food components, consumed in different combinations. It is therefore important to capture overall healthy food patterns that could be translated into dietary guidelines. Many data-driven food patterns referred to

as “Healthy” or “Prudent” have been associated with decreased risk of T2D (1–3). Diet is also an important factor for gut microbiota composition and richness (4), which have been found to be altered in many disease states including obesity and T2D (5–7), and a recent study indicated that the gut microbiota may play a causal role in the development of T2D via the effects on SCFA production from fermentable food components (8). Studies on overall dietary patterns in relation to gut microbiota are scarce and have mainly focused on associations with the Mediterranean dietary patterns or plant-based versus animal-based diets (9–13). Although diet has been identified as 1 of

the main determinants of gut microbiota composition, large observational studies are lacking regarding foods commonly consumed in modern societies and no study has examined data-driven food patterns in relation to gut bacterial composition. Moreover, the importance of gut microbiota in health and disease in a large number of individuals, or in population-based cohorts, have only recently been studied (4, 14).

Our aim was to identify data-driven food patterns using principal component analysis in 1726 men and women from the Malmö Offspring Study (MOS) without a previous diabetes diagnosis, and to examine whether the extracted food patterns were associated with prevalence of prediabetes at baseline, independently of other lifestyle factors. In addition, we wanted to examine the identified food patterns in relation to gut microbiota composition. Finally, the food pattern-related gut bacteria were examined in relation to prediabetes.

Methods

Study population and data collection

MOS is an ongoing population-based cohort study where children and grandchildren (aged >18 y) of participants in the Malmö Diet and Cancer Study—Cardiovascular Cohort are recruited (15, 16). Participants were invited via letter and visited the research clinic on 2 occasions with about a week in between the visits. There were no exclusion criteria. At the first visit, venous blood was drawn after an overnight fast, and anthropometrics and blood pressure were measured. The study participants were instructed on how to collect the fecal samples at home, how to record their food intake during 4 d (starting the day after the first visit), and how to fill in a web-based food propensity questionnaire and a comprehensive questionnaire on other lifestyle and socioeconomic factors (before the second visit). At the second visit the fecal samples were brought to the clinic. The study protocols were approved by the Ethics Committee of Lund University (protocol number DNR 2012/594) and all participants provided written informed consent.

From the start of the study in March 2013 until the end of April 2017, 2644 individuals participated in baseline examinations (47% of the eligible participants) (Supplemental Figure 1). Of the nonparticipants, 29% answered that they were not willing to take part, 28% did not reply, 26% did not come to their appointment, 5% died or moved before they had received an invitation, 4% wanted to participate later, 5% stated lack of time due to work or other activities, 1% were ill, and 2% had other reasons. In total, 1788 participants contributed dietary data by completing a web-based 4-d food record. Out of those, we excluded 62 individuals with prevalent diabetes (according to information from national and local registries and questionnaire data at baseline), leaving 1726 (55% women) individuals for the present study. For gut microbiota analyses, 1477 individuals with gut microbiota data were included. As use of antibiotics and probiotics are known to alter the gut microbiota composition, we performed sensitivity analysis on

the gut microbiota in a reduced sample ($n = 851$) after the exclusion of: 1) individuals reporting to have used antibiotics during the 6 mo previous to data collection ($n = 169$) or with missing data on use of antibiotics ($n = 177$), and/or 2) individuals reporting probiotic use >3 times per week ($n = 77$) or with missing data on the use of probiotics ($n = 363$).

Clinical measurements

Height (m), without shoes and hats, was measured to the nearest centimeter directly under the meter with the legs together looking straight ahead. Weight (kg) was measured in light clothing on a calibrated balance beam or digital scale. BMI (kg/m^2) was calculated from measurements of weight and height. Resting blood pressure (mmHg) was measured as a mean of 2 readings in the supine position after 10 min rest by use of an automatic device (Omron). Blood samples were analyzed at the Department of Clinical Chemistry, Malmö. Whole blood glycated hemoglobin (HbA1c) concentrations were measured within 4 h using the CapillaryS 3 Tera HbA1c kit (Sebia). Plasma glucose concentrations were measured directly using the HemoCue Glucose 201+ System (HemoCue AB). Total cholesterol, triglycerides, and HDL cholesterol plasma concentrations were measured within 4 h by enzymatic methods using the COBAS system (Roche Diagnostics). LDL cholesterol was calculated using the Friedewald equation.

Dietary data

Diet was assessed using a 4-d web-based food record, the Riksmaten2010, developed by the Swedish National Food Institute (17). The relative validity of the Riksmaten2010 method has been evaluated by comparing the reported energy intake to objectively measured total energy expenditure with the doubly labelled water technique ($r = 0.40$) (17). Estimated intakes of fiber sources with the Riksmaten2010 method have been compared with objective plasma biomarkers; the Pearson correlation coefficients for fruit and vegetable intake were 0.46 and 0.20, and for whole grain intake 0.30 and 0.29, in women and men, respectively (18). In addition, repeated 4-d records have been collected for 323 individuals indicating reliable data with Pearson correlation coefficients of 0.55, 0.40, 0.56, and 0.61 for energy-adjusted intakes of carbohydrates, fat, protein, and fiber, respectively (unpublished data).

The mean daily food intakes were calculated from frequency and portion estimates from the food records. Food intakes were converted into energy and nutrient intakes using the national food database; *Riksmaten vuxna* 2010, version 10–05-05. Food intakes were aggregated into 43 food groups considered to represent overall dietary intake. Our aim was to cover as many parts of the overall diet as possible, but to avoid an overly detailed level on foods consumed irregularly, which may not be satisfactorily captured on a 4-d basis. This was, for example, the reason that the intake of lean and fatty fish were grouped together. Characteristics related to both dietary behaviors and nutrient content were considered when aggregating the foods. In order to minimize the effects of misreporting, energy-adjusted intakes of the food groups were calculated by regressing the intakes on nonalcohol energy intake using the residual method (19).

Other variables

Lifestyle variables were based on answers from a web-based self-administered questionnaire on lifestyle and socioeconomic factors. Education was based on the participants highest level of completed education defined as primary (<9 y), secondary (9 y), upper secondary (12 y), and a university or college degree. Smoking status was defined as never-smoker, ex-smoker, irregular smoker, and regular smoker (based on the participant's own definition of regular smoking). Total physical activity level was estimated by summing 4-grade scale answers regarding physical activity at work (very light = 1, light = 2, moderately heavy = 3, and heavy/very heavy = 4) and leisure time (sedentary = 1, moderate activity = 2, moderate and regular activity = 3, and regular training = 4) into a 7-grade scale (2–8 points, e.g. 2 = 1 + 1 for very light physical activity at work and sedentary leisure time). Alcohol consumption was defined by a 7-category variable. Participants

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Address correspondence to UE (e-mail: ulrika.ericson@med.lu.se).

Abbreviations used: HbA1c, glycated hemoglobin; MOS, Malmö Offspring Study; OTU, operational taxonomical unit; T2D, type 2 diabetes.

reporting zero consumption during the 4-d record and reporting to never consume alcohol in the lifestyle questionnaire were categorized as zero-reporters. The second category was defined as no consumption during the 4-d record, but indication of alcohol consumption in the questionnaire, the other category ranges were ≤ 10 g/d, >10 – 20 g/d, >20 – 30 g/d, >30 – 40 g/d, and >40 g/d, according to reported consumption during the 4-d record. Use of antibiotics was based on the question “Have you used antibiotics during the previous 6 months?” Use of probiotics was defined as ≤ 3 times per week or >3 times per week based on information from the food propensity questionnaire.

