

# Metabolomic markers of glucose regulation after a lifestyle intervention in prediabetes

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## ABSTRACT

**Introduction** Disentangling the specific factors that regulate glycemia from prediabetes to normoglycemia could improve type 2 diabetes prevention strategies. Metabolomics provides substantial insights into the biological understanding of environmental factors such as diet. This study aimed to identify metabolomic markers of regression to normoglycemia in the context of a lifestyle intervention (LSI) in individuals with prediabetes.

**Research design and methods** We conducted a single-arm intervention study with 24 weeks of follow-up. Eligible study participants had at least one prediabetes criteria according to the American Diabetes Association guidelines, and body mass index between 25 and 45 kg/m<sup>2</sup>. LSI refers to a hypocaloric diet and >150 min of physical activity per week. Regression to normoglycemia (RNGR) was defined as achieving hemoglobin A1c (HbA1c) <5.5% in the final visit. Baseline and postintervention plasma metabolomic profiles were measured using liquid chromatography-tandem mass spectrometry. To select metabolites associated with RNGR, we conducted the least absolute shrinkage and selection operator-penalized regressions.

**Results** The final sample was composed of 82 study participants. Changes in three metabolites were significantly associated with regression to normoglycemia; N-acetyl-D-galactosamine (OR=0.54; 95% CI 0.32 to 0.82), putrescine (OR=0.90, 95% CI 0.81 to 0.98), and 7-methylguanine (OR=1.06; 95% CI 1.02 to 1.17), independent of HbA1c and weight loss. In addition, metabolomic perturbations due to LSI displayed enrichment of taurine and hypotaurine metabolism pathway ( $p=0.03$ ) compatible with biomarkers of protein consumption, lower red meat and animal fats and higher seafood and vegetables.

**Conclusions** Evidence from this study suggests that specific metabolomic markers have an influence on glucose regulation in individuals with prediabetes after 24 weeks of LSI independently of other treatment effects such as weight loss.

## INTRODUCTION

Type 2 diabetes (T2D) is a major threat to global health. T2D occurs at varying rates in people from different ethnic backgrounds.<sup>1</sup> Prediabetes is a prevalent condition that identifies individuals with hyperglycemia who

## WHAT IS ALREADY KNOWN ON THIS TOPIC

- Lifestyle interventions (LSI) provide benefits to glucose regulation in prediabetes.
- There is a considerable variation in the individuals to receive the benefits of a LSI.
- Metabolomics provides substantial insights into the interplay between disease, and environmental exposures to explain individual responses to treatment.

## WHAT THIS STUDY ADDS

- Three metabolites were significantly associated with regression to normoglycemia in prediabetes: N-acetyl-D-galactosamine, putrescine, and 7-methylguanine.
- In addition, changes in taurine and hypotaurine metabolism pathways were observed after a LSI.
- The metabolomic changes associated with a LSI are compatible with biomarkers of protein consumption, lower red meat and animal fats, and higher seafood and vegetables.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Metabolites associated with regression to normoglycemia can provide a potential route for therapeutic target discovery.
- Food profiles illuminated from these results can help to design more effective therapeutic strategies for diabetes prevention.

do not meet the criteria for T2D but whose glucose levels are higher than those considered normal.<sup>2</sup> Individuals with prediabetes are at increased risk of developing T2D and cardiovascular risk.<sup>3</sup> The seminal event in the progression from prediabetes to T2D is  $\beta$ -cell failure and can be reversible.<sup>4 5</sup> And among individuals with prediabetes, regression to normoglycemia is the most frequent outcome.<sup>6 7</sup>

Lifestyle interventions (LSI) can prevent future diagnosis of T2D.<sup>8</sup> Yet, patients at high risk of diabetes have considerable variation in their likelihood of seeing the benefit of



diabetes prevention treatments,<sup>9</sup> responding differently to the same diet. High-throughput metabolomics technologies have provided insights into the pathophysiological pathways and understanding T2D and prediabetes,<sup>10</sup> as well as markers of food and nutrient consumption that uncovered metabolic pathways that are potentially modified by diet.<sup>11</sup> There is evidence of differences in the lipidomic profile of high-density lipoprotein and low-density lipoprotein particles of individuals with prediabetes who regress to normoglycemia after ~2 years of follow-up without treatment.<sup>12</sup> However, the extent to which these or other metabolites are modified in a lifestyle intervention has not been described.

Metabotypes refer to the process of grouping similar individuals based on phenotypic or metabolomic profiles<sup>13</sup> and have helped to identify differential responses to dietary interventions<sup>14</sup> and weight loss.<sup>15</sup> Identifying the metabolomic profile of individuals with prediabetes who regress to normoglycemia can provide substantial insights into the dietetic and physiological modulators of glucose regulation during a LSI that could be nutritional intervention targets for T2D prevention. In this study, we aimed to identify metabolomic markers of regression to normoglycemia in the context of a LSI in individuals with prediabetes.

## RESEARCH DESIGN AND METHODS

### Study population and design

We conducted a single-arm intervention study with 24 weeks of follow-up. The study sample included individuals who responded to physical and electronic advertisements posted in the Research Unit of Metabolic Diseases (Unidad de Investigacion en Enfermedades Metabolicas (UIEM)) within the Instituto Nacional de Ciencias Medicas y Nutricion (INCMNSZ). Eligibility for study participation was first determined in a screening visit by the presence of at least one prediabetes criteria according to the American Diabetes Association: fasting glucose between 100 and 125 mg/dL, hemoglobin A1c (HbA1c) 5.7%–6.4%, and/or 2-hour blood sugar between 140 and 199 mg/dL after an oral load of 75 g of glucose.<sup>2</sup> Additional criteria were age between 18 and 69 years and body mass index (BMI) in the overweight or obesity categories (BMI 25–45 kg/m<sup>2</sup>). Exclusion criteria included using glucose-lowering drugs such as metformin or corticosteroids, pregnancy, and subjects under nutritional or physical activity counseling prescribed by a medical professional.

### Procedures

The study included four visits: screening, intervention, follow-up, and final. All visits took place at UIEM within the INCMNSZ. For the screening visit, subjects were asked to attend at 07:00 hours after an overnight fast. Participants underwent a 3-hour oral glucose tolerance test (OGTT) using 75 g of glucose. Anthropometric

measures were performed in the screening visit following standardized protocols. Subjects were evaluated in fasting with light clothes and without shoes on. Body weight was measured with Seca mBCA 514 (Hamburg, Germany) medical body composition analyzer. Height was measured with Seca 284 (Hamburg, Germany) stadiometer. Weight and height were used to compute BMI as the ratio of weight (kilograms nearest 0.01) to squared height (m<sup>2</sup>), computed with a Microsoft Excel formula. Waist and hip circumference (centimeters nearest 0.5) were measured at the midpoint between the lower ribs and the iliac crest and the level of the trochanter major, respectively. Both measures were used to calculate the waist-to-hip ratio.

Subjects with eligible criteria were asked to attend the intervention visit 1 week after the screening visit. During the intervention visit, participants underwent a body composition evaluation through dual-energy X-ray absorptiometry (General Electric, Lunar Prodigy, Madison, Wisconsin, USA).

### Lifestyle intervention

Standardized dietitians implemented the LSI. This intervention included a hypocaloric diet (500 kcal reduction of daily energy expenditure), distributed as follows: 45% of the total calorie daily intake of carbohydrates, 30% lipids, and 15% from protein sources. Individuals received instructions to organize their meals with an educational handbook containing food groups and personalized servings. In addition, the intervention included tailored physical activity recommendations to reach >150 min medium intensity per week. Goals were stabilized to reach >3% weight loss over the follow-up.<sup>15</sup> Participants were trained to log their food habits and physical activity three times per week. After 12 weeks, subjects attended a follow-up visit with the dietitian to reinforce knowledge and goals. The final visit took place 24 weeks after the intervention visit, and all measurements were repeated. Across visits, we evaluated participants' food and physical activity habits with a 24-hour food recall and a log of activities. This information helped to assess the patient's adherence to diet and physical activity modifications.

### Outcomes

Regression to normal glucose regulation (RNGR) was defined as HbA1c <5.5% in the final visit to avoid the regression to the mean bias,<sup>16</sup> frequently observed in single measurements such as fasting glucose and 2-hour glucose. Prediabetes maintenance was defined as having at least one of the prediabetes criteria in the last visit. Incident T2D was defined as fasting plasma glucose >126 mg/dL and/or glucose post load of >200 mg/dL and/or HbA1c >6.5 in any of the visits; these individuals were removed from the analysis to avoid comparison imbalance between groups since they represented a small proportion of the sample (n=6).

## Calculations

Daily energy, macronutrient and micronutrient intakes were assessed through a 24-hour food recall. Data were analyzed using ESHA's Food Processor Nutrition Analysis software (Salem, Oregon, USA). For measuring insulin secretion, we used the early insulin response, defined as the ratio of the 30 min change in insulin concentration to the 30 min change in glucose concentration after oral glucose loading, that is,  $(\text{Ins30} - \text{Ins0}) / (\text{Gluc30} - \text{Gluc0})^{17}$ ; and the oral disposition index defined as  $O = (\text{Ins0} - \text{Ins30}) / (\text{Gluc0} - \text{Gluc30} \times 1 / \text{Ins0})^{18}$ . Furthermore, insulin sensitivity was estimated with Matsuda index<sup>19</sup> as follows:

$$\text{ISI} = \frac{10^4}{\sqrt{(\text{G}_0 \times \text{I}_0 \times \text{m}_G \times \text{m}_I)^{1/2}}}, \text{ Oral Glucose Insulin Sensitivity Index (OGIS)}^{20}$$

through the website <http://webmet.pd.cnr.it/ogis/>, and homeostasis model assessment for insulin resistance that was computed as fasting glucose×fasting insulin/405.<sup>21</sup>

## Metabolomic analyses

The plasma metabolomics profiling was performed in the Metabolomics Platform at the Broad Institute of Harvard University and Massachusetts Institute of Technology (Cambridge, Massachusetts, USA) using high-throughput liquid standardization. A total of 219 named metabolites were qualified for primary analyses (online supplemental table 1). To reduce noise in the profiling data, we implemented a quality control (QC) pipeline and metabolite signals with noisy trends were removed from the study. The QC steps included: normalization with internal standards and pooled samples, removal of metabolites with >25% of missing values, missing value imputation, and normalization and covariate adjustment. Missing metabolites values were imputed using mice (<https://cran.r-project.org/web/packages/mice/index.html>) package.

