



Microbiota-Related Metabolites and the Risk of Type 2 Diabetes

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OBJECTIVE

Recent studies have highlighted the significance of the microbiome in human health and disease. Changes in the metabolites produced by microbiota have been implicated in several diseases. Our objective was to identify microbiome metabolites that are associated with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Our study included 5,181 participants from the cross-sectional Metabolic Syndrome in Men (METSIM) study that included Finnish men (age 57 ± 7 years, BMI 26.5 ± 3.5 kg/m²) having metabolomics data available. Metabolomics analysis was performed based on fasting plasma samples. On the basis of an oral glucose tolerance test, Matsuda ISI and disposition index values were calculated as markers of insulin sensitivity and insulin secretion. A total of 4,851 participants had a 7.4-year follow-up visit, and 522 participants developed type 2 diabetes.

RESULTS

Creatine, 1-palmitoleoylglycerol (16:1), urate, 2-hydroxybutyrate/2-hydroxyisobutyrate, xanthine, xanthurate, kynurenate, 3-(4-hydroxyphenyl)lactate, 1-oleoylglycerol (18:1), 1-myristoylglycerol (14:0), dimethylglycine, and 2-hydroxyhippurate (salicylurate) were significantly associated with an increased risk of type 2 diabetes. These metabolites were associated with decreased insulin secretion or insulin sensitivity or both. Among the metabolites that were associated with a decreased risk of type 2 diabetes, 1-linoleoylglycerophosphocholine (18:2) significantly reduced the risk of type 2 diabetes.

CONCLUSIONS

Several novel and previously reported microbial metabolites related to the gut microbiota were associated with an increased risk of incident type 2 diabetes, and they were also associated with decreased insulin secretion and insulin sensitivity. Microbial metabolites are important biomarkers for the risk of type 2 diabetes.

Type 2 diabetes is a major global health concern. It is caused by genetic risk variants in interplay with environmental and lifestyle factors. The two main pathophysiological disturbances in this disease are impaired insulin secretion and insulin resistance (1). Understanding the pathophysiology of type 2 diabetes is crucial for its prevention and treatment. It is especially important to identify early biomarkers for the risk of type 2 diabetes. Advances in metabolomics allowing studies of small molecules, metabolites, have opened an emerging technology for biomarker studies and precision medicine (2).

A growing number of studies have shown that the microbiota is likely to play an important role in human health and disease (3). The microbiota consists of various

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microorganisms, mainly bacteria, but also viruses, protozoa, and fungi. It is responsible for food digestion, modulation of immune responses, and the generation of different metabolites resulting from microbial metabolic activities (4). Imbalances or disturbances in the homeostasis between the microbiota and host environment may play an important role in the pathogenesis of many disorders, such as liver, gastrointestinal, and metabolic diseases, including obesity, type 2 diabetes, lipid disorders, and cardiovascular disease (5). Moreover, the microbiome produces metabolites that could be pathogenic or beneficial to the host. These metabolites and their end products may play crucial roles in host biosynthetic and metabolic networks as well as various immunological and neurobiological processes (4).

The microbiota regulates several important metabolic mechanisms of the body, and is associated with metabolic diseases and other disorders. Previous studies have suggested that patients with type 2 diabetes show evidence of changes in the intestinal microbiota composition although the results have been conflicting (6). The gut microbiota is able to ferment indigestible carbohydrates resulting in the release of metabolites, such as short chain fatty acids, acetate, propionate, and butyrate. These metabolites may have a beneficial effect on weight control, inflammation, insulin sensitivity, and glucose homeostasis (7).