Definition of prediabetes

In total, 260 cases were classified as having prediabetes. The classification was based on fasting plasma glucose ≥ 6.0 mmol/L and/or HbA1c ≥ 42 mmol/L at baseline (2013–2017) ($n = 258$) and/or individuals diagnosed with T2D during follow-up according to register data ($n = 12$) [follow-up until 31 December 2016, mean follow-up = 1.6 y (0–3.8)]. Register data were obtained from national and regional registries (3).

Gut microbiota

Fecal samples were collected without preservatives at home in sterile plastic tubes (80.9924.014 polypropylene, Sarstedt) and stored in a home freezer until they were brought to the clinic where they were stored at -80°C . The microbial DNA was extracted using the QIAamp column Stool Kit and the V1–V3 region (300bp \times 2) of the 16S rRNA gene was amplified and sequenced on a HiSeq Illumina at the GATC Biotech (Constance). The fastq files were then aligned by FLASH and binned together to operational taxonomical units (OTUs) using QIIME 1 (Quantitative Insight Into Microbial Ecology) (20, 21). The sequences were matched with the reference database Greengene (v.13.8). OTUs with $<0.01\%$ counts assigned to them (out of the pool of all counts assigned to all OTUs) and OTUs occurring in <3 individuals were removed, leaving 64 bacteria characterized at genus level and belonging to 8 microbial phyla, for further analysis. All association analyses were performed using normalized absolute abundancies, i.e. counts that were normalized by cumulative sum scaling (CSS) in R using the *metagenomicSeq* package. In addition, to descriptively illustrate percentage abundances of the bacterial genera in the study population, relative abundances were used. The Shannon diversity index was calculated using *diversity* within the R package *vegan*.

Statistical analysis

The SPSS statistical computer package (version 24.0; IBM Corporation) was used for all statistical analyses. All food variables were log transformed (e-log) to normalize the distribution before analysis. To handle log transformation of zero intakes, we added a very small amount (0.01 g). All food intakes were energy adjusted with the residual method.

We used principal component analysis (eigenvalues >1 and varimax rotation) to reduce 43 energy-adjusted food groups into factors representing food patterns. From the obtained scree plots (Supplemental Figure 2), we decided to retain and rotate the 2 factors with eigenvalues >2 , that explained most of the variance in the data (6.8% and 5.2%, respectively). These factors were possible to interpret and translate into food patterns based on their loadings for the initial food group variables. In addition, these factors were found to be similar in men and women, indicating robust patterns. Reported characteristics of the patterns were based on food group loadings <-0.25 or $>+0.25$. All individuals were assigned scores for each of the 2 factors that represented food patterns, corresponding to the agreement of their diet to the patterns.

We examined baseline characteristics according to prediabetes status and quintiles of the factors representing food patterns with the general linear model for continuous variables (adjusted for age and sex) and with the chi-square test for categorical variables. Food patterns in relation to prediabetes were examined using logistic regression.

The basic model included adjustments for age, sex, and total energy intake. A second multivariable model also included physical activity level, smoking, alcohol consumption, and level of education, and a third model additionally included BMI. Missing data for the potential confounding variables were treated as separate categories. We examined Spearman's correlations between retained food patterns and microbiota genera. In addition, we examined microbiota genera across quintiles of the food patterns with the general linear model adjusted for age, sex, physical activity level, smoking, and alcohol consumption, as well as with additional adjustments for BMI and fiber intake. Finally, the gut bacterial genera found to associate with food patterns, after adjustment for lifestyle factors including BMI, were examined in relation to prediabetes with logistic regression. Tests for interactions between gender and food patterns on prediabetes and gut bacterial composition were performed [gender \times quintile of food pattern (treated as continuous variables)].

In sensitivity analysis, we only included individuals who had not used antibiotics during the previous 6 mo and those who did not use probiotics >3 times per week.

All statistical tests were 2-sided. Statistical nominal significance was assumed at $P < 0.05$.

To correct for multiple testing, when analyzing dietary patterns in relation to 64 gut bacterial genera, the Bonferroni correction was applied and therefore statistical significance was assumed at $P < 8 \times 10^{-4}$ (0.05/64).

Results

We retained 2 factors from the principal component analysis. The first derived factor explained 6.8% of the variance in the data. We called it the Health-conscious food pattern, because it was characterized by (loadings <-0.25 or $>+0.25$) high intakes of fruits and berries, nuts and seeds, legumes, other vegetables (nonlegumes), plain yogurt, fresh cheese, tea, animal replacement foods, breakfast cereals, cooked grains such as bulgur, oil-based dressings, fish and fiber-rich bread, and by low intakes of sugar-sweetened beverages, red and processed meat, white bread and fried/deep-fried potatoes (Figure 1A). The second food pattern, named the Sugar and High-Fat Dairy pattern, explained 5.2% of the variance and was characterized by high intakes of pastry and desserts, high-fat milk, cream, traditional sauces, jam and sugar, white bread, boiled potatoes, cheese, butter, eggs, processed meat, and sweets, and by low intake of food replacement products, such as weight loss powders (Figure 1B).

The factors showed strongest loadings for the same foods in women and men (data not shown), with the main exception that the Sugar and High-Fat Dairy pattern did not show a loading above 0.25 for cream in men (loading = 0.16).

Baseline characteristics

The study participants with high adherence to the Health-conscious food pattern were more often women and they were characterized by higher age and level of education, compared with those with low adherence to that food pattern (Table 1). In addition, those adhering to the Health-conscious pattern reported less sedentary leisure time, less heavy work, less smoking, and lower energy intake, and they had a lower BMI, blood pressure, fasting glucose, triglycerides, and higher HDL cholesterol. Their diets contained more polyunsaturated fat and fiber, but less sucrose, and they reported somewhat higher alcohol consumption. Those adhering to the Sugar and High-Fat Dairy pattern were also more often women and of higher age, level of education, and energy intake, compared with those with low adherence to the Sugar and High-Fat Dairy pattern.