## Statistical analysis

Differences in baseline clinical characteristics, as well as differences before and after treatment, were analyzed with a t-test and Mann-Whitney U test according to the variable's distribution. Metabolomic fold changes (FC) were computed using a paired approach using the limma R package (<https://bioconductor.org/packages/limma/>). Generalized estimating equation models were used to account for HbA1c at baseline. Metabolites were log-transformed and adjusted by age and sex. Residuals of the regression were normalized by inverse normal transformation. The changes of the metabolites before versus after the intervention were computed using an FC analysis setting a cut-off point of an FC > 0.25 and a p value adjusted for multiple comparisons < 0.05 as significant (q value). For metabolite selection, we conducted the least absolute shrinkage and selection operator-penalized regressions (LASSO) with 10-fold cross-validation to select metabolites before the intervention (baseline) and after intervention accounting for baseline abundances ( $\log_2 \text{FC}$ ). We used Rngr as outcome variable b giving minimum mean cross-validated error 9. We repeated

this process 100 times and accumulated the selection frequency across 100 iterations for each metabolite separately and used a threshold of 10 selections to prioritize the top metabolites selected. This analysis was performed using the glmnet (<https://cran.r-project.org/web/packages/glmnet/index.html>) package implemented in R V.3.6.1 program (<https://www.r-project.org/>). We considered two-sided  $p < 0.05$  to denote evidence against the null hypothesis.

## Pathway analysis

We used MetaboAnalyst V.4.0<sup>22</sup> to identify enriched metabolic pathways for the set of prioritized metabolites in two time-points, baseline, and treatment, and used the Kyoto Encyclopedia of Genes and Genomes<sup>23</sup> database to map the signatures with their physiological pathways.

## RESULTS

A total of 205 individuals were screened, of which 138 were confirmed prediabetes cases in the screening visit, and 131 attended the intervention visit (online supplemental figure 1). The retention rate at the end of the study was 67.1%. The most frequently reported cause of withdrawal was the lack of time to attend the visits. Subjects who did not complete the study were younger than those who completed the study ( $45.02 \pm 11$  vs  $49.45 \pm 11$ ,  $p=0.035$ ).

After 24 weeks of LSI, 27 (30.6%) individuals regressed to normal glucose regulation, 55 (62.5%) maintained their prediabetes status and 6 individuals (6.8%) progressed to T2D. The characteristics at baseline overall and by groups are shown in table 1. Rngr subjects were younger ( $44.33 \pm 13.87$  vs  $52.23 \pm 10.88$ ,  $p=0.008$ ). Surrogates of insulin secretion and sensitivity did not show significant differences at baseline among groups ( $p>0.05$ ), indicating that Rngr was not entirely driven by relatively better glycemic health at baseline (online supplemental table 2).

## Intervention effectiveness

After 24 weeks of LSI, the mean weight loss was  $2.46 \pm 3.48$  kg, with 47.5% ( $n=39$ ) of the subjects reaching the >3% weight loss goal. The intervention was effective in reducing weight, waist circumference, body fat, fasting glucose, HbA1c, and increasing insulin sensitivity measured by OGIS ( $p<0.05$ ) (online supplemental table 3). Participants displayed favorable changes in their dietary behavior: significant reduction in total energy and sugar intake, and increased protein intake ( $p<0.01$ ) (figure 1). The increment of protein consumption was associated with a lower probability of maintenance (HR 0.97, 95% CI 0.94 to 0.99) independent of age, sex, HbA1c at baseline, and weight loss (table 2). Increased protein consumption increased after the intermediate visit (online supplemental figure 2).

**Table 1** Baseline characteristics of the study population according to their final status (n=82)

	Overall (n=82)	Prediabetes maintenance (n=52)	Regression to normal glucose tolerance (n=30)	P value
Age (years)	49.34±12.13	52.23±10.88	44.33±13.87	0.008
Sex				
Male, n, (%)	21 (25.6)	13 (25)	8 (26.6)	0.868
Diagnostic criteria at baseline				
Impaired fasting glucose only, n (%)	6 (7)	2 (3.8)	4 (13.3)	
Impaired HbA1c% only, n (%)	25 (30)	15 (28.8)	10 (33.3)	
Impaired glucose tolerance only, n (%)	2 (2)	1 (2)	1 (3.3)	0.117
Two criteria	36 (44)	22 (42.3)	14 (46.6)	
Three criteria	13 (16)	12 (23)	1 (3.3)	
T2D family history, n (%)	45 (56%)	25 (48)	20 (66.6)	0.123
Fasting glucose (mg/dL)	99.16±9.18	98.35±10.32	100.57±6.71	0.214
Fasting insulin (U/mL)	9.2 (6.20–13.40)	9 (6–11.50)	9.35 (6.7–14.47)	0.371
HbA1c%	5.89±0.26	6±0.21	5.71±0.24	0.0001
Glucose 120' (mg/dL)	129.41±30.43	131.33±31.23	125.97±29.17	0.442
Aix75%	36.5 (26–45.75)	38.5 (30–46.25)	30.5 (24.25–39.75)	0.039
Aix%	39.5 (33–50)	42.5 (34–51)	37 (32–43)	0.087
Aortic augmentation index (mg Hg)	16.5 (11–21)	18 (11–23)	14.5 (11.25–18.75)	0.116
PWV (m/s)	6.21±0.95	6.21±0.89	6.21±1.07	0.985
BMI				
Male	30.97±4.37	30.42±4.63	32.86±4.05	0.462
Female	30.55±3.74	30.37±3.88	30.88±3.53	0.601
Waist circumference				
Male	101.96±11.69	98.90±10.95	106.91±11.82	0.141
Female	95.10±10.30	95.69±10.49	93.97±10.11	0.547
Waist-to-hip ratio				
Male	0.95±0.06	0.94±0.07	0.95±0.06	0.693
Female	0.88±0.06	0.90±0.06	0.85±0.05	0.001
Body fat %				
Male	33.31±4.84	31.90±4.55	35.24±4.82	0.148
Female	43.07±4.79	42.83±5.15	43.48±4.66	0.597
Free fat mass index				
Male	19.44±2.03	19.37±2.43	19.54±1.46	0.855
Female	15.62±3.60	15.14±4.30	16.46±3.57	0.095
Visceral adipose tissue volume				
Male	1308±741.17	1172.73±780.74	1491.75±687.88	0.355
Female	1121±468.01	1164.14±490.29	1049.41±429.20	0.447
Estimate daily caloric consumption (kcal)	2114.43±901.19	1988.64±778.4	2332.46±1060.69	0.127
Fat consumption percentage (%)	31.81±12.40	31.75±12.35	31.92±12.69	0.951
Protein consumption percentage (%)	16.50 (13.15–21.93)	17.20 (24.78–37.80)	15.40 (13.77–22.53)	0.736
Carbohydrate consumption percentage (%)	50.65±13.67	50.76±13.50	50.45±14.19	0.923
Daily sugar consumption (g)	56.50 (27.69–94.62)	52.78 (25.52–87.08)	60.11 (37.74–109.72)	0.238
Daily fiber consumption (g)	13.10 (6.95–21.21)	14.14 (9.07–22.91)	9.02 (6.40–18.14)	0.05

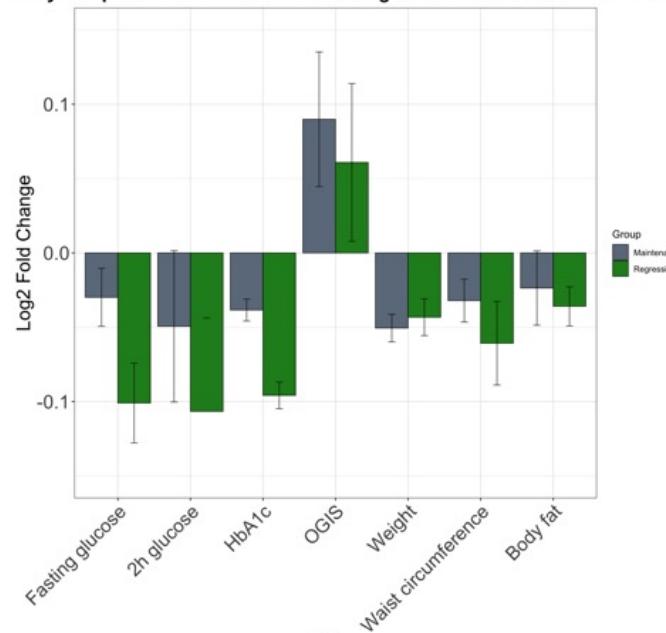
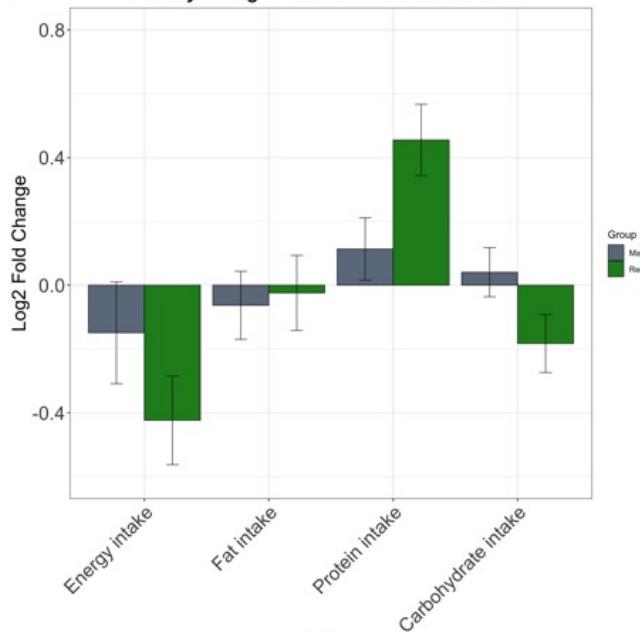
Differences between the two groups were computed with  $\chi^2$  test for qualitative variables, t-test for parametric variables and Wilcoxon test for non-parametric variables.

BMI, body mass index; HbA1c, hemoglobin A1c; PWV, pulse wave velocity; T2D, type 2 diabetes.

### Metabolites associated with RNGR

Before the intervention (baseline), three metabolites were selected at least 10 times in the LASSO regression

(online supplemental table 4): PS (P-36.1)/PS (O.36.2) (OR=0.51; 95% CI 0.28 to 0.90), C5.1 carnitine (OR=0.58; 95% CI 0.32 to 0.97), and Cer (d18:1/24:1) (OR=0.59;

**Body composition and biochemical changes after 24 weeks of treatment****Dietary changes after 24 weeks of treatment**

**Figure 1** Changes after 24 weeks of lifestyle intervention in body composition, biochemical (A) and dietary traits (B). Changes are displayed as Log2 fold change and SE. The intervention effectively reduced fasting glucose, HbA1c, weight, waist circumference, and energy intake ( $p<0.001$ ). After adjusting for confounders, subjects regressed to normal values of HbA1c and increased their protein consumption ( $p=0.023$ ). Before and after significance values were computed with paired t-test. Significances with covariate adjustment (age, age $^2$ , sex, and BMI (in non-anthropometric variables)) were computed in a logistic regression model. BMI, body mass index; HbA1c, hemoglobin A1c; OGIS, Oral Glucose Insulin Sensitivity Index.

95% CI 0.33 to 0.98). Nevertheless, these metabolites appeared to be affected by BMI and HbA1c since neither of them maintained the significance level after the adjustment.