Recent advances in high-throughput technologies have facilitated large-scale biomarker studies applying a metabolomics approach. This has made it possible to investigate the association of all known metabolites from the gut microbiota with the risk of type 2 diabetes compared with previous studies that included only selected metabolites. Furthermore, previous studies have often been cross-sectional and small in size and, therefore, underpowered. Large prospective randomly selected population-based cohorts having metabolomics data available are needed to obtain reliable results. Therefore, we performed a study including 5,169 participants of the Metabolic Syndrome in Men (METSIM) study having a follow-up of 7.4 years to investigate the role of microbiota-based metabolites as risk factors for type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

The METSIM study comprises 10,197 Finnish men, aged from 45 to 73 years, randomly selected from the population register of Kuopio town, Eastern Finland. The cross-sectional and follow-up studies of the METSIM cohort were performed using identical protocols and similar clinical and laboratory measurements. Height was measured to the nearest 0.5 cm, and weight was measured using a calibrated digital scale (Seca 877; Seca, Hamburg, Germany) to the nearest 0.1 kg. BMI was calculated as the weight in kilograms divided by the height in meters squared. Glucose tolerance was evaluated with a 2-h oral glucose tolerance test (75 g glucose). We measured glucose and insulin levels at 0, 30, and 120 min, and we determined glucose tolerance status according to the American Diabetes Association criteria at both baseline and follow-up studies (8). Altogether 1,412 participants were diagnosed with type 2 diabetes at the baseline study, and they were excluded from all statistical analyses. A total of 5,169 men without diabetes at baseline (age 57 ± 7 years, BMI 26.5 ± 3.5 kg/m², mean \pm SD) having the metabolomics data were included in the current study. Their plasma fasting glucose (FG) was 5.6 ± 0.4 mmol/L, and 2-h glucose (2HG) was 5.9 ± 1.6 mmol/L. A total of 4,851 men participated in the follow-up study (mean follow-up time 7.4 ± 2.9 years). We have previously shown that this subset of men had clinical and laboratory characteristics similar to those of the entire METSIM population without diabetes, and therefore, this subsample can be considered to represent the entire METSIM cohort (9). During the follow-up, 522 participants developed incident type 2 diabetes. The study was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital, Kuopio, Finland. All study participants gave written informed consent.

Laboratory Measurements

We measured plasma glucose using an enzymatic hexokinase photometric assay (Konelab Systems Reagents; Thermo Fisher Scientific, Vantaa, Finland). Insulin was determined by immunoassay (ADVIA Centaur Insulin IRI no. 02230141; Siemens Medical Solutions Diagnostics,

Tarrytown, NY). We measured metabolites as part of Metabolon's untar-getted Discovery HD4 platform using ultra-high-performance liquid chromatography–tandem mass spectroscopy, as previously described in detail (9). The determination of metabolites was performed in three batches. Batch one included 999 samples with 717 metabolites identified; batch two included 1,231 samples with 778 metabolites; and batch three included 3,000 samples with 843 metabolites identified. All metabolites having >50% missing values, including mainly those with data available exclusively from a single batch, were omitted from the statistical analyses. Among a total of 857 unique metabolites, 86 metabolites related to gut microbiota were included in current statistical analysis (Supplementary Table 1).

Calculations

We calculated the Matsuda ISI as previously published (9), and insulin secretion index (area under the curve [AUC]; $\text{InsAUC}_{0-30}/\text{GluAUC}_{0-30}$) as follows: (insulin at 0 min + insulin at 30 min)/(glucose at 0 min + glucose at 30 min). We have previously validated the Matsuda ISI as the best index for ISI as compared with the M value of the euglycemic hyperinsulinemic clamp, and $\text{InsAUC}_{0-30}/\text{GluAUC}_{0-30}$ as the best marker of insulin secretion as compared with insulin secretion during a frequently sampled intravenous glucose tolerance test (10). Disposition index (DI), a measure of insulin secretion adjusted for prevailing insulin sensitivity, was calculated as Matsuda ISI \times ($\text{InsAUC}_{0-30}/\text{GluAUC}_{0-30}$) (10).

Statistical Analysis

We conducted statistical analyses using IBM SPSS Statistics, version 25. We log-transformed all continuous traits with the exception of age and follow-up time to correct for their skewed distribution. We applied Cox regression to associate the levels of metabolites with incident type 2 diabetes, and we present the results as hazard ratios (HR) and 95% CIs. We tested Cox proportionality assumption for the metabolites using *survival* and *survminer* packages in R, and we found that a fitted Cox regression model adequately described the data. $P < 5.8 \times 10^{-5}$ (corrected for 857 metabolites) was considered as statistically significant and $P < 0.05$ as nominally significant. We

used the area under the receiver operating characteristic (ROC) curve to assess the accuracy of the clinical risk factors (age, BMI, smoking, and physical activity) and metabolites in detecting incident type 2 diabetes. We examined the association of the metabolites with Matsuda ISI, DI, and glucose levels with linear regression adjusted for batch effect, follow-up time, and corresponding baseline values. We present the results as standardized regression coefficients (β and SE).