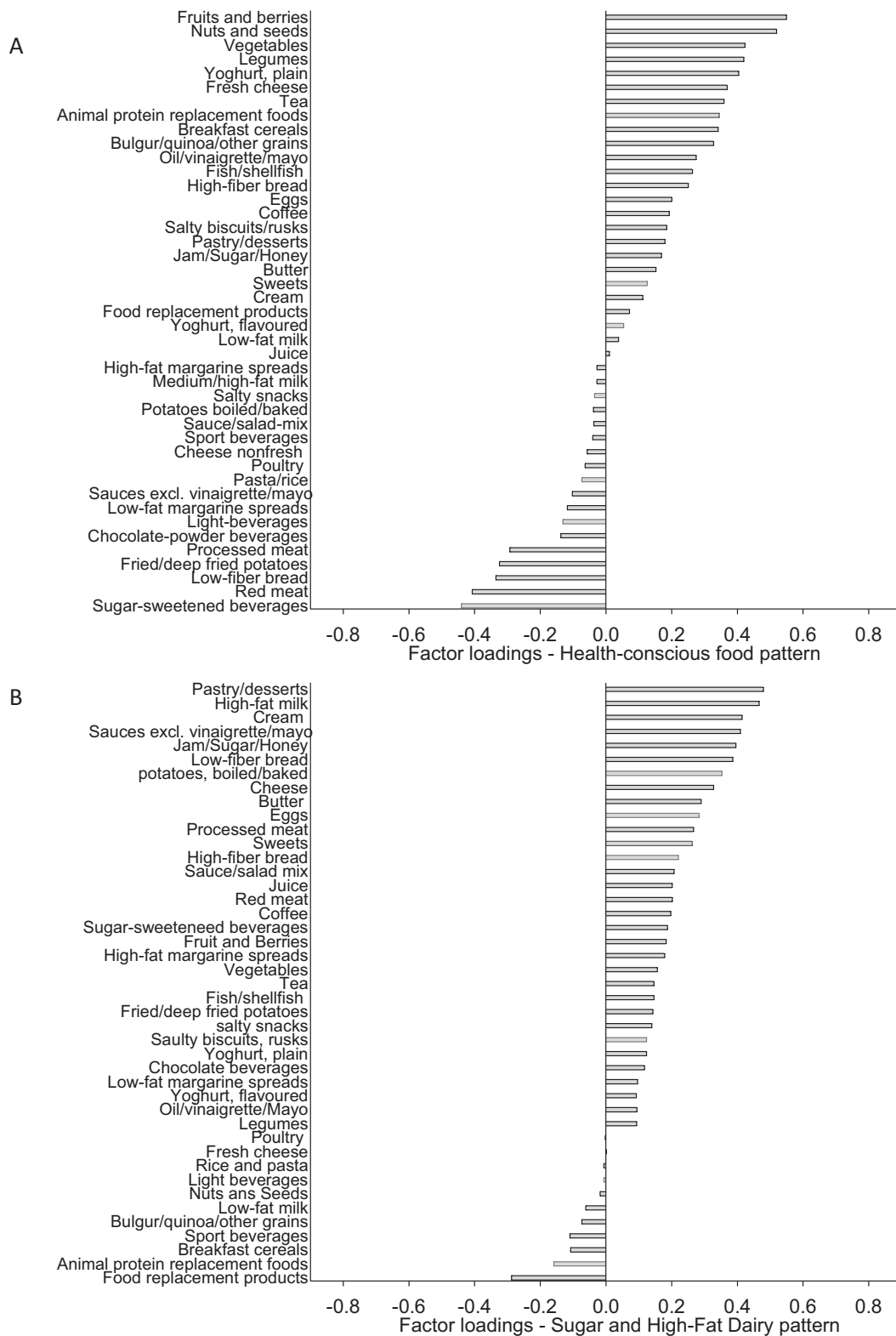


FIGURE 1 The Health-conscious food pattern explained most of the variance in the data (6.8%) in 1726 individuals without prevalent diabetes from the Malmö Offspring Study (panel A) and the second retained food pattern, the Sugar and High-Fat Dairy pattern (panel B), explained 5.2% of the variance in the data.

In addition, they had a more sedentary leisure time and their diet contained more saturated fat and sucrose. Finally, they reported much higher alcohol consumption during the 4-d food record.

Individuals identified as having prediabetes were older and had a higher BMI, waist circumference, and blood pressure compared with those without prediabetes (**Supplemental Table 1**). They were also found to have a more sedentary leisure time,

lower LDL cholesterol and HDL cholesterol concentrations, and they reported lower alcohol consumption during the 4-d food record period.

The most abundant bacterial genus in MOS was *Bacteroides*, followed by an unclassified genus in the family Ruminococcaceae, another in the family Rikenellaceae, and 1 in the order Clostridiales, as well as *Faecalibacterium*, all with relative abundances of over 5% in the feces samples collected at baseline (Figure 2).

Food patterns and prediabetes

High adherence to the Health-conscious food pattern was associated with a lower prevalence of prediabetes, both in the basic model and after adjustment for several lifestyle factors (P for trend across quintiles of the food pattern = 0.03) (Table 2). However, after additional adjustment for BMI the association did not remain significant (P -trend = 0.19). We did not observe

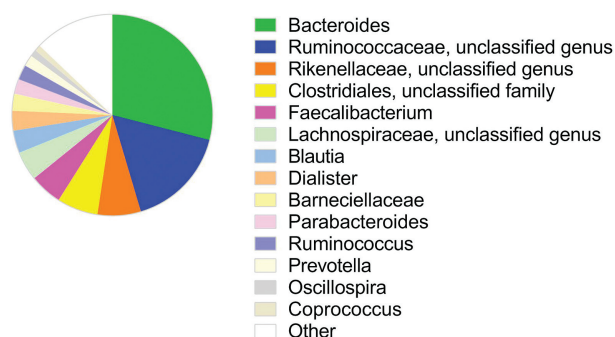


FIGURE 2 Mean relative abundance (%) of bacterial genera in feces samples collected in 1477 individuals at baseline of the Malmö Offspring Study. Other refers to the 50 identified genera not specified in the figure (Supplemental Table 2).

TABLE 1 Baseline characteristics across quintiles of food patterns in 1726 individuals from the Malmö Offspring Study without prevalent diabetes

Baseline characteristics	n	β^2	Quintile of the Health-conscious food pattern ¹					P-trend ³
			1 (n = 345)	2 (n = 345)	3 (n = 346)	4 (n = 345)	5 (n = 345)	
Age, y	1726	+2.2 ± 0.24	33.8 ± 0.73	38.5 ± 0.72	41.8 ± 0.72	42.2 ± 0.73	43.1 ± 0.74	<0.001
BMI, kg/m ²	1726	-0.7 ± 0.08	26.9 ± 0.23	25.8 ± 0.23	25.8 ± 0.23	25.0 ± 0.23	23.9 ± 0.23	<0.001
Systolic BP, mmHg	1710	-1.0 ± 0.22	118 ± 0.69	117 ± 0.67	116 ± 0.66	115 ± 0.67	114 ± 0.68	<0.001
Diastolic BP, mm Hg	1710	-1.2 ± 0.15	74 ± 0.44	72 ± 0.43	72 ± 0.43	70 ± 0.44	69 ± 0.44	<0.001
FPG, mmol/L	1725	-0.04 ± 0.01	5.46 ± 0.04	5.37 ± 0.04	5.36 ± 0.04	5.35 ± 0.04	5.29 ± 0.04	<0.001
B-HbA1c, mmol/mol	1060	-0.03 ± 0.11	33.8 ± 0.34	33.4 ± 0.33	34.0 ± 0.32	33.6 ± 0.33	33.5 ± 0.33	0.79
Total P-cholesterol, mmol/L	1722	+0.01 ± 0.02	4.96 ± 0.05	4.99 ± 0.05	4.94 ± 0.05	4.92 ± 0.05	4.96 ± 0.05	0.65
P-LDL-C, mmol/L	1721	-0.02 ± 0.02	3.20 ± 0.05	3.18 ± 0.04	3.15 ± 0.04	3.13 ± 0.05	3.10 ± 0.05	0.11
P-HDL-C, mmol/L	1722	+0.04 ± 0.01	1.54 ± 0.02	1.64 ± 0.02	1.61 ± 0.02	1.63 ± 0.02	1.75 ± 0.02	<0.001
P-TG, mmol/L	1712	-0.07 ± 0.01	1.22 ± 0.03	1.15 ± 0.03	1.11 ± 0.03	1.03 ± 0.03	0.93 ± 0.03	<0.001
Energy intake, MJ/d	1726	+0.28 ± 0.05	8.0 ± 0.14	8.2 ± 0.13	8.6 ± 0.13	8.7 ± 0.14	9.2 ± 0.14	<0.001
Protein, E%	1726	+0.06 ± 0.07	17.3 ± 0.21	17.8 ± 0.21	17.7 ± 0.20	17.7 ± 0.21	17.6 ± 0.21	0.38
Fat, E%	1726	+0.39 ± 0.12	36.3 ± 0.38	37.0 ± 0.37	37.3 ± 0.37	37.4 ± 0.37	38.0 ± 0.38	0.002
SFA, E%	1726	-0.06 ± 0.06	13.9 ± 0.19	14.5 ± 0.18	14.4 ± 0.18	14.2 ± 0.18	13.8 ± 0.19	0.31
PUFA, E%	1726	+0.22 ± 0.04	5.7 ± 0.11	5.8 ± 0.11	5.9 ± 0.11	6.2 ± 0.11	6.6 ± 0.11	<0.001
Carbohydrate, E%	1726	-0.45 ± 0.14	46.4 ± 0.41	45.2 ± 0.40	45.0 ± 0.40	44.9 ± 0.40	44.3 ± 0.41	0.001
Fiber, g/MJ	1726	+0.25 ± 0.01	1.9 ± 0.04	2.1 ± 0.04	2.2 ± 0.04	2.5 ± 0.04	2.9 ± 0.04	<0.001
Sucrose, E%	1726	-0.44 ± 0.08	9.7 ± 0.24	8.6 ± 0.23	8.4 ± 0.23	8.2 ± 0.23	7.7 ± 0.24	<0.001
Alcohol, ⁴ g/d	1726	+0.48 ± 0.32	11.9 ± 0.98	14.7 ± 0.96	14.6 ± 0.95	14.6 ± 0.96	14.5 ± 0.98	<0.001 ⁵
								Pvalue ⁶
Gender, female, %	1726		32.5	43.5	55.2	65.2	75.4	<0.001
Smokers, current, %	1594		21.8	14.6	14.2	9.8	6.3	<0.001
Higher education, ⁷ %	1590		23.4	34.5	38.9	45.4	56.1	<0.001
LPAL, sedentary/low %	1585		55.1	49.5	48.6	38.7	30.4	<0.001
Work AL, very light/light %	1502		51.1	56.8	60.0	66.3	69.6	<0.001