After 6 months of intervention, 40 metabolites measured by their FC were prioritized 10 or more times in the LASSO regression (online supplemental figure 3, online supplemental table 5). The profile was composed mainly by changes in amino acids (N(6)-methyllysine, thyroxine, creatine, N-acetylalanine, glutamine), lysophosphatidylcholines (LPC (18.3), LPC (14.0), LPC (16.0)), carnitines (C18 carnitine, C10:2:carnitine, C5-DC:carnitine, C12:1:carnitine), sphingolipids (Sd18.1.22.1, SM[d18:1/14.0],

SM[d18.1.20.0]), and organic compounds (1,7-dimethyluric acid, trigonelline, tryptophan, allantoin, N-carbamoyl-beta-alanine, 1-methylhistamine, 2-methylguanosine, 7-methylguanine). This profile displays metabolites biomarkers of vegetables, fruits, and legumes (trigonelline, tryptophan, allantoin, N-carbamoyl-beta-alanine) consumption, as well as sources of protein and fat (amino acid, fatty acid, acylcarnitines, and glycerophospholipid). After adjusting for HbA1c and weight loss, only three metabolites remained significant: N-acetyl-D-galactosamine (OR=0.54; 95% CI 0.32 to 0.82), 7-methylguanine (OR=1.06; 95% CI 1.02 to 1.17), and putrescine (OR=0.90, 95% CI 0.81 to 0.98) (figure 2).

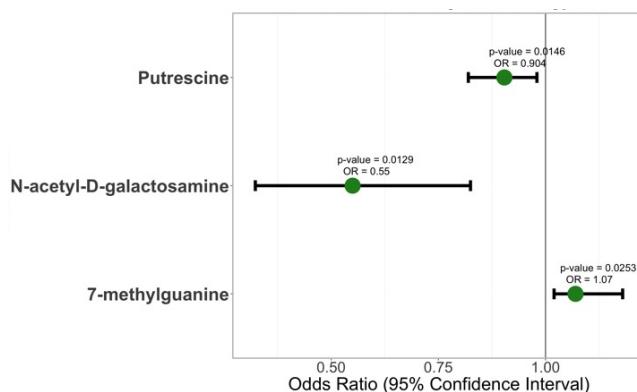
**Table 2** Clinical variables associated with regression to normal glucose tolerance

Outcome: maintenance to normal glucose tolerance				
	HR	SE	95% CI	P value
Age	1.15	0.10	0.93 to 1.42	0.17
Age $^2$	0.99	0.00	0.99 to 1.00	0.24
Sex	0.81	0.35	0.40 to 1.63	0.56
$\Delta$ weight	0.96	0.49	0.87 to 1.06	0.49
$\Delta$ protein	0.97	0.01	0.94 to 0.99	0.039
HbA1c	14.59	0.68	3.77 to 56.3	0.0001

Likelihood ratio test 30.65 p=0.00003; Wald test 23.43 p=0.0007; score (logrank) test=25.27 p=0.004.  
HbA1c, hemoglobin A1c.

### Metabolomic changes after LSI

Finally, we sought to identify metabolites with substantial change after the LSI. Fourteen metabolites displayed significant changes before and after LSI ( $\log_2\text{FC}>0.25$  and  $q<0.05$ ) (figure 3, online supplemental table 6). After the LSI, we observed lower levels of C14 carnitine ( $\log_2\text{FC} -0.46$  and  $q=0.013$ ), PS/P36 ( $\log_2\text{FC} -0.30$  and  $q=0.013$ ), inosine ( $\log_2\text{FC} -0.45$  and  $q=0.013$ ), thyroxine ( $\log_2\text{FC} -0.35$  and  $q=0.01$ ), myristoleic acid ( $\log_2\text{FC} -0.29$  and  $q=0.01$ ), and niacinamide ( $\log_2\text{FC} -0.44$  and  $q=0.02$ ). Our sample also displayed increased levels of cystine ( $\log_2\text{FC} 0.56$  and  $q=0.001$ ), hypotaurine ( $\log_2\text{FC} 0.48$   $q=0.002$ ), sphinganine ( $\log_2\text{FC} 0.64$  and  $q=0.003$ ), taurine ( $\log_2\text{FC} 0.45$  and  $q=0.01$ ), 5-hydroxytryptophan ( $\log_2\text{FC} 0.46$  and  $q=0.02$ ), serotonin ( $\log_2\text{FC} 0.46$  and  $q=0.02$ ), glycine



**Figure 2** Metabolites associated with regression to normoglycemia. Metabolite abundances were adjusted by age and sex and normalized using inverse normal transformation. Values fold change before versus intervention. Logistic regression model was computed using regression to normoglycemia as outcome adjusted by hemoglobin A1c and Δ weight.

(log<sub>2</sub>FC 0.25 and q=0.02), and pro-glycine (log<sub>2</sub>FC 0.47 and q=0.02) (figure 3A). These metabolites enriched the taurine and hypotaurine metabolism pathway (p=0.03) (figure 3B). The metabolomic profile after LSI reflects activity in biomarkers of protein consumption, lower red meat and animal fats (C14 carnitine, inosine, myristoleic acid), and higher seafood and vegetables (cystine, sphinganine, taurine, serotonin).

## DISCUSSION

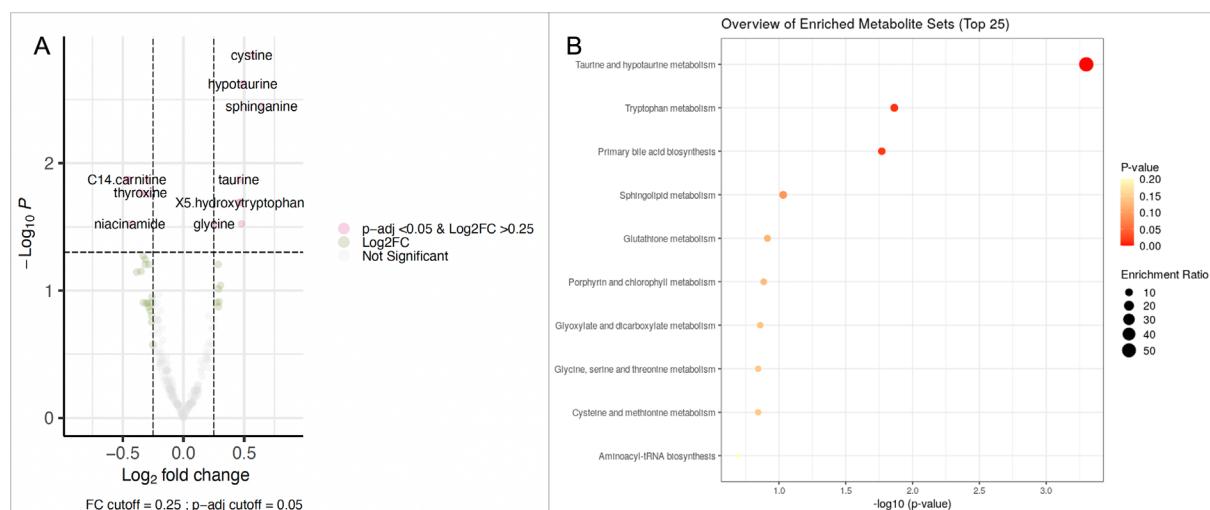
This study set out with the aim of identifying metabolomic markers of regression to normoglycemia in the context of a 24 weeks of LSI in individuals with prediabetes. Changes in three metabolites (N-acetyl-D-galactosamine, putrescine, and 7-methylguanine) were significantly associated with regression to normoglycemia independent of other treatment factors such as HbA1c at baseline and

weight loss. In addition, the metabolomic changes associated with a LSI were represented by activity in biomarkers of protein consumption, lower red meat and animal fats, and higher seafood and vegetables (cystine, sphinganine, taurine, serotonin). These metabolites enriched the taurine and hypotaurine metabolism pathway (p=0.03).

In our study, low concentrations of N-acetyl-D-galactosamine were associated with a higher probability to regress to normoglycemia; this is consistent with previous reports that associate higher concentrations of N-acetyl-D-galactosamine with incident cardiovascular disease,<sup>24</sup> diabetes mellitus,<sup>25</sup> as well as the longitudinal risk of all-cause, cardiovascular, and cancer mortality after 20.5 years among initially healthy individuals.<sup>26</sup> N-acetyl-D-galactosamine identifies glycan residues on circulating glycoproteins, the majority of which are acute phase reactants. A large proportion of the potential risk associated with elevated N-acetyl-D-galactosamine would relate to systemic inflammatory pathways.<sup>26</sup> Further studies are needed to explore potential modulators and causes of N-acetyl-D-galactosamine concentrations.

Low levels of putrescine were associated with a higher probability of regression to normoglycemia in our study. Putrescine is a polyamine. In mouse models, the dysregulation of polyamine metabolism impacts glucose, lipid, and energy homeostasis.<sup>27-28</sup> Fernandez-Garcia *et al* reported high levels of serum putrescine in patients with T2D and a positive correlation with HbA1c.<sup>29</sup> This is consistent with the deleterious role of putrescine in glucose regulation found in our study. External sources of putrescine include fruits, vegetables, and seeds, which might reflect the changes in the pattern of consumption of the participants.

Finally, 7-methylguanine was positively associated with regression to normoglycemia. 7-Methylguanine is a hypoxanthine. In previous studies, this metabolite has shown a positive association with T2D.<sup>30</sup> Differences



**Figure 3** (A) Metabolites associated with lifestyle intervention. (B) Enrichment analysis of the significantly modified metabolites (log<sub>2</sub>FC >0.25 and q=0.05) after 24 weeks of intervention. FC, fold change.



between these findings and our findings could be related to sample size differences and longer follow-ups.

In this study, subjects who increased their protein consumption were more likely to regress to normal glucose tolerance than those who maintained it. Stentz *et al*<sup>31</sup> found that a high protein diet had 100% remission of prediabetes compared with only 33% on a high carbohydrate diet with similar weight loss. This association may partly be explained by increases in incretin hormones gastric inhibitory polypeptide, glucagon-like peptide-1, and ghrelin in a high protein diet, which improves insulin sensitivity and β-cell function.<sup>32</sup>

Finally, taurine and hypotaurine increased their abundance in plasma after the LSI. These metabolites are antioxidant and anti-inflammatory agents.<sup>33</sup> The main natural dietary sources are foods of animal origin, particularly seafood.<sup>34</sup> Regarding its effect on glucose metabolism, taurine and hypotaurine have been shown to lower blood glucose levels and improve hyperglycemia and hyperglycemia-induced insulin resistance in mouse models.<sup>35</sup>

The findings in this study are limited by the sample size and lack of replication in an independent cohort. Also, the method used to assess diet in this study, 24 hours recall, can be subject to substantial reporting bias and misreporting. Furthermore, a recent report<sup>36</sup> described the importance of classifying individuals with prediabetes based on their diagnosis criteria since this heterogeneity is crucial for their treatment response. In our study, we included individuals with any prediabetes diagnostic criteria to maximize our power, and our results might be limited by not accounting for this heterogeneity. Further studies, which consider these limitations, and longer follow-ups to measure outcomes associated with T2D incidence are recommended.