RESULTS

We investigated the association of 86 microbiome-based metabolites (Microbiome panel; Metabolon Inc.), representing established microbiome metabolites, novel microbially derived metabolites, host metabolites, and xenobiotics/dietary metabolites with incident type 2 diabetes (Supplementary Table 1).

Associations With Increased Risk of Type 2 Diabetes

Figure 1 and Supplementary Table 2 show the associations of the metabolites with incident diabetes. Creatine had the most significant association with the risk of type 2 diabetes (HR 1.43, 95% CI 1.30–1.56). Monoacylglycerols, 1-palmitoleoylglycerol (16:1) (HR 1.41, 95% CI 1.28–1.55), 1-oleoylglycerol (18:1) (HR 1.29, 95% CI 1.29–1.41), and 1-myristoylglycerol (HR 1.23, 95% CI 1.13–1.34) were among the seven metabolites significantly associated with incident type 2 diabetes after adjustment for batch effect, baseline age, BMI, smoking, and physical activity. Other metabolites significantly associated with incident type 2 diabetes after adjustment for confounding factors were urate (HR 1.39, 95% CI 1.27–1.52), 2-hydroxybuturate/2-hydroxyisobutyrate (HR 1.33, 95% CI 1.21–1.46), kynurenate (HR 1.31, 95% CI 1.20–1.42), xanthine (HR 1.31, 95% CI 1.21–1.42), xanthurenate (HR 1.31, 95% CI 1.18–1.44), 3-(4-hydroxyphenyl)lactate (HR 1.30, 95% CI 1.20–1.42), dimethylglycine (HR 1.20, 95% CI 1.12–1.28), and 2-hydroxyhippurate (HR 1.19, 95% CI 1.10–1.30). Kynurenate, *N*-acetyltryptophan, taurochenodeoxycholate, glycocholate, and glycochenodeoxycholate were significantly associated with incident diabetes after adjustment for batch effect, but after further adjustment for age, BMI, smoking, and physical activity, they were only nominally associated with incident diabetes. Fifteen

other metabolites were nominally associated ($P < 0.05$) with incident diabetes after adjustment for batch effect and other confounding factors.

Associations With Decreased Risk for Type 2 Diabetes

Ten metabolites decreased the risk of diabetes either significantly or nominally. We found that 1-linoleoyl-glycerophosphocholine (GPC) (18:2) lowered the risk of type 2 diabetes significantly and 1-lignoceroyl-GPC (24:0), 1-linolenoyl-GPC (18:3), 1-stearoyl-GPC (18:0), and indolepropionate nominally after the adjustment for all confounding factors. We also found that 3-phenylpropionate, Hippurate, and spermidine lowered the risk of type 2 diabetes nominally (Fig. 1 and Supplementary Table 2).

Associations With FG and 2HG Levels

All metabolites significantly associated with an increase in incident type 2 diabetes were significantly or nominally associated with increases in FG and/or 2HG except for 2-hydroxyhippurate. Metabolites significantly decreasing the risk of incident type 2 diabetes had decreases in FG and/or 2HG levels (Table 1).

Associations With DI and Matsuda ISI

To investigate the mechanisms behind the risk of type 2 diabetes and hyperglycemia, we calculated DI (insulin secretion adjusted for insulin sensitivity) and Matsuda ISI (Table 1). We found significant or nominally significant decreases in DI for 11 metabolites that were significantly associated with incident type 2 diabetes, whereas insulin sensitivity was decreased only for 5 metabolites who developed incident diabetes during the follow-up. Correspondingly, insulin sensitivity and insulin secretion were increased in individuals whose risk of type 2 diabetes was decreased during the follow-up.