Baseline characteristics	n	β^2	Quintile of the Sugar and High-Fat Dairy pattern ¹					P-trend ³
			1 (n = 345)	2 (n = 345)	3 (n = 346)	4 (n = 345)	5 (n = 345)	
Age, y	1726	+1.06 ± 0.24	37.3 ± 0.74	39.0 ± 0.74	40.9 ± 0.74	40.1 ± 0.74	42.0 ± 0.74	<0.001
BMI, kg/m ²	1726	-0.12 ± 0.07	25.9 ± 0.23	25.5 ± 0.23	25.4 ± 0.23	25.4 ± 0.23	25.3 ± 0.23	0.10
Systolic BP, mm Hg	1710	-0.36 ± 0.21	117 ± 0.67	117 ± 0.67	116 ± 0.67	115 ± 0.67	116 ± 0.67	0.09
Diastolic BP, mm Hg	1710	-0.01 ± 0.14	72 ± 0.44	71 ± 0.44	71 ± 0.44	72 ± 0.44	71 ± 0.44	0.94
FPG, mmol/L	1725	-0.01 ± 0.01	5.41 ± 0.04	5.36 ± 0.04	5.38 ± 0.04	5.30 ± 0.04	5.40 ± 0.04	0.42
B-HbA1c, mmol/mol	1060	0.14 ± 0.10	33.3 ± 0.33	33.8 ± 0.32	33.6 ± 0.32	33.5 ± 0.33	34.2 ± 0.34	0.18
Total P-cholesterol, mmol/L	1722	-0.01 ± 0.02	4.93 ± 0.05	5.00 ± 0.05	4.96 ± 0.05	4.96 ± 0.05	4.93 ± 0.05	0.81
P-LDL-C, mmol/L	1721	-0.01 ± 0.12	3.14 ± 0.04	3.20 ± 0.04	3.14 ± 0.04	3.15 ± 0.04	3.14 ± 0.05	0.76
P-HDL-C, mmol/L	1722	1 × 10 ⁻⁴ ± 0.01	1.61 ± 0.02	1.64 ± 0.02	1.66 ± 0.02	1.64 ± 0.02	1.61 ± 0.02	0.99
P-TG, mmol/L	1712	0.004 ± 0.01	1.10 ± 0.03	1.10 ± 0.03	1.04 ± 0.03	1.10 ± 0.03	1.12 ± 0.03	0.72
Energy intake, MJ/d	1726	+0.6 ± 0.04	7.3 ± 0.13	8.0 ± 0.13	8.6 ± 0.13	9.0 ± 0.13	9.8 ± 0.13	<0.001
Protein, E%	1726	-0.82 ± 0.06	19.9 ± 0.19	17.9 ± 0.19	17.2 ± 0.19	16.9 ± 0.19	16.2 ± 0.20	<0.001
Fat, E%	1726	+0.77 ± 0.12	35.7 ± 0.36	36.5 ± 0.36	37.0 ± 0.36	38.1 ± 0.36	38.7 ± 0.37	<0.001
SFA, E%	1726	+0.43 ± 0.06	13.2 ± 0.18	13.9 ± 0.18	14.2 ± 0.18	14.5 ± 0.18	15.0 ± 0.18	<0.001
PUFA, E%	1726	+0.12 ± 0.03	5.9 ± 0.11	5.8 ± 0.11	6.0 ± 0.11	6.3 ± 0.11	6.3 ± 0.11	0.001
Carbohydrate, E%	1726	+0.05 ± 0.13	44.4 ± 0.40	45.7 ± 0.40	45.8 ± 0.40	45.0 ± 0.40	45.0 ± 0.40	0.69
Fiber, g/MJ	1726	-0.12 ± 0.01	2.6 ± 0.04	2.4 ± 0.04	2.3 ± 0.04	2.2 ± 0.04	2.1 ± 0.04	<0.001

(Continued)

TABLE 1 (Continued)

Baseline characteristics	n	β^2	Quintile of the Sugar and High-Fat Dairy pattern ¹					P-trend ³
			1 (n = 345)	2 (n = 345)	3 (n = 346)	4 (n = 345)	5 (n = 345)	
Sucrose, E%	1726	+0.48 ± 0.07	7.5 ± 0.23	8.1 ± 0.23	8.8 ± 0.23	8.8 ± 0.23	9.5 ± 0.23	<0.001
Alcohol, ⁴ g/d	1726	+2.1 ± 0.30	9.4 ± 0.94	11.5 ± 0.94	15.5 ± 0.94	15.6 ± 0.94	18.1 ± 0.95	<0.001 ⁵
								P-value ⁶
Gender, female, %	1726		44.6	54.5	53.8	56.8	62.0	<0.001
Smokers, current, %	1594		17.4	12.5	12.2	11.8	12.8	0.24
Higher education, ⁷ %	1590		32.7	36.7	36.9	42.8	49.8	0.001
LPAL, sedentary/low %	1585		37.7	41.0	46.0	45.6	50.8	0.01
Work AL, very light/light %	1502		57.6	56.3	64.8	62.1	63.8	0.13

¹Values are means ± SEs or percentage distribution.

² β indicates mean difference per intake quintile ± SEs.

³Calculated with the general linear model. Adjusted for age and sex (continuous) when appropriate.