## CONCLUSIONS

The evidence from this study suggests that changes in three metabolites, N-acetyl-D-galactosamine, putrescine, and 7-methylguanine, were significantly associated with regression to normoglycemia independent of other treatment factors such as HbA1c at baseline and weight loss. In addition, our results show that increased protein consumption could positively impact the treatment of individuals with prediabetes. The findings of this study have several practical implications for treating individuals at high risk of T2D.

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**Contributors** CAA-S and MdRS-G designed research, and MdRS-G, AD, and CBC conducted research. MdRS-G performed the statistical analysis, and MdRS-G, KEW, and AKM wrote the paper. MdRS-G 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. All authors read and approved the final manuscript.

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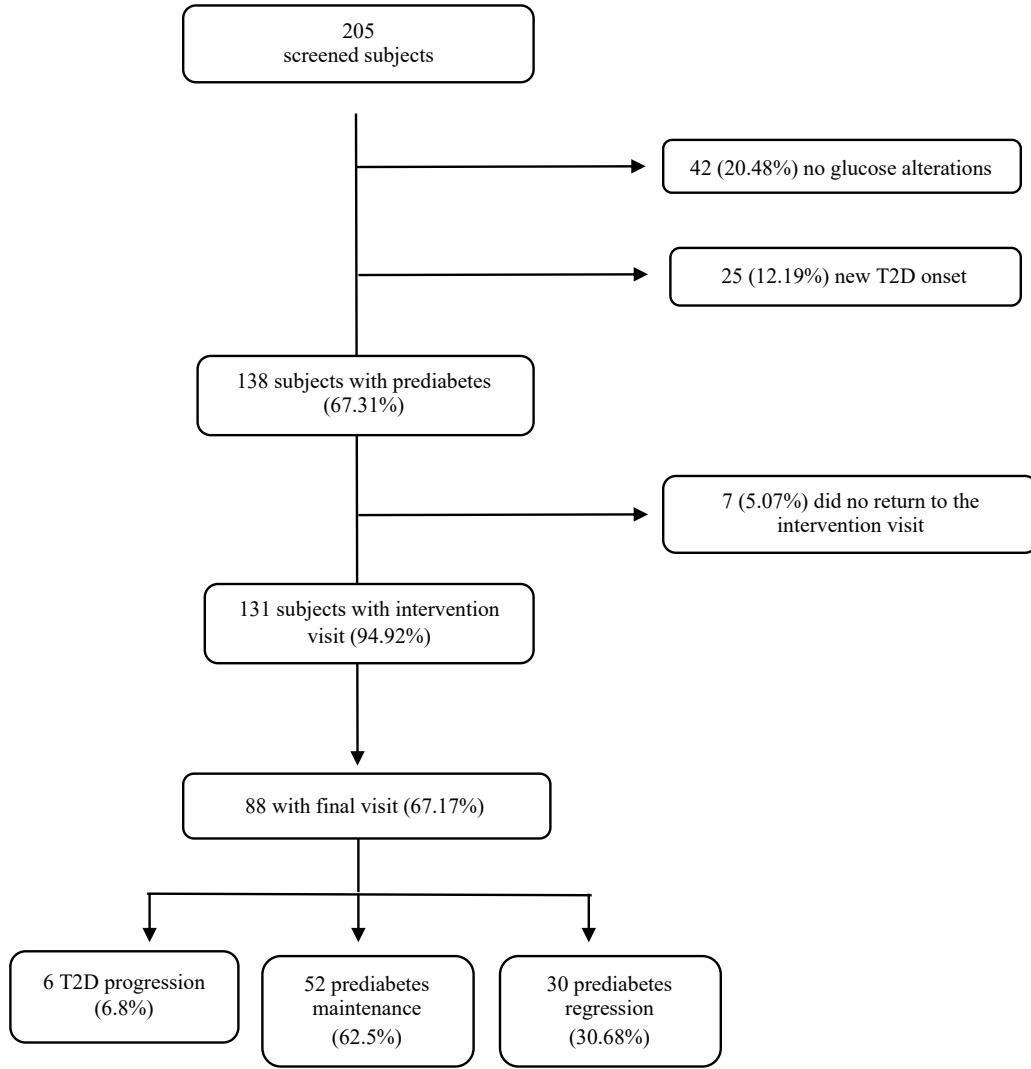


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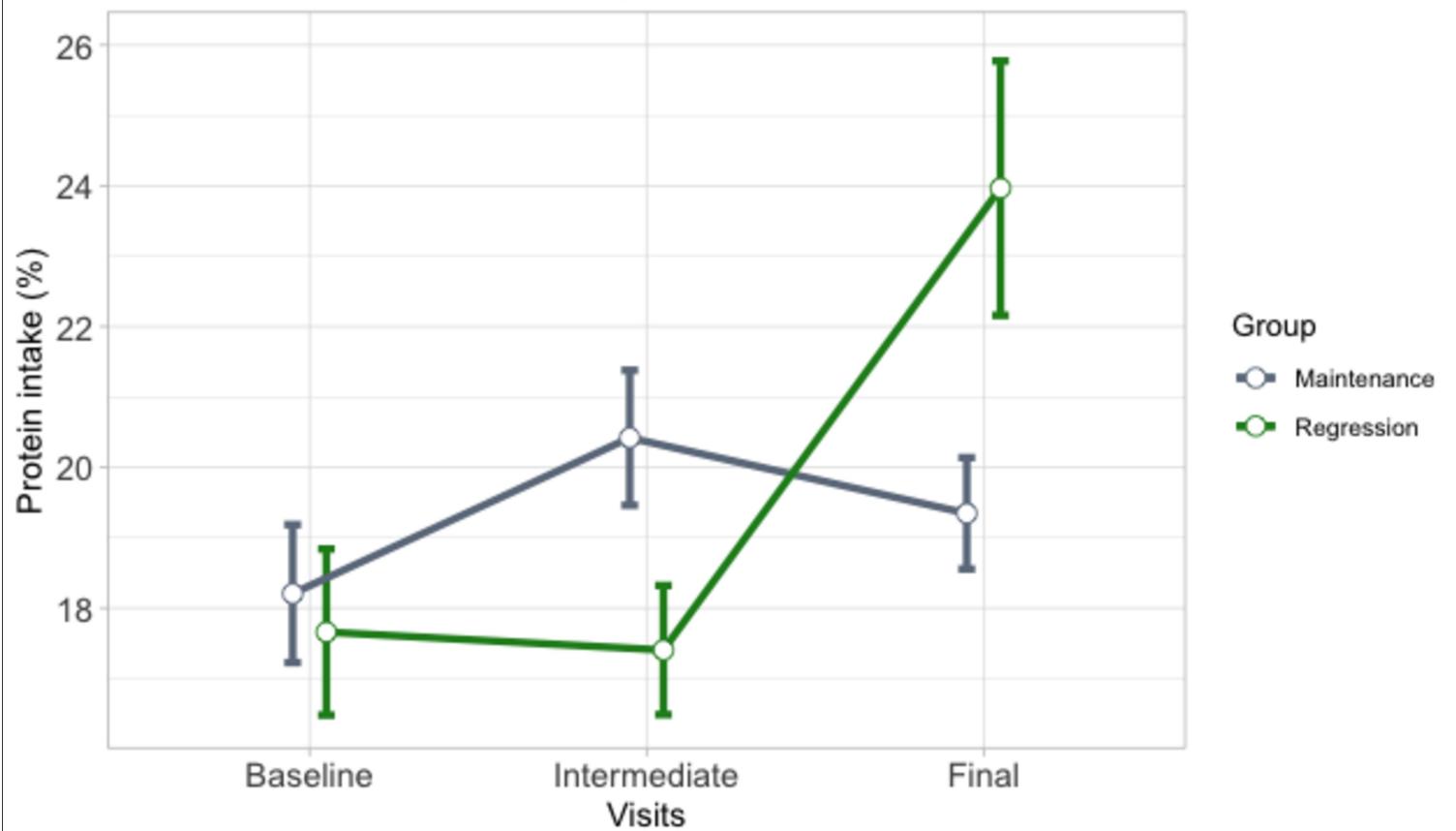
**Supplementary figure 1.** Flow chart of participants' selection process. T2D: Type 2 diabetes

**Supplementary figure 2.** Changes in protein consumption across visits

**Supplementary figure 3.** Heatmap of Metabolites selected by LASSO at least 10 times for their association with regression to normoglycemia (RGNG).



# Protein consumption across visits





**Supplementary table 1. Metabolites panel studied**

	<b>Method</b>	<b>Compound ID</b>	<b>MZ</b>	<b>RT</b>	<b>HMDB_ID</b>	<b>Metabolite</b>
<b>1</b>	HILIC-pos	QI7675	174.1363	6.85	internalstandard	phenylalanine_d8
<b>2</b>	HILIC-pos	QI4035	126.1367	7.54	internalstandard	valine_d8
<b>3</b>	HILIC-pos	QI2153	197.0669	3.75	HMDB0011103	1.7_dimethyluric_acid
<b>6</b>	HILIC-pos	QI4213	282.1193	7.82	HMDB0003331	1_methyladenosine
<b>7</b>	HILIC-pos	QI2873	166.0722	5.33	HMDB0003282	1_methylguanine
<b>8</b>	HILIC-pos	QI8555	298.1143	5.21	HMDB0001563	1_methylguanosine
<b>9</b>	HILIC-pos	QI6451	126.1027	9.89	HMDB0000898	1_methylhistamine
<b>11</b>	HILIC-pos	QI6850	137.0708	8.37	HMDB0000699	1_methylnicotinamide
<b>14</b>	HILIC-pos	QI7706	146.1174	6.8	HMDB0094649	2_aminoheptanoic_acid
<b>15</b>	HILIC-pos	TF1	104.0706	7.87	HMDB0001906	2_aminoisobutyric_acid
<b>16</b>	HILIC-pos	QI7791	160.1331	6.55	HMDB0000991	2_aminooctanoate
<b>20</b>	HILIC-pos	QI9472	196.0605	2.71	HMDB0000840	2_hydroxyhippurate
<b>23</b>	HILIC-pos	TF2	298.1146	5.38	HMDB0005862	2_methylguanosine
<b>30</b>	HILIC-pos	QI6419	170.0923	10.03	HMDB0000479	3_methylhistidine
<b>31</b>	HILIC-pos	QI9271	167.0562	3.44	HMDB0001886	3_methylxanthine
<b>34</b>	HILIC-pos	QI9260	146.081	3.53	HMDB0003681	4_acetamidobutanoate
<b>35</b>	HILIC-pos	QI1685	195.0765	2.44	HMDB0001867	4_aminohippuric_acid
<b>37</b>	HILIC-pos	QI7403	146.0921	7.45	HMDB0003464	4_guanidinobutanoic_acid
<b>42</b>	HILIC-pos	QI8464	199.0825	5.39	HMDB0004400	5_acetylaminogluturacil
<b>47</b>	HILIC-pos	QI7918	221.092	6.31	HMDB0000472	5_hydroxytryptophan
<b>52</b>	HILIC-pos	QI3176	258.1083	5.83	HMDB0000982	5_methylcytidine
<b>58</b>	HILIC-pos	QI8170	166.0722	5.89	HMDB0000897	7_methylguanine
<b>59</b>	HILIC-pos	QI2180	167.0562	3.81	HMDB0001991	7_methylxanthine
<b>62</b>	HILIC-pos	TF3	328.1027	5.49	HMDB0010316	acetaminophen glucuronide