ROC Curves Predicting of Incident Type 2 Diabetes

Figure 2 shows for the ROC curve for a model including known risk factors for type 2 diabetes (age, BMI, smoking, and physical activity) giving the AUC of 0.686. When three metabolites (creatine, 1-palmitoleoylglycerol [16:1], and urate) were included in the model the AUC increased up to 0.723 (95% CI 0.698–0.748). However, this increase was not statistically significant ($P = 0.051$).

CONCLUSIONS

Recent studies have indicated that the composition of the microbiota affects systemic metabolism. Changes in the composition of the microbiota, referred to as microbiota dysbiosis, have been linked to several diseases, including obesity, diabetes, and cardiovascular disease (11). Our large population-based METSIM study gives further evidence that microbiota metabolites increase the risk of incident type 2 diabetes.

Metabolites Increasing Significantly the Risk of Type 2 Diabetes

We found that among the metabolites investigated, creatine was most strongly associated with the risk of type 2 diabetes, which is a new finding. Creatine increases muscle mass, and as a dietary supplement combined with exercise, it improves insulin sensitivity (12). However, long-term effects of creatine on the risk of type 2 diabetes in middle-aged or elderly individuals have not been previously reported. In our study, creatine significantly reduced insulin sensitivity but only nominally reduced insulin secretion. In agreement with our findings, animal studies have reported that prolonged creatine supplementation decreases insulin sensitivity (13). Bacteria and fungi capable of degrading creatine have been identified in the human colon (14,15).

We found that three monoacylglycerols, 1-palmitoleoylglycerol (16:1), 1-oleoylglycerol (18:1), and 1-myristoylglycerol (14:0), indirectly controlled by gut microbiota, increased significantly the risk of incident type 2 diabetes by 41%, 29%, and 24%, respectively, as well as FG and 2HG levels. Bile and enzymes emulsify triacylglycerides in the intestinal lumen to form free fatty acids and monoacylglycerols that are taken up by intestine. No previous studies have reported that monoacylglycerols, except for monoacylglyceride 18:2 (16), increase the risk of type 2 diabetes or glucose levels. Also, 1-palmitoleoylglycerol (16:1), 1-oleoylglycerol (18:1), and 1-myristoylglycerol (14:0) significantly decreased insulin secretion but not insulin sensitivity.

Uric acid was associated with a 39% increased risk of type 2 diabetes in our study confirming the results of a previous study (17). Similarly, xanthine, a precursor of uric acid, was found to be associated