⁴Alcohol consumption assessed with the *Riksmaten2010* method (17).

⁵P value calculated from ln-transformed values.

⁶Chi-square test.

⁷University degree.

AL, activity level; B-HbA1c, blood glycosylated hemoglobin; BP, blood pressure; FPG, fasting plasma glucose; LPAL, leisure time physical activity level; P-cholesterol, plasma cholesterol; P-HDL-C, plasma HDL-C; P-LDL-C, plasma LDL-C; P-TG, plasma triglycerides.

any interaction between the Health-conscious food pattern and gender ($P = 0.72$).

The Sugar and High-Fat Dairy pattern did not indicate any overall association with the prevalence of prediabetes. However, we observed a statistical interaction with gender (P -interaction = 0.03); no significant association was seen in men (P -trend = 0.40), but women adhering to the Sugar and High-Fat Dairy pattern were more likely to have prediabetes (OR comparing the highest quintile with the lowest: 2.16; 95% CI: 1.02, 4.54; P -trend = 0.03) (Table 3) and this association remained significant after adjustment for BMI (P -trend = 0.04).

Food patterns and gut microbiota

Adherence to the Health-conscious food pattern was significantly correlated to the abundance of 19 bacterial genera after the Bonferroni correction (Supplemental Table 2). After adjustment for lifestyle factors, a high adherence to the pattern was associated with a higher abundance of 4 bacterial genera and lower abundance of 6 genera. After additional adjustment for BMI, the higher abundance of *Lachnospira* and *Roseburia* genus in the RF39 order (Figure 3A), and the lower abundance of *Blautia*, *Anaerotruncus*, and *Eubacterium* (Figure 3B), remained significant. After additional adjustment for fiber intake, only the higher abundances of *Lachnospira* and *Roseburia*, and the lower abundance of *Eubacterium* remained significant. In a sensitivity analysis, restricted to individuals reporting no use of antibiotics during the previous 6 mo and consumption of probiotics ≤ 3 times per week, the higher abundances of *Lachnospira* ($\beta = 0.22$; P -trend across quintiles of the food pattern = 1×10^{-4}) and *Roseburia* ($\beta = 0.25$; P -trend = 1×10^{-4}) with a higher adherence to the Health-conscious food pattern, and the lower abundance of *Eubacterium*, remained similar as in the whole study sample. However, the lower abundance of *Eubacterium* was only nominally significant ($\beta = -0.33$, P -trend = 0.002). In addition, higher adherence to the Health-conscious food pattern was associated with a significantly lower abundance of *Anaerotruncus* ($\beta = -0.25$; P -trend = 3×10^{-4}) in the sensitivity analysis.

Adherence to the Sugar and High-Fat Dairy pattern correlated to a genus within the family Christensenellaceae and a genus within the order SFA98, but it did not significantly associate with any bacterial genera after adjustment for

potential confounders, although a few nominally significant associations were observed (Supplemental Table 3).

We did not observe any significant association between the food patterns and α -diversity (Shannon index across the food pattern quintiles; P -trend ≥ 0.16).

Gender was not found to significantly modify the association between the food patterns and gut bacteria (data not shown).

Food pattern related gut bacteria and prediabetes

The abundance of *Roseburia* was inversely associated with the prevalence of prediabetes (OR per quintile of gut bacterial abundance: 0.86; 95% CI: 0.76–0.96; P -trend = 0.01) (Figure 4). The association remained significant after adjustment for adherence to the Health-conscious food pattern. The other 5 gut bacterial genera that associated with the Health-conscious food pattern were not associated with the prevalence of prediabetes, after adjustment for lifestyle factors including BMI (Table 4).

Adjusting for gut bacteria associating with both diet and prediabetes

In line with the result of the whole study cohort (Figure 5A), adherence to the Health-conscious food pattern tended to associate with a lower prevalence of prediabetes in the sample only including those with data on gut microbiota ($n = 1477$) (OR per quintile: 0.88; 95% CI: 0.79–1.00; P -trend = 0.056 after adjustment for potential confounders) (Figure 5B). The association was also attenuated after adjustment for BMI (OR: 0.93; 95% CI: 0.82–1.06; P -trend = 0.30) (Figure 5C). When additionally adjusting for the abundance of *Roseburia*, the association between the Health-conscious food pattern and prevalence of prediabetes was further attenuated (OR: 0.96; 95% CI: 0.84–1.10; P -trend = 0.56) (Figure 5D). The association was also slightly attenuated when adjusting for *Roseburia* but not BMI (OR: 0.91; 95% CI: 0.80–1.04; P -trend = 0.15).

Discussion

In this large observational study with data on diet and microbiota, 2 food patterns were extracted from principal component analysis. Participants adhering to the first food pattern, characterized by Health-conscious food choices, such

TABLE 2 ORs with CIs for prediabetes across quintiles of food patterns in 1726 individuals from the Malmö Offspring Study without prevalent diabetes

Food patterns	β^1	Quintile of food pattern					P-trend
		1 OR	2 OR (95% CI)	3 OR (95% CI)	4 OR (95% CI)	5 OR (95% CI)	
Health conscious							
Cases/controls		60/285	48/297	49/297	53/292	50/295	
Basic model ²	- 0.10 ± 0.05	1.00	0.64 (0.42, 0.98)	0.58 (0.38, 0.90)	0.64 (0.41, 0.99)	0.59 (0.38, 0.93)	0.049
Multivariable model ³	- 0.13 ± 0.06	1.00	0.70 (0.44, 1.14)	0.60 (0.37, 0.96)	0.63 (0.39, 1.03)	0.54 (0.32, 0.92)	0.03
Multivariable model with BMI ⁴	- 0.08 ± 0.06	1.00	0.76(0.47, 1.23)	0.65 (0.40, 1.05)	0.72 (0.44, 1.18)	0.68 (0.40, 1.17)	0.19
P for interaction with gender							0.72
Sugar and High-Fat Dairy							
Cases/controls		53/292	50/295	52/294	45/300	60/285	
Basic model ²	+0.02 ± 0.05	1.00	0.92 (0.60, 1.41)	0.92 (0.60, 1.42)	0.83 (0.53, 1.30)	1.17 (0.75, 1.82)	0.19
Multivariable model ³	+0.07 ± 0.06	1.00	0.95 (0.58, 1.56)	0.95 (0.58, 1.56)	1.01 (0.61, 1.66)	1.35 (0.82, 2.23)	0.22
Multivariable model with BMI ⁴	+0.08 ± 0.06	1.00	1.00 (0.61, 1.65)	1.00 (0.61, 1.64)	1.05 (0.63, 1.74)	1.41 (0.85, 2.34)	0.33
P for interaction with gender							0.03

¹ β indicates mean difference per intake quintile ± SEs.²Adjusted for age, sex, and total energy intake.³Adjusted for age, sex, total energy intake, level of education, smoking, alcohol intake, and level of physical activity.⁴Adjusted for age, sex, total energy intake, level of education, smoking, alcohol intake, level of physical activity, and BMI.

as fiber-rich plant foods, were less likely to have prediabetes. Several gut bacterial genera correlated with adherence to this Health-conscious food pattern. A higher abundance of *Roseburia* was found to associate both with a higher adherence to the Health-conscious food pattern and with a lower prevalence of prediabetes. The association between the Health-conscious food pattern and prevalence of prediabetes was attenuated after adjustment for BMI and abundance of *Roseburia*, suggesting possible underlying pathways. Women adhering to the pattern characterized by foods high in sugar and high-fat dairy were more likely to have prediabetes, independently of BMI, and this pattern did not show any strong associations with gut bacteria, indicating other underlying mechanisms, such as stronger glucose response by sugar-rich foods or impaired insulin sensitivity due to the high content of saturated fat in dairy products (22–24).