<b>63</b>	HILIC-pos	QI7126	146.1173	7.88	HMDB000089 5	acetylcholine
<b>65</b>	HILIC-pos	QI8342	222.0971	5.59	HMDB000021 2	acetyl_galactos amine
<b>69</b>	HILIC-pos	QI8264	185.1284	5.72	HMDB000158 4	acisoga
<b>70</b>	HILIC-pos	QI8587	268.1039	5.15	HMDB000005 0	adenosine
<b>74</b>	HILIC-pos	TF4	203.1502	9.92	HMDB000155 0	ADMA
<b>78</b>	HILIC-pos	QI7103	90.055	7.94	HMDB000010 1	alanine
<b>79</b>	HILIC-pos	QI9228	159.0511	3.72	HMDB000040 2	allantoin
<b>84</b>	HILIC-pos	QI5100	258.1099	10.7	HMDB000008 6	alpha_glycerop hosphocholine
<b>93</b>	HILIC-pos	QI4787	175.1188	9.33	HMDB000051 7	arginine
<b>95</b>	HILIC-pos	QI7015	133.0608	8.05	HMDB000010 8	asparagine
<b>101</b>	HILIC-pos	QI6640	118.0864	9.11	HMDB000004 3	betaine
<b>102</b>	HILIC-pos	QI10871	585.2705	1.74	HMDB000005 4	bilirubin
<b>103</b>	HILIC-pos	QI10050	583.255	2.05	HMDB000100 9	biliverdin
<b>106</b>	HILIC-pos	QI6671	146.1174	8.91	HMDB003903 0	butyrobetaine
<b>110</b>	HILIC-pos	QI1984	195.0877	2.96	HMDB000184 7	caffeine
<b>113</b>	HILIC-pos	QI6661	162.1122	8.98	HMDB000006 2	carnitine
<b>114</b>	HILIC-pos	QI6707	204.1228	8.8	HMDB000020 1	C2_carnitine
<b>115</b>	HILIC-pos	QI6841	218.1385	8.44	HMDB000082 4	C3_carnitine
<b>117</b>	HILIC-pos	QI4626	262.1283	8.69	HMDB001313 3	C3_DC_CH3_c arnitine
<b>118</b>	HILIC-pos	QI4407	232.1542	8.12	HMDB000201 3	C4_carnitine
<b>119</b>	HILIC-pos	QI6664	248.149	8.96	HMDB001312 7	C4_OH_carniti ne
<b>120</b>	HILIC-pos	QI4248	246.1698	7.88	HMDB000068 8	C5_carnitine
<b>121</b>	HILIC-pos	QI4299	244.1541	7.98	HMDB000250 6	C5_1_carnitine
<b>122</b>	HILIC-pos	QI4544	276.1439	8.46	HMDB001313 0	C5_DC_carniti ne
<b>123</b>	HILIC-pos	QI7270	260.1854	7.66	HMDB000070 5	C6_carnitine
<b>124</b>	HILIC-pos	QI4020	274.201	7.53	HMDB001525 8	C7_carnitine
<b>125</b>	HILIC-pos	QI7402	288.2166	7.45	HMDB000079 1	C8_carnitine
<b>126</b>	HILIC-pos	QI3884	302.2322	7.31	HMDB001528 9	C9_carnitine
<b>127</b>	HILIC-pos	QI7513	316.2478	7.24	HMDB000065 1	C10_carnitine
<b>128</b>	HILIC-pos	QI7464	312.2165	7.36	HMDB001332 5	C10_2_carnitin e
<b>129</b>	HILIC-pos	QI7580	344.2791	7.05	HMDB000225 0	C12_carnitine
<b>130</b>	HILIC-pos	QI3802	342.2634	7.08	HMDB001332 6	C12_1_carnitin e
<b>131</b>	HILIC-pos	QI3736	372.3103	6.91	HMDB000300 6	C14_carnitine
<b>132</b>	HILIC-pos	QI7638	370.2947	6.91	HMDB000201 4	C14_1_carnitin e
<b>133</b>	HILIC-pos	QI7611	368.2791	6.97	HMDB001333 1	C14_2_carnitin e

<b>134</b>	HILIC-pos	QI7716	400.3417	6.79	HMDB000022 2	C16_carnitine
<b>136</b>	HILIC-pos	QI3625	428.3729	6.68	HMDB000084 8	C18_carnitine
<b>137</b>	HILIC-pos	QI3631	426.3573	6.7	HMDB000506 5	C18_1_carnitin e
<b>139</b>	HILIC-pos	QI3646	424.3417	6.77	HMDB000646 9	C18_2_carnitin e
<b>141</b>	HILIC-pos	QI3616	448.3417	6.65	HMDB000645 5	C20_4_carnitin e
<b>142</b>	HILIC-pos	QI3493	540.4989	6.37	HMDB000634 7	C26_carnitine
<b>150</b>	HILIC-pos	QI4527	104.1069	8.4	HMDB000009 7	choline
<b>152</b>	HILIC-pos	QI9486	206.0812	2.68	HMDB001162 1	cinnamoylglyci ne
<b>153</b>	HILIC-pos	QI6816	176.1029	8.49	HMDB000090 4	citrulline
<b>154</b>	HILIC-pos	QI1496	363.2162	2.07	HMDB000000 3	cortisol
<b>155</b>	HILIC-pos	QI1454	361.2006	2.06	HMDB000280 2	cortisone
<b>156</b>	HILIC-pos	QI2788	177.1021	5.16	HMDB000104 6	cotinine
<b>158</b>	HILIC-pos	QI6837	132.0768	8.45	HMDB000000 4	creatine
<b>159</b>	HILIC-pos	QI7777	114.0663	6.6	HMDB000050 2	creatinine
<b>160</b>	HILIC-pos	QI3576	100.1121	6.54	HMDB003140 4	cyclohexylamin e
<b>163</b>	HILIC-pos	QI4749	241.0309	9.19	HMDB000019 2	cystine
<b>165</b>	HILIC-pos	QI3462	112.0506	6.32	HMDB000063 0	cytosine
<b>170</b>	HILIC-pos	QI6291	287.2439	10.56	HMDB000217 2	diacetylspermin e
<b>173</b>	HILIC-pos	QI4617	104.0706	8.67	HMDB000009 2	dimethylglycin e
<b>174</b>	HILIC-pos	TF6	202.1186	8.1	HMDB024021 2	DMGV
<b>180</b>	HILIC-pos	QI6645	143.0813	9.06	NA	ectoine
<b>182</b>	HILIC-pos	TF7	104.0706	7.76	HMDB000011 2	GABA
<b>183</b>	HILIC-pos	QI3821	172.133	7.16	HMDB000501 5	gabapentin
<b>186</b>	HILIC-pos	QI7201	148.0602	7.74	HMDB000014 8	glutamate
<b>187</b>	HILIC-pos	QI4433	147.0762	8.17	HMDB000064 1	glutamine
<b>190</b>	HILIC-pos	QI4363	76.0393	8.06	HMDB000012 3	glycine
<b>191</b>	HILIC-pos	QI8458	466.3161	5.39	HMDB000015 9	glycocholate
<b>193</b>	HILIC-pos	QI2279	450.3211	4.23	HMDB000063 1	glycodeoxychol ate_glycocheno deoxycholate
<b>196</b>	HILIC-pos	QI4308	118.0613	7.99	HMDB000012 8	guanidinoacetic _acid
<b>203</b>	HILIC-pos	QI1880	180.0654	2.66	HMDB000071 4	hippurate
<b>206</b>	HILIC-pos	QI6530	156.0767	9.54	HMDB000017 7	histidine
<b>208</b>	HILIC-pos	QI4797	189.1345	9.36	HMDB000007 0	homoarginine
<b>209</b>	HILIC-pos	QI6818	190.1185	8.47	HMDB000007 9	homocitrulline
<b>210</b>	HILIC-pos	QI3895	136.0424	7.33	HMDB000074 2	homocysteine

<b>212</b>	HILIC-pos	QI2758	193.0972	5.1	HMDB000139 0	hydroxycotinin e
<b>214</b>	HILIC-pos	QI4324	132.0656	8.01	HMDB000072 5	hydroxyproline
<b>215</b>	HILIC-pos	TF8	110.027	8.13	HMDB000090 5	hypotaurine
<b>222</b>	HILIC-pos	QI8657	269.0878	5.02	HMDB000019 5	inosine
<b>224</b>	HILIC-pos	TF9	132.1019	7.21	HMDB000017 2	isoleucine
<b>227</b>	HILIC-pos	QI8481	190.0498	5.35	HMDB000071 5	kynurenic_acid
<b>230</b>	HILIC-pos	TF10	132.1019	7.08	HMDB000068 7	leucine
<b>233</b>	HILIC-pos	QI10093	324.2894	2.05	HMDB001225 2	linoleoyl_ethan olamide
<b>235</b>	HILIC-pos	QI4851	147.1126	9.56	HMDB000018 2	lysine
<b>239</b>	HILIC-pos	QI3819	150.0582	7.16	HMDB000069 6	methionine
<b>240</b>	HILIC-pos	QI6717	166.0532	8.78	HMDB000200 5	methionine_sul foxide
<b>243</b>	HILIC-pos	QI6655	141.0657	9.01	HMDB000282 0	methylimidazol eacetic_acid
<b>251</b>	HILIC-pos	QI10914	227.2005	1.72	HMDB000200 0	myristoleic_aci d
<b>252</b>	HILIC-pos	QI6423	188.1757	10.01	HMDB000127 6	N1_acetylsper midine
<b>254</b>	HILIC-pos	QI2375	153.0657	4.43	HMDB000419 3	N1_methyl_2_ pyridone_5_car boxamide
<b>255</b>	HILIC-pos	QI2967	312.1299	5.47	HMDB000482 4	N2.N2_dimeth ylguanosine
<b>256</b>	HILIC-pos	QI2352	286.1031	4.39	HMDB000592 3	N4_acetylcytidi ne
<b>258</b>	HILIC-pos	QI6453	161.1283	9.87	HMDB000203 8	N6_methyllysin e
<b>259</b>	HILIC-pos	QI6308	175.144	10.45	HMDB001328 7	N6.N6_dimeth yllysine
<b>260</b>	HILIC-pos	QI6174	189.1597	11.44	HMDB000132 5	N6.N6.N6_trim ethyllysine
<b>261</b>	HILIC-pos	QI6812	189.1233	8.5	HMDB000020 6	N6_acetyllysin e
<b>262</b>	HILIC-pos	QI2202	132.0656	3.92	HMDB000076 6	N_acetylalanin e
<b>263</b>	HILIC-pos	QI8888	176.0553	4.67	HMDB000081 2	N_acetylasparti c_acid
<b>266</b>	HILIC-pos	QI4558	198.0849	8.49	HMDB003205 5	N_acetylhistidi ne
<b>270</b>	HILIC-pos	QI4581	175.1076	8.54	HMDB000335 7	N_acetylornithi ne
<b>272</b>	HILIC-pos	QI7034	131.118	8.03	HMDB000206 4	N_acetylputres cine
<b>276</b>	HILIC-pos	QI4491	217.1294	8.29	HMDB000462 0	N_alpha_acetyl arginine
<b>277</b>	HILIC-pos	QI2280	133.0609	4.23	HMDB000002 6	N_carbamoyl_ betaAlanine