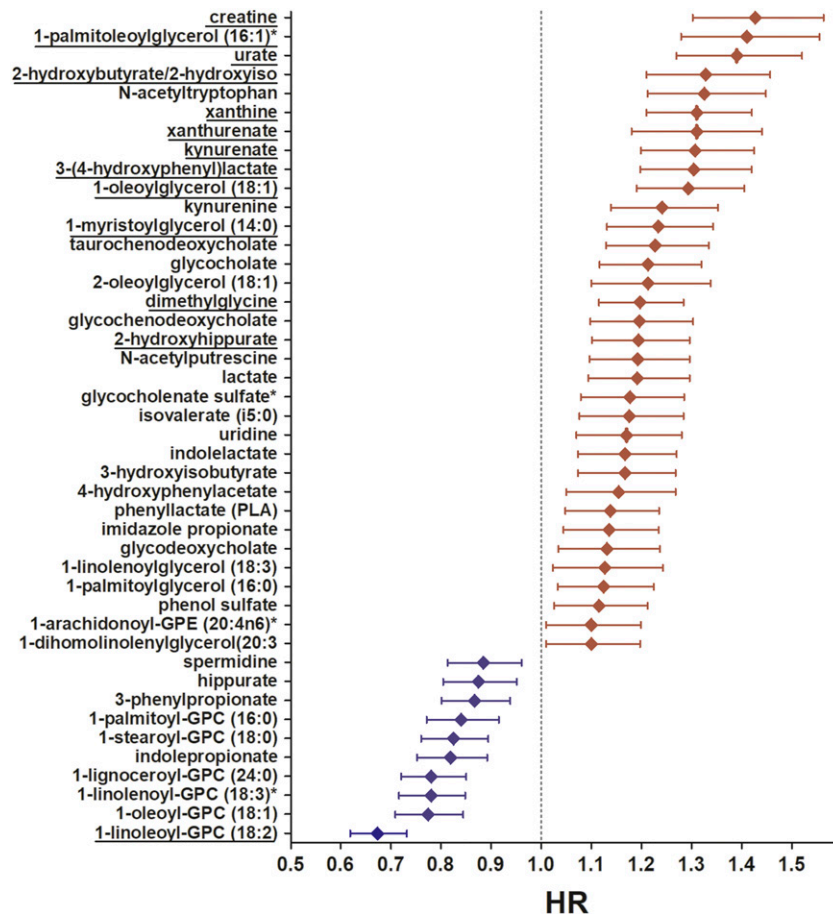


Figure 1—Metabolites significantly (bold and underlining) or nominally (bold) associated with increased or decreased risk of type 2 diabetes (HR and their 95% CIs) adjusted for confounding factors (age, BMI, smoking, and physical activity) are marked by bold and underlining.

with an increased risk of type 2 diabetes in our study, as previously reported (18). A novel finding in our study was that xanthine was also associated with impaired insulin secretion.

Also, 2-hydroxybutyrate, produced by threonine and methionine, significantly increased the risk of type 2 diabetes by 32%. It is a good marker of early stage hyperglycemia and insulin resistance and the risk of type 2 diabetes, as previously published (19,20). In our study, 2-hydroxybutyrate was significantly associated with the risk of incident type 2 diabetes and insulin resistance. Our novel finding was that 2-hydroxybutyrate was also significantly associated with reduced insulin secretion.

We found that 3-(4-hydroxyphenyl)-lactate, a lactobacillus breakdown product of phenylalanine (21), increased significantly the risk of type 2 diabetes by 32% and increased 2HG and, nominally, FG. It also decreased insulin secretion significantly but did not have

effects on insulin sensitivity. No previous studies are available on the effects of 3-(4-hydroxyphenyl) lactate on incident diabetes, glucose levels, insulin secretion, or insulin sensitivity.

Our study is the first to show that kynurenate and xanthurenate, products of the metabolism of amino acid tryptophan, significantly increased the risk of incident type 2 diabetes by 30%. A previous study showed that kynurenate and xanthurenate levels were increased in type 2 diabetes (22). Another study reported that kynurenate increased insulin resistance but did not increase the risk of type 2 diabetes (23). Our study showed that kynurenate significantly decreased insulin secretion but only nominally, whereas xanthurenate significantly decreased both insulin secretion and insulin sensitivity. Kynurenine, an upstream metabolite of kynurenate in the tryptophan pathway, was nominally associated with increases in incident type 2 diabetes and FG but significantly, with decreases both

in insulin secretion and insulin sensitivity. Kynurenine is involved in tryptophan metabolism, which is in direct or indirect control of microbiota. Moreover, indoleamine 2,3-dioxygenase 1, the enzyme responsible for conversion of tryptophan to kynurenine has been shown to be regulated by microbiota (24). Tryptophan metabolites have been shown to inhibit both proinsulin synthesis and glucose- and leucine-induced insulin release from rat pancreatic islets in agreement with our findings (25). Tryptophan is also a precursor for serotonin synthesis in the gut mucosa (26). Interestingly, in our study, serotone nominally decreased the risk of type 2 diabetes, FG, and 2HG and increased insulin secretion, in agreement with a previous study in human and mouse islets (27).

The 2-hydroxyhippurate (salicylurate) microbial metabolite is an aryl glycine conjugate formed by the body to eliminate excess salicylates, including aspirin. This metabolite was significantly associated with a 20% increase in the risk of type 2 diabetes but did not have significant effects on FG or 2HG levels, insulin secretion, or insulin sensitivity. A previous study demonstrated that aspirin reduces the risk of developing diabetes probably attributable to inhibition of inflammation (28).

Dimethylglycine, a derivative of glycine, increased the risk of type 2 diabetes by 20% in our study confirming the results of a previous study (29). Additionally, dimethylglycine increased 2HG level and reduced insulin sensitivity. High levels of dimethylglycine have been previously associated with an increase in HbA_{1c} in a prospective study (30).