Our observation showing that individuals adhering to the Health-conscious food pattern were less likely to have prediabetes is in line with earlier results regarding healthy/prudent dietary patterns and incident diabetes, in other populations (1, 2), and with our previously reported findings from the Malmö

Diet and Cancer study (3), where the index individuals (first generation) to those enrolled in MOS were included. However, in the present study cohort we also had the opportunity to examine food patterns in relation to the gut microbiota. Although we observed the Health-conscious pattern to be related to several bacterial genera, the association with *Roseburia* is of special interest, as the food pattern was so strongly associated with *Roseburia* and as *Roseburia* per se was found to associate with a lower prevalence of prediabetes, independently of adherence to the Health-conscious food pattern.

As *Roseburia* is known to be a butyrate-producing genera, dependent on fermentable carbohydrates in the diet (25), it is biologically plausible that *Roseburia* could affect diabetes development, as butyrate and other SCFAs act as signal substances with beneficial effects on glucose metabolism (20, 26). Moreover, a recent study indicated that the butyrate-producing activity of the gut may causally affect the glucose-stimulated insulin response and that *Roseburia* was among the bacteria showing the strongest correlation to such activity (8). Since different types of dietary fibers are utilized as substrates in bacterial SCFA production, it is possible that potential effects

TABLE 3 ORs with CIs for prediabetes across quintiles of the Sugar and High-Fat Dairy food pattern in women ($n = 938$) and men ($n = 788$) from the Malmö Offspring Study without prevalent diabetes

Sugar and high-fat dairy pattern	β^1	Quintile of food pattern					P-trend
		1 OR	2 OR (95% CI)	3 OR (95% CI)	4 OR (95% CI)	5 OR (95% CI)	
Women							
Cases/controls		18/136	26/162	27/159	27/169	39/175	
Basic model ²	+0.12 ± 0.07	1.00	1.21 (0.62, 2.33)	1.21 (0.62, 2.34)	1.28 (0.66, 1.50)	1.42 (0.90, 3.31)	0.10
Multivariable model ³	+0.18 ± 0.08	1.00	1.33 (0.62, 2.82)	1.28 (0.60, 2.71)	1.61 (0.76, 3.42)	2.16 (1.02, 4.54)	0.03
Multivariable model with BMI ⁴	+0.17 ± 0.08	1.00	1.42 (0.66, 3.05)	1.32 (0.61, 2.83)	1.61 (0.74, 3.48)	2.21 (1.04, 4.72)	0.04
Men							
Cases/controls		35/156	24/133	25/135	18/131	21/110	
Basic model ²	- 0.09 ± 0.08	1.00	0.77 (0.43, 1.38)	0.77 (0.42, 1.39)	0.56 (0.29, 1.08)	0.80 (0.42, 1.55)	0.27
Multivariable model ³	- 0.08 ± 0.09	1.00	0.71 (0.35, 1.42)	0.72 (0.36, 1.45)	0.59 (0.28, 1.24)	0.78 (0.36, 1.65)	0.40
Multivariable model with BMI ⁴	- 0.06 ± 0.09	1.00	0.73 (0.37, 1.47)	0.76 (0.38, 1.56)	0.63 (0.30, 1.33)	0.82 (0.38, 1.78)	0.52

¹ β indicates mean difference per intake quintile ± SEs.²Adjusted for age, sex, and total energy intake.³Adjusted for age, sex, total energy intake, level of education, smoking, alcohol intake, and level of physical activity.⁴Adjusted for age, sex, total energy intake, level of education, smoking, alcohol intake, level of physical activity, and BMI.

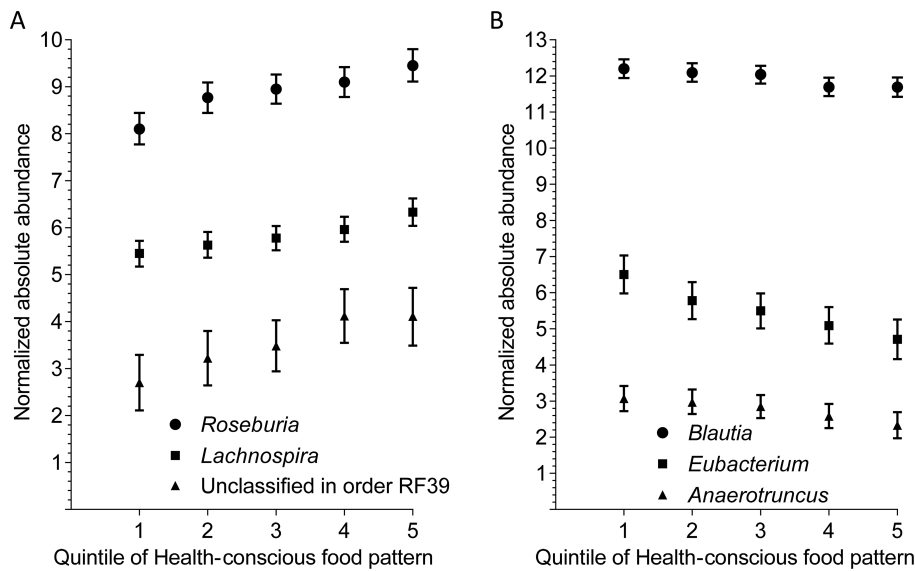


FIGURE 3 High adherence to the Health-conscious food pattern was significantly associated with a higher abundance of *Lachnospira*, *Roseburia*, and a genus in the RF39 order (panel A), and with a lower abundance of *Blautia*, *Anaerotruncus*, and *Eubacterium* (panel B) after adjustment for lifestyle factors including BMI (P values for trend across quintiles of the food pattern $< 8 \times 10^{-4}$) in 1477 individuals from the Malmö Offspring Study. Data points show mean normalized absolute abundances (with 95% CIs) of the bacterial genera in quintiles (Q) of the food pattern. Q1, $n = 286$; Q2, $n = 292$; Q3, $n = 306$; Q4, $n = 293$; Q5, $n = 300$.

of higher fiber intake on the abundance of *Roseburia* partly explain our observed association between adherence to the Health-conscious pattern and lower prevalence of prediabetes. This food pattern was indeed characterized by high intakes of different fiber-rich foods and especially by high intakes of fruits and berries, nuts and seeds, and vegetables. Further