280	HILIC-pos	QI2193	123.0557	3.88	<sup>6</sup> HMDB000140	niacinamide
283	HILIC-pos	QI1731	258.2062	2.45	<sup>2</sup> HMDB001327	N_lauroylglycine
285	HILIC-pos	QI6731	130.0863	8.74	NA	N_methylproline
287	HILIC-pos	QI6533	189.1345	9.54	<sup>6</sup> HMDB002941	NMMA
292	HILIC-pos	QI4840	133.0972	9.54	<sup>4</sup> HMDB000021	ornithine
295	HILIC-pos	QI2040	220.1178	3.17	<sup>0</sup> HMDB000021	pantothenate
298	HILIC-pos	QI2534	265.1118	4.73	<sup>4</sup> HMDB000634	phenylacetylglutamine
300	HILIC-pos	TF11	166.0863	6.83	<sup>0</sup> HMDB000015	phenylalanine
305	HILIC-pos	QI4395	130.0863	8.1	<sup>0</sup> HMDB000007	pipecolic_acid
306	HILIC-pos	QI1180	286.1435	2.02	<sup>7</sup> HMDB002937	piperine
308	HILIC-pos	QI6768	173.092	8.61	<sup>8</sup> HMDB001117	pro_gly
309	HILIC-pos	QI4460	116.0708	8.23	<sup>2</sup> HMDB000010	proline
310	HILIC-pos	QI4799	144.1017	9.36	<sup>7</sup> HMDB000482	proline_betaine
311	HILIC-pos	QI2329	245.0767	4.34	<sup>7</sup> HMDB000070	pseudouridine
313	HILIC-pos	QI6515	89.1073	9.58	<sup>4</sup> HMDB000141	putrescine
319	HILIC-pos	QI2027	259.0923	3.12	<sup>4</sup> HMDB000088	ribothymidine
324	HILIC-pos	TF12	203.1502	9.82	<sup>4</sup> HMDB000335	SDMA
325	HILIC-pos	QI4205	106.0499	7.8	<sup>7</sup> HMDB000018	serine
326	HILIC-pos	QI7862	177.1021	6.4	<sup>0</sup> HMDB000025	serotonin
336	HILIC-pos	QI3402	126.0222	6.22	<sup>1</sup> HMDB000025	taurine
337	HILIC-pos	QI6460	265.1115	9.84	<sup>5</sup> HMDB000025	thiamine
338	HILIC-pos	QI4177	120.066	7.75	<sup>7</sup> HMDB000016	threonine
340	HILIC-pos	QI3152	777.6926	5.79	<sup>8</sup> HMDB000024	thyroxine
341	HILIC-pos	QI4723	138.0548	9.03	<sup>5</sup> HMDB000087	trigonelline
343	HILIC-pos	QI6911	76.0757	8.23	<sup>5</sup> HMDB000092	trimethylamine_N_oxide
344	HILIC-pos	QI716	121.1016	1.8	<sup>3</sup> HMDB001373	trimethylbenzene
347	HILIC-pos	TF13	205.0972	6.63	<sup>0</sup> HMDB000092	tryptophan
349	HILIC-pos	TF14	182.0812	6.94	<sup>8</sup> HMDB000015	tyrosine
350	HILIC-pos	QI2362	169.0354	4.41	<sup>0</sup> HMDB000028	urate
353	HILIC-pos	QI2452	139.05	4.61	<sup>1</sup> HMDB000030	urocanic_acid
354	HILIC-pos	TF15	118.0863	7.53	<sup>3</sup> HMDB000088	valine
355	HILIC-pos	QI1391	436.2328	2.06	<sup>3</sup> HMDB001452	valsartan
357	HILIC-pos	QI328	269.2262	1.61	<sup>5</sup> HMDB000030	vitamin_A
359	HILIC-pos	QI9174	153.0406	3.91	<sup>2</sup> HMDB000029	xanthine
361	HILIC-pos	QI2348	285.0794	4.38	<sup>0</sup> HMDB000029	xanthosine
364	HILIC-pos	QI7037	468.3082	8.03	<sup>0</sup> HMDB001037	LPC_14_0
365	HILIC-pos	QI7091	494.3241	7.95	<sup>3</sup> HMDB001038	LPC_16_1
366	HILIC-pos	QI4252	496.3395	7.89	<sup>2</sup> HMDB001038	LPC_16_0

372	HILIC-pos	QI7118	518.322	7.91	HMDB001038 7	LPC_18_3
375	HILIC-pos	QI7129	520.3397	7.87	HMDB001038 6	LPC_18_2
377	HILIC-pos	QI7161	522.3554	7.82	HMDB000281 5	LPC_18_1
381	HILIC-pos	QI4189	524.3711	7.77	HMDB001038 4	LPC_18_0
383	HILIC-pos	QI7131	542.3221	7.87	HMDB001039 7	LPC_20_5
384	HILIC-pos	QI7169	544.3396	7.8	HMDB001039 5	LPC_20_4
387	HILIC-pos	QI7282	546.354	7.64	HMDB001039 4	LPC_20_3
389	HILIC-pos	QI7260	550.3871	7.67	HMDB001039 1	LPC_20_1
390	HILIC-pos	QI7193	568.3391	7.76	HMDB001040 4	LPC_22_6
391	HILIC-pos	QI4190	570.3552	7.77	HMDB001040 3	LPC_22_5
394	HILIC-pos	QI7200	480.3446	7.74	HMDB001040 7	LPC_P_16_0_
						LPC_O_16_1
395	HILIC-pos	QI7102	508.376	7.94	HMDB001312 2	LPC_P_18_0_
						LPC_O_18_1
401	HILIC-pos	QI7855	454.2926	6.42	HMDB001150 3	LPE_16_0
403	HILIC-pos	QI7849	476.2763	6.43	HMDB001147 8	LPE_18_3
404	HILIC-pos	QI3516	478.2926	6.41	HMDB001150 7	LPE_18_2
405	HILIC-pos	QI3499	480.3083	6.37	HMDB001150 6	LPE_18_1
407	HILIC-pos	QI7893	482.3239	6.35	HMDB001151 0	LPE_18_0
409	HILIC-pos	QI3484	502.2927	6.36	HMDB001151 7	LPE_20_4
410	HILIC-pos	QI7128	508.3398	7.87	HMDB001151 2	LPE_20_1
411	HILIC-pos	QI7153	510.3554	7.83	HMDB001151 1	LPE_20_0
412	HILIC-pos	QI7902	526.293	6.33	HMDB001152 6	LPE_22_6
413	HILIC-pos	QI7703	706.5372	6.81	HMDB000780 0	PC_30_0
414	HILIC-pos	QI3651	730.5374	6.79	HMDB000787 4	PC_32_2
415	HILIC-pos	QI3628	754.5366	6.69	HMDB000788 3	PC_34_4
416	HILIC-pos	QI7732	756.5524	6.76	HMDB000800 6	PC_34_3
422	HILIC-pos	TF5	786.5984	6.68	HMDB000805 0	PC_36_2
423	HILIC-pos	QI3624	742.5739	6.68	HMDB001121 1	PC_P_34_2_P
						C_O_34_3
424	HILIC-pos	QI7781	766.573	6.59	HMDB001122 0	PC_P_36_4_P
						C_O_36_5
425	HILIC-pos	QI3585	790.5733	6.57	HMDB001122 9	PC_P_38_6_P
						C_O_38_7
426	HILIC-pos	QI3584	792.5889	6.56	HMDB001131 9	PC_P_38_5_P
						C_O_38_6
427	HILIC-pos	QI3059	716.5217	5.64	HMDB000892 8	PE_34_2
428	HILIC-pos	QI3658	720.5532	6.79	HMDB000892 5	PE_34_0
429	HILIC-pos	QI8353	740.5219	5.58	HMDB000893 7	PE_36_4
430	HILIC-pos	QI3037	744.5533	5.61	HMDB000899 4	PE_36_2
431	HILIC-pos	QI8361	764.521	5.56	HMDB000910 2	PE_38_6
432	HILIC-pos	QI8370	768.5524	5.54	HMDB000900 3	PE_38_4
433	HILIC-pos	QI2995	792.5527	5.52	HMDB000901 9	PE_40_6

<b>434</b>	HILIC-pos	QI3031	700.5266	5.59	HMDB001134 3	PE_P_34_2_P E_O_34_3
<b>435</b>	HILIC-pos	QI3029	702.5433	5.59	HMDB000895 2	PE_P_34_1_P E_O_34_2
<b>436</b>	HILIC-pos	QI8379	724.5268	5.52	HMDB001141 0	PE_P_36_4_P E_O_36_5
<b>437</b>	HILIC-pos	QI3015	728.5586	5.56	HMDB001144 1	PE_P_36_2_P E_O_36_3
<b>438</b>	HILIC-pos	QI8386	748.526	5.5	HMDB001142 0	PE_P_38_6_P E_O_38_7
<b>439</b>	HILIC-pos	QI2983	750.5417	5.5	HMDB001138 7	PE_P_38_5_P E_O_38_6
<b>441</b>	HILIC-pos	QI8393	752.5574	5.49	HMDB001138 6	PE_P_38_4_P E_O_38_5
<b>443</b>	HILIC-pos	QI2966	776.5578	5.47	HMDB001139 4	PE_P_40_6_P E_O_40_7
<b>444</b>	HILIC-pos	QI3692	772.5472	6.83	NA	PS_P_36_2_PS _O_36_3
<b>445</b>	HILIC-pos	QI7669	774.5632	6.86	NA	PS_P_36_1_PS _O_36_2
<b>446</b>	HILIC-pos	QI3909	675.5423	7.36	HMDB001209 7	SM_d18_1_14 _0
<b>447</b>	HILIC-pos	QI3891	701.5583	7.33	HMDB024061 3	SM_d18_1_16 _1
<b>449</b>	HILIC-pos	QI7488	703.5733	7.31	HMDB001016 9	SM_d18_1_16 _0
<b>453</b>	HILIC-pos	QI7508	729.5897	7.25	HMDB001210 1	SM_d18_1_18 _1
<b>454</b>	HILIC-pos	QI3856	731.6054	7.24	HMDB000134 8	SM_d18_1_18 _0
<b>456</b>	HILIC-pos	QI7554	759.636	7.16	HMDB001210 2	SM_d18_1_20 _0
<b>457</b>	HILIC-pos	QI7567	785.6517	7.12	HMDB001210 4	SM_d18_1_22 _1
<b>458</b>	HILIC-pos	QI3807	787.6675	7.12	HMDB001210 3	SM_d18_1_22 _0
<b>462</b>	HILIC-pos	QI8162	300.2893	5.9	HMDB000025 2	sphingosine
<b>465</b>	HILIC-pos	QI8149	302.305	5.92	HMDB000026 0	sphinganine
<b>466</b>	HILIC-pos	QI963	538.5196	1.92	HMDB000494 9	Cer_d18_1_16 _0
<b>467</b>	HILIC-pos	QI867	648.6285	1.87	HMDB000495 3	Cer_d18_1_24 _1
<b>469</b>	HILIC-pos	QI11015	612.5558	1.64	HMDB000710 2	C34_1_DAG_o r_TAG_NH4_a dduct