Bile acids (taurochenodeoxycholate, glycocholate, and glycochenodeoxycholate) were nominally associated with an increased risk of type 2 diabetes after the adjustment for confounding factors, and they also increased both FG and 2HG levels, and decreased insulin secretion (except for glycochenodeoxycholate). Bile acids are metabolized by the microbiota in the lower part of the small intestine and colon, and they facilitate the absorption of dietary fat-soluble molecules (4,31). Impaired bile acid signaling contributes to hyperglycemia and progression to type 2 diabetes through multiple mechanisms, including farnesoid X receptor and G protein-coupled bile acid receptor TGR5 (32).

Table 1—Association of the metabolites with FG, 2HG, DI, and ISI during 7.4-year follow-up visit

Metabolite*	FG	2HG	DI	ISI
Creatine	↑↑	↑↑	↓	↓↓
1-palmitoleoylglycerol (16:1)	↑↑	↑↑	↓↓	±
Urate	↑↑	↑↑	↓↓	↓
2-hydroxybutyrate/2-hydroxyisobutyrate	↑↑	↑	↓↓	↓↓
N-acetyltryptophan	↑↑	↑↑	↓↓	↓
Xanthine	↑↑	↑↑	↓↓	↓↓
Xanthurenate	↑↑	↑↑	↓↓	↓
Kynurenate	↑↑	↑↑	↓↓	↓
3-(4-hydroxyphenyl)lactate	↑	↑↑	↓↓	±
1-oleoylglycerol (18:1)	↑	↑	↓↓	±
Kynurenine	↑	↑↑	↓↓	↓↓
1-myristoylglycerol (14:0)	↑	↑	↓↓	±
Taurochenodeoxycholate	↑	↑↑	↓	↓
Glycocholate	↑	↑	↓	±
2-oleoylglycerol (18:1)	±	±	↓	±
Dimethylglycine	±	↑	±	↓
Glycochenodeoxycholate	±	↑	±	±
2-hydroxyhippurate (salicylurate)	±	±	±	↑
N-acetylputrescine	↑	↑	↓	±
Lactate	↑	↑	±	↑↑
Glycochenolate sulfate	↑	±	↓	±
Isovalerate (i5:0)	↑	±	↓	↓
Uridine	↑↑	±	↓↓	↓
Indolelactate	↑	↑	↓	±
3-hydroxyisobutyrate	↑↑	↑	↓	↓
4-hydroxyphenylacetate	±	±	±	±
Phenyllactate	±	↑	±	±
Imidazole propionate	±	±	±	±
Glycodeoxycholate	↑	↑	±	±
1-linolenoylglycerol (18:3)	±	±	±	↑
1-palmitoylglycerol (16:0)	±	±	↓	±
Phenol sulfate	±	±	±	±
1-arachidonoyl-GPE (20:4n6)	±	±	±	↑
1-dihomo-linolenoylglycerol (20:3)	±	±	↓	±
Spermidine	±	±	±	±
Hippurate	↓	↓	↑	↑↑
3-phenylpropionate (hydrocinnamate)	↓	↓	↑↑	↑
1-palmitoyl-GPC (16:0)	±	±	±	↑↑
1-stearoyl-GPC (18:0)**	±	↓	↑	↑↑
Indolepropionate**	↓↓	↓	↑	↑↑
1-lignoceroyl-GPC (24:0)**	↓	↓↓	↑↑	↑↑
1-linolenoyl-GPC (18:3)**	↓	↓	↑↑	↑↑
1-oleoyl-GPC (18:1)**	↓↓	↓↓	↑↑	↑↑
1-linoleoyl-GPC (18:2)**	↓↓	↓↓	↑↑	↑↑

GPE, glycerophosphoethanolamine. *Bold and underlining, metabolites having statistically significant ($P < 5.8 \times 10^{-5}$) association with incident diabetes after adjustment for batch effect, baseline age, BMI, smoking, and physical activity. Bold, metabolites were nominally associated with incident type 2 diabetes after adjustment for confounding factors (qFig. 1). The linear regression analysis for FG, 2HG, DI, and ISI adjusted for follow-up time and baseline measurements. ↑↑, statistically significant increase ($P < 5.8 \times 10^{-5}$); ↑, nominally significant ($P < 0.05$) increase; ↓↓, statistically significant decrease ($P < 5.8 \times 10^{-5}$); and ↓, nominally significant ($P < 0.05$) decrease. **Metabolites having preventive effect on incident type 2 diabetes.