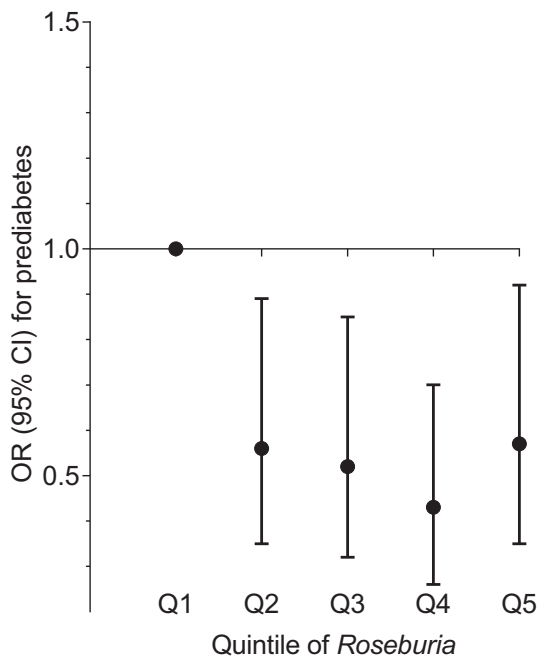


FIGURE 4 The prevalence of prediabetes was found to be lower in individuals with a higher abundance of *Roseburia* (OR per quintile of gut bacterial abundance: 0.86; 95% CI: 0.76–0.96; P -trend = 0.01) in the Malmö Offspring Study ($n = 1477$). Data points show ORs (with 95% CIs) of prediabetes in quintiles (Q) of bacterial abundance (normalized absolute abundance of *Roseburia*). Q1, $n = 295$; Q2, $n = 296$; Q3, $n = 295$; Q4, $n = 296$; Q5, $n = 295$.

support of this is the fact that the association between the Health-conscious food pattern and prevalence of prediabetes was slightly attenuated after adjustment for *Roseburia*. On the other hand, the association between adherence to the Health-conscious food pattern and higher abundance of *Roseburia*, although attenuated, remained significant after adjustment for fiber intake, indicating that the overall food pattern per se, or components other than fiber, may also be of importance. In addition, a meta-analysis on fiber interventions and gut bacterial abundances did not find *Roseburia* to be significantly changed after fiber supplementation by itself (21). Other foods characterizing the Health-conscious pattern may affect growth of *Roseburia* indirectly, due to the potential effects on transit time, and digestion or absorption of fiber-rich foods, and fat intake has also been indicated to potentially influence the abundance of *Roseburia* (27). Moreover, the high content of yogurt in the Health-conscious pattern may also contribute to decreased development of diabetes via beneficial effects on gut bacterial composition as earlier described (28). In line with our results, *Roseburia* has been associated with another overall healthy dietary pattern, i.e. the Mediterranean pattern, and identified as a marker of health (25), and a lower abundance of *Roseburia* has been observed in individuals with cardiometabolic diseases including T2D (29, 30).

The abundance of *Lachnospira*, which like *Roseburia* belongs to the Lachnospiraceae family, as with *Roseburia*, was found to be higher upon greater adherence to the Health-conscious food pattern in all statistical models, as well as in the sensitivity analysis. *Lachnospira* can use pectin (a type of dietary fiber mainly found in fruits and vegetables) for acetate production, which in turn can be used by other bacteria for butyrate production (31). The abundance of *Lachnospira* was not associated with the prevalence of prediabetes, but interestingly, a bacterial cluster characterized by *Lachnospira* and *Roseburia* has previously been associated with a vegetable-based dietary pattern (10). *Eubacterium* is another butyrate-producing genera (32) that was found to consistently associate with the Health-conscious pattern, but in contrast to *Roseburia*,

TABLE 4 ORs with CIs for prevalence of prediabetes across quintiles of diet-related gut bacteria in 1477 individuals from the Malmö Offspring Study without prevalent diabetes

Gut bacterial genus	β^1	Quintile of gut bacterial abundance					P-trend
		1 OR	2 OR (95% CI)	3 OR (95% CI)	4 OR (95% CI)	5 OR (95% CI)	
<i>Roseburia</i>							
Cases/controls		58/237	46/250	45/250	36/260	44/251	
Basic model ²	-0.11 ± 0.05	1.00	0.65 (0.42, 1.00)	0.68 (0.44, 1.06)	0.50 (0.31, 0.79)	0.68 (0.44, 1.06)	0.04
Multivariable model ³	-0.16 ± 0.06	1.00	0.59 (0.37, 0.93)	0.54 (0.33, 0.87)	0.43 (0.26, 0.71)	0.56 (0.35, 0.92)	0.01
Multivariable model with BMI ⁴	-0.16 ± 0.06	1.00	0.56 (0.35, 0.89)	0.52 (0.32, 0.85)	0.43 (0.26, 0.70)	0.57 (0.35, 0.92)	0.01
<i>Blautia</i>							
Cases/controls		52/243	41/255	46/249	37/259	53/242	
Basic model ²	0.03 ± 0.05	1.00	0.82 (0.52, 1.28)	1.02 (0.65, 1.59)	0.77 (0.48, 1.23)	1.20 (0.78, 1.85)	0.53
Multivariable model ³	0.06 ± 0.06	1.00	0.80 (0.49, 1.32)	0.95 (0.58, 1.54)	0.82 (0.49, 1.35)	1.30 (0.82, 2.07)	0.32
Multivariable model with BMI ⁴	0.04 ± 0.06	1.00	0.79 (0.48, 1.30)	0.92 (0.56, 1.50)	0.77 (0.46, 1.27)	1.14 (0.71, 1.84)	0.53
<i>Lachnospira</i>							
Cases/controls		50/245	49/247	40/255	40/256	50/245	
Basic model ²	-0.03 ± 0.05	1.00	0.97 (0.63, 1.51)	0.74 (0.47, 1.18)	0.76 (0.48, 1.20)	0.98 (0.63, 1.51)	0.58
Multivariable model ³	0.001 ± 0.06	1.00	1.22 (0.75, 1.98)	0.92 (0.55, 1.54)	1.01 (0.61, 1.67)	1.10 (0.68, 1.79)	0.99
Multivariable model with BMI ⁴	-0.01 ± 0.06	1.00	1.22 (0.75, 2.00)	0.94 (0.56, 1.57)	1.02 (0.62, 1.69)	1.06 (0.65, 1.73)	0.88
<i>Anaerotruncus</i>							
Cases/controls		63/315	34/179	39/256	47/249	46/249	
Basic model ²	0.02 ± 0.05	1.00	0.99 (0.63, 1.58)	0.86 (0.55, 1.33)	1.12 (0.74, 1.71)	1.04 (0.68, 1.59)	0.71
Multivariable model ³	-0.002 ± 0.06	1.00	0.96 (0.58, 1.58)	0.85 (0.53, 1.35)	0.96 (0.59, 1.54)	1.01 (0.64, 1.59)	0.97
Multivariable model with BMI ⁴	0.02 ± 0.05	1.00	1.01 (0.61, 1.68)	0.87 (0.54, 1.40)	0.98 (0.61, 1.58)	1.05 (0.66, 1.67)	0.74
<i>Eubacterium</i>							
Cases/controls		40/255	47/249	41/254	53/243	48/247	
Basic model ²	+0.03 ± 0.05	1.00	1.28 (0.81, 2.04)	1.03 (0.64, 1.66)	1.44 (0.92, 2.27)	1.11 (0.70, 1.77)	0.54
Multivariable model ³	+0.08 ± 0.06	1.00	1.15 (0.69, 1.93)	1.04 (0.61, 1.75)	1.44 (0.88, 2.35)	1.32 (0.82, 2.23)	0.17
Multivariable model with BMI ⁴	+0.06 ± 0.06	1.00	1.13 (0.67, 1.90)	0.98 (0.58, 1.67)	1.37 (0.84, 2.25)	1.22 (0.74, 2.02)	0.28
Genus within the order RF39							
Cases/controls		70/375	41/216	45/214	33/225	40/218	
Basic model ²	-0.05 ± 0.05	1.00	0.98 (0.67, 1.58)	0.76 (0.69, 1.59)	0.91 (0.43, 1.06)	0.45 (0.62, 1.46)	0.33
Multivariable model ³	-0.05 ± 0.06	1.00	0.92 (0.57, 1.47)	1.04 (0.65, 1.63)	0.65 (0.39, 1.06)	0.91 (0.57, 1.45)	0.33
Multivariable model with BMI ⁴	-0.04 ± 0.06	1.00	0.92 (0.57, 1.47)	1.03 (0.65, 1.63)	0.70 (0.43, 1.16)	1.00 (0.62, 1.61)	0.53

¹ β indicates mean difference per quintile of normalized absolute abundance ± SEs.