**Supplementary table 2.** Surrogat

	<b>Overall</b>	<b>Prediabetes maintenance (PDM)</b>
	<b>(n=82)</b>	<b>(n=52)</b>
Matsuda index (ISI)	0.91 (0.52 – 1.41)	0.95 (0.55 -1.38)
OGIS	381.45 ± 79.01	384.66 + 84.77
Oral disposition index	0.11 (0.06- 0.20)	0.11 (0.05 -0.15)
Early insulin response 30'	1.06 (0.53 -1.61)	0.92 (0.49 -1.49)
HOMA-IR	2.15 (1.50 -3.48)	1.95 (1.40 -3.00)
HOMA-B	29.14 (19.79 -43.75)	29.31 (19.24 – 36.67)
tAUC glucose	24351.45 ± 4459	24637.50 ± 4386.86
iAUC glucose	1413.04 ± 1845	1479.18 ± 1823.80
tAUC insulin	11707.5 (7215.00 - 18720.00)	10528.5 (6891 – 18795)
iAUC insulin	9511.5 (6304 – 16020)	8982 (5955 – 15157)

Data are presented as mean and standard deviation, and median and interquartile range according to the Curve. Differences between the two groups were computed with Chi2 for qualitative variables.

tes of insulin secretion and sensitivity

Prediabetes regression	<i>p</i> value	<i>P</i> value adjusted by age, sex, age <sup>2</sup> and BMI
<b>(n=30)</b>		
0.78 (0.52 – 1.41)	0.564	0.414
375.71 ± 68.63	0.757	0.879
0.11 (0.08 -0.25)	0.174	0.131
1.36 (0.74 -2.34)	0.047	0.338
2.40 (1.52 -3.68)	0.378	0.281
28.87 (21.38 -46.68)	0.488	0.281
24329 ± 4688.62	0.646	0.844
1283.40 ± 1917.49	0.37	0.645
13382.25 (8683 – 18432)	0.362	0.504
11436 (7113.38 – 16399)	0.393	0.552

o distribution. HOMA-IR. Homeostasis Model Assessment. TG. Triglycerides. AUC. Area Under the curve. *p*-value, t-test for parametric variables, and Wilcoxon for non-parametric variables.

Supplementary Table 3. Changes in metabolic par

	<b>Overall (N=82)</b>	<b>Prediabetes Maintenance (PDM) (n=52)</b>
△		
Fasting glucose (mg/dL)	4.00 (-9.00 - 2.00)	-2.00 (-6.00 - 3.00)
△		
2h glucose(mg/dL)	23.32	26.32
△		
Hba1c %	-0.01	0.06
△ Fasting insulin		
	-1.15 (-3.55 – 1.65)	-0.95 (-2.55 – 1.55)
△ ODI		
	0.01 (-0.07 – 0.06)	0.01 (-0.04 -0.07)

$\triangle$  OGIS $21.05 \pm 80.11$        $23.44 \pm 81.49$ 

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 $\triangle$ 

## Weight (kg)

1.01      0.79

---

 $\triangle$ 

## Waist circumference (cm)

-10.12      4.5

---

 $\triangle$ 

## Body fat mass

-0.76 (-2.3 – 0.49)      -0.80 (-2.45 - 0.5)

---

 $\triangle$ 

## Free fat mass index

-0.2 (-0.54 - 0.12)      -0.12 (0.47 – 0.17)

---

 $\triangle$ VAT ( $\text{cm}^3$ )

-56.50 (-204.25 - 90.50)      -54 (-224 – 106)

---

 $\Delta$ 

Energy intake (kcal)	-288.06 (-1043.70 - 241.88)	-223.39 326.15)	(-994.37)	-
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 $\Delta$ 

Fat consumption (%)	13.13	13.31
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 $\Delta$ 

Protein consumption (%)	4.70 (-1.60- 8.32)	1.20 (-2.40 - 6.90)
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 $\Delta$ 

Carbohydrate consumption	14.17	16.46
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 $\Delta$  (%)

Sugar daily consumption	-6.75 (-48.24 - 18.18)	0.00 (-42.67 – 21.44)
-------------------------	------------------------	-----------------------

(gr)  
 HbA1c. Hemoglobin A1c. ODI. Oral disposition index. OGIS. Oral glucose insulin sensit and standard deviation or median and interquartile range according to distribution. D difference between baseline vs post-intervention P2 value was computed comparing I adjusting by age, sex, and BMI

ameters after 24 weeks of treatment

**Regression to normal  
glucose tolerance (RNGT) P value 1 P value 2**

**(n=30)**

-6.50 (-9.75 - -1.00) 0.0006\* 0.101

18.04 0.062 0.487

-0.17 0.00001\* <0.001\*

-2.20 (-4.25 – 2.25) 0.522 0.475

-0.01 (-0.12 – 0.04) 0.778 0.114

16.79 ± 78.89                  0.022\*                  0.742

---

1.45                  0.00001\*                  0.327

---

-12.3                  0.0016\*                  0.542

---

-0.70 (-2.0 – 0.37)                  0.055                  0.859

---

-0.32 (-0.57 - -0.02)                  0.585                  0.34

---

-66 (-171.5 – 49.5)                  0.493                  0.99

---

-599.28 (-1139.65 – 116.13) 0.003\* 0.1

---

13.04 0.484 0.74

---

5.65 (0.67 – 9.43) 0.002\* 0.027\*

---

9.56 0.32 0.175

---

-23.21 (-63.50 - 2.05) 0.108 0.118

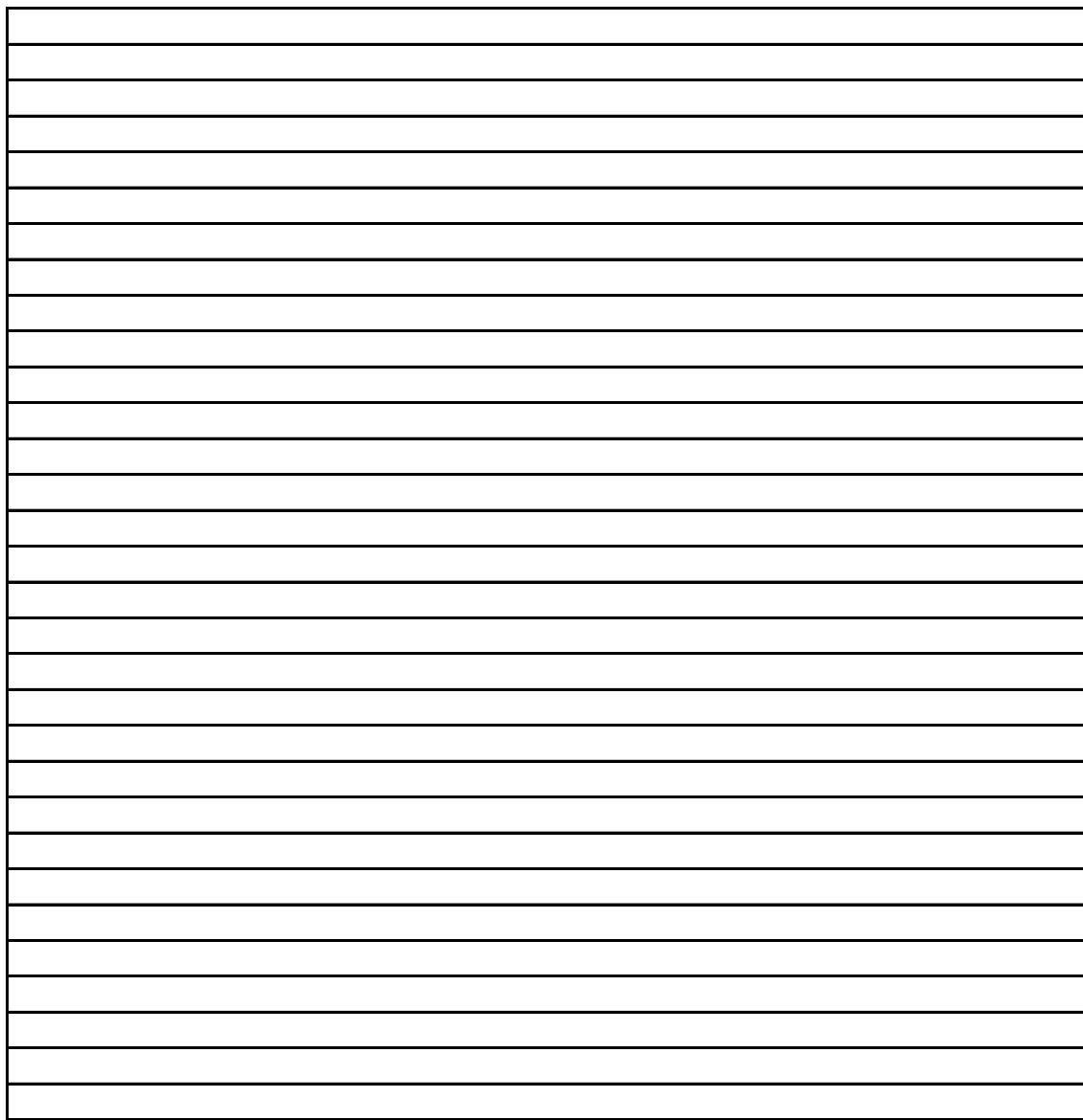
TIVITY INDEX. VAT. VISCEAL ADIPOSE TISSUE. Data are presented as mean  
delta was computed final visit results/baseline visit. P1 represents the  
prediabetes maintenance and regression to normal tolerance

Supplementary table 4. Metabolites selected at least 10 times at baseline for the analysis.