N-acetyltryptophan, a derivative of tryptophan, was nominally associated with type 2 diabetes but significantly with increases in FG and 2HG. It was associated with a decrease in insulin secretion, whereas insulin sensitivity was only nominally decreased. No previous population-based studies are available investigating the role of N-acetyltryptophan with respect to the risk of type 2 diabetes.

Lactate was nominally associated with incident type 2 diabetes, and increases in FG and 2HG, and a decrease in insulin secretion. Previously the Atherosclerosis Risk in Communities (ARIC) Study has shown that lactate levels are associated with a 20% increased risk of type 2 diabetes (33). Thirteen other metabolites had nominally significant associations with type 2 diabetes without and after adjustments for confounding factors. We did not find significant association of trimethylamine N-oxide with type 2 diabetes, although a recent meta-analysis found that association (34). However, the causality of this association needs further studies (35).

The ROC curve analysis indicated that the three best metabolites predicting incident type 2 diabetes improved slightly but not significantly ($P = 0.051$) the prediction of incident type 2 diabetes compared with the model including known risk factors for type 2 diabetes (age, BMI, smoking, and physical activity).

Metabolites Decreasing the Risk of Type 2 Diabetes

1-Linoleoylglycerophosphocholine had a preventive effect of 38% on the risk of type 2 diabetes after the adjustment for confounding factors, in agreement with a previous publication (21). Similarly, 1-oleoylglycerophosphocholine was associated with a 22% increased risk of type 2 diabetes confirming a previous study (36), but after the adjustment for confounding factors, the association was only nominally significant. Our novel finding was that lignoceroylglycerophosphocholine decreased the risk of type 2 diabetes by 23%. All the metabolites mentioned previously were also associated with the elevation of FG and 2HG levels, and increases in insulin secretion and insulin sensitivity. Lysophospholipids have a role in lipid signaling by acting on lysophospholipid receptors, and they can stimulate glucose-dependent