² Calculated with the general linear model. Adjusted for age and sex (continuous) when appropriate.

³ Adjusted for age, sex, smoking, alcohol intake, and physical activity.

⁴ Adjusted for age, sex, smoking, alcohol intake, physical activity, and BMI.

the abundance of *Eubacterium* was lower at higher adherence to the pattern and was not found to associate with prediabetes. Different bacteria could compete for dietary substrates for butyrate production (33) and this may explain the contrasting associations. The potentially pathogenic *Anaerotruncus* (34) has, in accordance with our results showing a lower abundance of this genera at higher adherence to the Health-conscious pattern, been negatively correlated to the colonic content of SCFA in pigs (35).

Although it is reasonable to believe that overall dietary quality and especially fiber intake may beneficially affect glucose metabolism and diabetes development, via effects on gut bacterial composition including abundance of *Roseburia*, dietary components also act via other pathways, which may partly have contributed to our observations. The association between the Health-conscious food pattern and prevalence of prediabetes was, for example, attenuated after adjustment for BMI, suggesting that obesity-related dietary qualities might also be of importance. Although *Roseburia* was associated with the prevalence of prediabetes independently of BMI, dietary fiber components may, for example, affect BMI and subsequently diabetes development via beneficial effects on satiety. Potentially

favorable effects of antioxidants in fruits and vegetables characterizing the Health-conscious food pattern could also have contributed to the observed lower prevalence of prediabetes, as antioxidants may affect glucose metabolism (36).

The strengths of this study are the large size, when considering studies with data on gut microbiota, detailed dietary data, and that only few observational studies have reported associations between dietary patterns and microbiota. Our intention was to study overall food patterns instead of single foods or nutrients. This approach has several advantages; food intakes are correlated and it may be challenging to disentangle their individual importance, cumulative effects of several foods may also be easier to detect compared with those of single foods or nutrients, and foods are consumed together and may interact. For example, it is possible that foods with probiotic components, such as some fermented dairy products, may have greater effects if accompanied by foods with prebiotic qualities such as fiber-rich foods (37, 38). In addition, it cannot be excluded that a pattern indicating Health-conscious food choices might be a better marker of long-term fiber intake than assessed fiber intake per se reported during 4 specific days. On the other hand, fiber consumption close to feces sampling is also

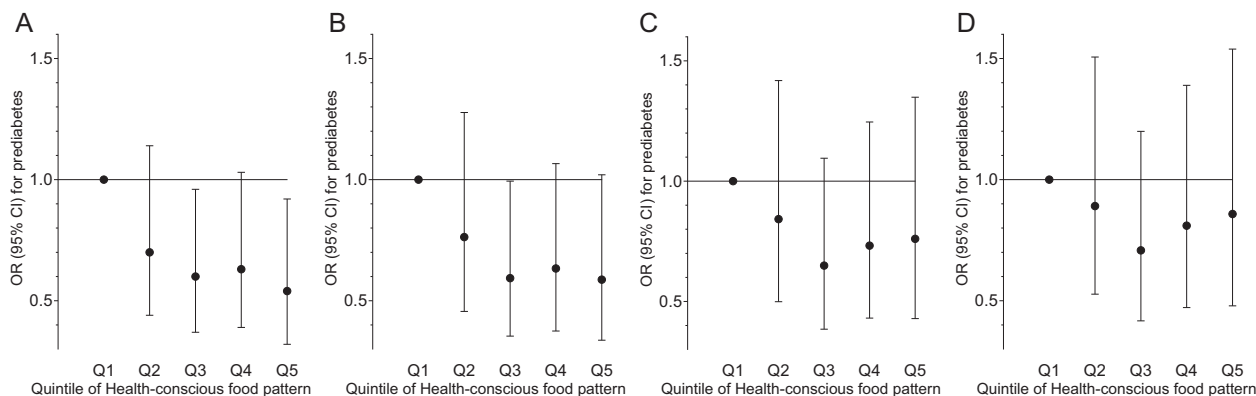


FIGURE 5 Adherence to the Health-conscious food pattern was associated with a lower prevalence of prediabetes, after adjustment for lifestyle factors, in 1726 individuals without prevalent diabetes from the Malmö Offspring Study (P -trend across quintiles of the food pattern = 0.03) (panel A), and the association remained similar in the sample only including individuals with data on gut microbiota ($n = 1477$) (P -trend = 0.06) (panel B). In line with observations in the whole study sample the association was attenuated after adjustment for BMI (P -trend = 0.30) (panel C). When additionally adjusting for the abundance of *Roseburia*, the Health-conscious food pattern was further attenuated (P -trend = 0.56) (panel D). Data points show ORs (with 95% CIs) of prediabetes in quintiles of the food pattern.

of importance, as the changes in fiber intake can rapidly affect the gut bacterial composition (39).

A limitation of our study is that we could not examine incident T2D, as the participants were followed for only a few years resulting in very few incident cases. Instead, we could examine the prevalence of prediabetes, defined according to baseline concentrations of fasting glucose and HbA1c or the development of T2D during the short follow-up. However, the observed association between the Health-conscious food pattern and the butyrate-producing genera *Roseburia* is biologically plausible, considering that the pattern was characterized by fiber-rich foods that provide substrates for SCFA production, which in turn affect glucose metabolism. We also need to acknowledge that measurement error is a major problem in studies with self-reported diet and 1 consequence might have been unsatisfactory adjustment for fiber intake. Moreover, we cannot completely exclude the risk of overadjustment, when including fiber intake in the same model as the Health-conscious food pattern. Another drawback is that both diet and microbiota were only measured at baseline in the whole study sample. Nevertheless, data from a subsample of our study population with repeat measurements of diet indicate acceptable agreement between the measurements; with Pearson correlation coefficients of 0.6 and 0.7 for energy-adjusted intakes of fiber and fat, respectively (40). Unfortunately, we do not have any repeat measurements of microbiota. Another concern is that our results may not be generalizable to other populations; different dietary patterns may, for example, occur in other study settings. In addition, dietary patterns could represent overall lifestyle, and despite adjustment for several confounders, we cannot completely rule out residual confounding. On the other hand, the fact that the Health-conscious pattern was characterized by foods that per se have been found to be associated with cardiometabolic disease in other studies (41–48) makes our findings more credible. Finally, a lack of power may be an important issue in the sensitivity analyses, as almost half the study sample was removed.

To conclude, our findings suggest that the association between the Health-conscious food pattern and lower prevalence of prediabetes may partly be explained by links between healthy

diet, BMI-status, and abundance of *Roseburia*, as the included fiber-rich foods may constitute substrates for gut bacterial SCFA production and thereby affect glucose metabolism. The association between adherence to the Sugar and High-Fat Dairy pattern and higher prevalence of prediabetes may be explained by more direct links to glucose metabolism. Future studies are warranted to replicate our findings in other populations and to evaluate their importance in experimental settings.

Acknowledgments

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