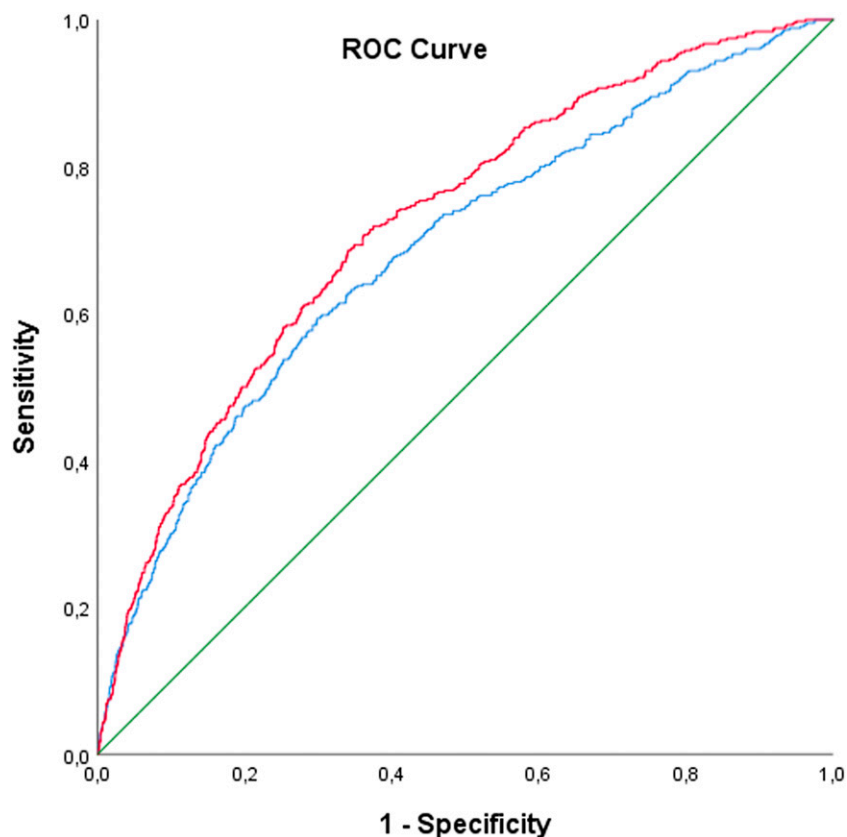


Figure 2—Area under the ROC curve in the prediction of incident type 2 diabetes in the METSIM study. The first ROC curve (blue) includes age, BMI, smoking, and physical activity as predictors for type 2 diabetes (model 1, AUC = 0.686) and the second ROC curve (red) includes model 1 and three metabolites, creatine, 1-palmitoleoylglycerol (16:1), and urate as predictors of type 2 diabetes (AUC = 0.723 [95% CI 0.698–0.748]). Number of participants in statistical analyses: with type 2 diabetes ($n = 420$) and without type 2 diabetes ($n = 3,751$). Change in AUC among the two models was not significant ($P = 0.051$).

insulin release (37) through lysophospholipid receptors such as G-protein-coupled receptor 119, localized in pancreatic β -cells.

As previously published indolepropionate (38), a microbial metabolite of tryptophan, reduced the risk of type 2 diabetes in our study by 18%, but after the adjustment for confounding factors, this association was only nominally significant. The mechanism for the preventive effect of indolepropionate is the stimulation of glucagon-like peptide 1 from intestinal enteroendocrine L cells which stimulate insulin secretion and increase insulin sensitivity as demonstrated also in our study (39).

Metabolites Affecting Insulin Secretion and Insulin Sensitivity

As expected, metabolites strongly associated with incident diabetes and elevated levels of FG and 2HG exhibited decreases in insulin secretion and insulin

sensitivity. Correspondingly, metabolites associated with a reduced risk of incident diabetes had improvements both in insulin secretion and insulin sensitivity. However, there were also metabolites increasing the risk of incident diabetes without having a significant effect on insulin secretion or insulin sensitivity. Dimethylglycine and 2-hydroxyhippurate increased the risk of incident diabetes significantly, but their effects on insulin secretion and insulin sensitivity were only modest. This may indicate that there are other less known mechanisms leading to type 2 diabetes.

Strengths and Limitations

The strengths of this study include the large and homogeneous METSIM study population. We applied validated methods to measure insulin sensitivity and secretion (10). Additionally, we used a very conservative threshold for statistical significance to increase credibility of our

findings. The limitations of the study are that only middle-aged and elderly Finnish men were included in our study, and therefore, we do not know whether the results are valid for women, different age groups, and other ethnic and racial groups. We are not aware of any unselected population sample where a large set of microbiota metabolites had been measured and where insulin secretion and insulin sensitivity were evaluated using similar validated insulin secretion and insulin sensitivity markers. Therefore, we could not replicate our findings in other populations. Other limitations of our study are that information regarding the diet and medication affecting microbiota was not available and that we did not have microbiota samples. Finally, the exact location of the source of the metabolites requires future studies, especially with respect to the contribution of oral versus gut microbiota.

In conclusion, we have identified several new microbiota metabolites increasing the risk of type 2 diabetes. We also demonstrated that the conversion to diabetes was associated with increased FG and 2HG levels and decreased insulin secretion during the follow-up, but the association with decreased insulin sensitivity was not as strong. This demonstrates the crucial role of insulin secretion in the conversion to type 2 diabetes. We also demonstrated that the metabolites that were associated with a reduced risk of type 2 diabetes had increased insulin sensitivity and quite often increased insulin secretion. However, not all metabolites increasing the risk of type 2 diabetes were associated with insulin secretion and insulin sensitivity, indicating that there may be other less known mechanisms increasing the risk of type 2 diabetes.

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Author Contributions. J.V. conceived the study, performed metabolomics and genetic data analyses, and wrote and revised the manuscript. L.F.S. and T.K. performed metabolomics data analyses and revised the manuscript. U.S. contributed to the discussion and revised the manuscript. M.L. conceived the study, wrote and reviewed the manuscript, and supervised the entire study. M.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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