Metabolites	times selected			
PS.P.36.1.PS.O.36.2	15			
C5.1.carnitine	11			
Cer.d18.1.24.1	10			
phenylacetylglutamine	9			
myristoleic.acid	9			
adenosine	8			
cyclohexylamine	7			
X7.methylxanthine	6			
PE.P.40.6.PE.O.40.7	6			
thyroxine	5			
Sd18.1.22.0	5			
piperine	4			
N.acetylhistidine	4			
ectoine	4			
X4.acetamidobutanoate	3			
X1.7.dimethyluric.acid	3			
riboflavin	3			
PC.P.38.6.PC.O.38.7	3			
C6.carnitine	3			
C12.1.carnitine	3			
betaine	3			
arginine	3			
acetylcholine	3			
X5.acetylamino.6.amino.3.methyluracil	2			
vitamin.A	2			
trimethylamine.N.oxide	2			
Sd18.1.18.0	2			
PE.P.38.4.PE.O.38.5	2			
PC.P.38.5.PC.O.38.6	2			
LPE.20.0	2			
hydroxyproline	2			
cortisol	2			
citrulline	2			
Cer.d18.1.16.0	2			
C4.carnitine	2			
C12.carnitine	2			
alpha.glycerophosphocholine	2			
X2.hydroxyhippurate	1			
X2.aminooctanoate	1			
X2.aminoheptanoic.acid	1			
valsartan	1			

urocanic.acid	1			
trimethylbenzene	1			
sphinganine	1			
Sd18.1.18.1	1			
Sd18.1.16.0	1			
PS.P.36.2.PS.O.36.3	1			
proline.betaine	1			
pro.gly	1			
N6.acetyllysine	1			
N4.acetylcytidine	1			
N1.methyl.2.pyridone.5.carboxamide	1			
N.methylproline	1			
N.carbamoyl.beta.alanine	1			
N.acetylaspartic.acid	1			
methylimidazoleacetic.acid	1			
LPE.18.0	1			
LPC.P.18.0.LPC.O.18.1	1			
LPC.18.0	1			
homocitrulline	1			
glycodeoxycholate.glycochenodeoxycholate	1			
glycine	1			
glutamate	1			
gabapentin	1			
GABA	1			
cystine	1			
creatinine	1			
creatine	1			
cinnamoylglycine	1			
C5.DC.carnitine	1			
biliverdin	1			
xanthosine	1			

ieir association with regression to normoglycemia



Supplementary table 5. Metabolites (fold change) selected their association

Metabolites	times selecte	HMDB
vitamin.A	100	HMDB0000305
Cer.d18.1.16.0	54	HMDB0004949
X1.7.dimethyluric.acid	47	HMDB0011103
Sd18.1.22.1	41	HMDB0012104
PC.P.38.5.PC.O.38.6	39	HMDB0011387
Sd18.1.14.0	38	HMDB0012097
LPC.18.3	37	HMDB0010387
acetyl.galactosamine	35	HMDB0000212
trigonelline	34	HMDB0000875
tryptophan	34	HMDB0000929
GABA	27	HMDB0000112
allantoin	26	HMDB0000462
N.carbamoyl.beta.alanine	26	HMDB0000026
carnitine	25	HMDB0000062
X1.methylhistamine	22	HMDB0000898
C18.carnitine	21	HMDB0000848
LPC.18.1	21	HMDB0002815
N6.N6.N6.trimethyllysine	21	HMDB0001325
Sd18.1.20.0	21	HMDB0012102
C10.2.carnitine	20	HMDB0000651
N6.methyllysine	20	HMDB0002038
LPC.18.0	19	HMDB0010384
X2.methylguanosine	19	HMDB0005862
C5.DC.carnitine	18	HMDB0013130
X7.methylguanine	17	HMDB0000897
LPC.P.16.0.LPC.O.16.1	16	HMDB0010382
thyroxine	16	HMDB0000248
C4.OH.carnitine	14	HMDB0013127
PE.40.6	14	HMDB0009012
putrescine	14	HMDB0001414
creatine	13	HMDB0000064
LPC.14.0	12	HMDB0010379
LPC.16.0	12	HMDB0010382
N.acetylalanine	12	HMDB0000766
alpha.glycerophosphocholine	11	HMDB0000086
PE.P.40.6.PE.O.40.7	11	HMDB0011394
C3.carnitine	10	HMDB0000824
glutamine	10	HMDB0000641
hydroxyproline	10	HMDB0000725
PE.P.36.2.PE.O.36.3	10	HMDB0011343
C12.1.carnitine	9	

C18.1.carnitine	9		
C18.2.carnitine	9		
N.acetyltornithine	9		
N.acetylputrescine	9		
PC.34.3	9		
PS.P.36.1.PS.O.36.2	9		
acisoga	8		
C14.2.carnitine	8		
LPE.20.4	8		
N1.methyl.2.pyridone.5.carboxamide	8		
PE.36.2	8		
urate	8		
citrulline	7		
dimethylglycine	7		
methionine.sulfoxide	7		
methylimidazoleacetic.acid	7		
N.acetylaspartic.acid	7		
NMMA	7		
cystine	6		
gabapentin	6		
PC.32.2	6		
PC.P.36.4.PC.O.36.5	6		
PE.P.38.5.PE.O.38.6	6		
trimethylamine.N.oxide	6		
X2.hydroxyhippurate	6		
adenosine	5		
arginine	5		
bilirubin	5		
guanidinoacetic.acid	5		
homocysteine	5		
PC.P.34.2.PC.O.34.3	5		
X4.aminohippuric.acid	5		
X5.acetylamino.6.amino.3.methyluracil	5		
X5.methylcytidine	5		
X7.methylxanthine	5		
C20.4.carnitine	4		
LPC.18.2	4		
LPC.22.5	4		
LPE.18.3	4		
N.lauroylglycine	4		
PE.38.4	4		
pipecolic.acid	4		
SDMA	4		

sphingosine	4		
threonine	4		
butyrobetaine	3		
C16.carnitine	3		
C6.carnitine	3		
caffeine	3		
cinnamoylglycine	3		
cortisol	3		
cyclohexylamine	3		
diacetylspermine	3		
DMGV	3		
glycine	3		
isoleucine	3		
LPC.20.3	3		
LPE.18.1	3		
LPE.20.0	3		
myristoleic.acid	3		
ornithine	3		
PC.30.0	3		
PC.34.4	3		
PE.P.38.4.PE.O.38.5	3		
phenylacetylglutamine	3		
pro.gly	3		
Sd18.1.18.1	3		
serotonin	3		
trimethylbenzene	3		
X3.methylxanthine	3		
betaine	2		
C7.carnitine	2		
cytosine	2		
ectoine	2		
hydroxycotinine	2		
kynurenic.acid	2		
leucine	2		
LPC.16.1	2		
methionine	2		
PC.36.2	2		
PE.P.38.6.PE.O.38.7	2		
pseudouridine	2		
Sd18.1.16.0	2		
Sd18.1.18.0	2		
Sd18.1.22.0	2		
thiamine	2		

urocanic.acid	2		
valsartan	2		
X2.aminoheptanoic.acid	2		
acetylcholine	1		
asparagine	1		
C10.carnitine	1		
C12.carnitine	1		
C26.carnitine	1		
C4.carnitine	1		
C5.carnitine	1		
choline	1		
glycocholate	1		
glycodeoxycholate.glycochenodeoxycholate	1		
id_number.xpred-141	1		
id_number.xpred-151	1		
id_number.xpred-172	1		
linoleoyl.ethanolamide	1		
LPC.20.1	1		
LPC.20.4	1		
LPC.22.6	1		
lysine	1		
N6.acetylysine	1		
phenylalanine	1		
piperine	1		
proline.betaine	1		
PS.P.36.2.PS.O.36.3	1		
Sd18.1.16.1	1		
serine	1		
taurine	1		
valine	1		
X2.aminooctanoate	1		
X5.hydroxytryptophan	1		
xanthine	1		



Supplementary table 6. Metabolites

	logFC	t	P.Value	adj.P.Val
cystine	0.56118248	4.77221763	6.52E-06	0.00140743
hypotaurine	0.48828891	4.46364171	2.20E-05	0.00237235
sphinganine	0.64354987	4.25049618	4.95E-05	0.0035622
C14.carnitine	-0.4657375	-3.7291174	0.00032516	0.01344287
PS.P.36.2.PS.O.36.3	-0.3024346	-3.717713	0.00033824	0.01344287
inosine	-0.4511111	-3.6468866	0.00043139	0.01344287
taurine	0.45433261	3.64400476	0.00043565	0.01344287
thyroxine	-0.3533607	-3.5358338	0.00062784	0.01695174
myristoleic.acid	-0.2996608	-3.4909908	0.00072901	0.01749613
X5.hydroxytryptophan	0.46336451	3.39322308	0.00100529	0.02027298
serotonin	0.46188273	3.38503854	0.00103242	0.02027298
glycine	0.25241144	3.20554411	0.00183138	0.02987869
pro.gly	0.47810208	3.20113902	0.00185683	0.02987869
niacinamide	-0.4422621	-3.1876935	0.00193658	0.02987869
pantothenate	-0.3319995	-2.9743375	0.00371403	0.05348201
Sd18.1.18.0	-0.3143789	-2.9312361	0.00422043	0.05697575
N.acetylputrescine	-0.282156	-2.8686727	0.00506929	0.06234547
LPC.P.16.0.LPC.O.16.1	0.2857317	2.84691281	0.00539951	0.06234547
PS.P.36.1.PS.O.36.2	-0.3150078	-2.8415356	0.00548409	0.06234547
C2.carnitine	-0.3490149	-2.7804263	0.00653444	0.0705719
C16.carnitine	-0.384517	-2.7581464	0.00696101	0.07159898
hippurate	0.3050117	2.65520279	0.009281	0.09112258
LPC.20.1	0.28787271	2.61565566	0.01034465	0.09714976
PE.40.6	-0.2033433	-2.5630324	0.0119306	0.10737538
X1.methyladenosine	-0.2581428	-2.5321417	0.01296052	0.11197888
LPC.18.2	0.29400911	2.48404863	0.01472349	0.1223182
caffeine	-0.2624567	-2.455292	0.01587744	0.1243893
LPC.18.1	0.27483372	2.44486538	0.0163154	0.1243893
X1.methylnicotinamide	-0.3300825	-2.4359033	0.01670041	0.1243893
C6.carnitine	-0.2902056	-2.4053435	0.01807492	0.12564457
X7.methylguanine	-0.2183316	-2.3963808	0.01849669	0.12564457
xanthine	-0.3056972	-2.3939201	0.01861401	0.12564457
N2.N2.dimethylguanosine	-0.240678	-2.3740194	0.01958735	0.12820814
bilirubin	0.28852147	2.34437845	0.02112083	0.13417942
N.lauroylglycine	-0.2474167	-2.3205182	0.02243146	0.13538592
PC.30.0	-0.2849873	-2.3181688	0.02256432	0.13538592
C7.carnitine	-0.2746426	-2.2813599	0.02473838	0.14340302
PE.38.6	-0.1884646	-2.2734601	0.02522831	0.14340302
LPC.20.4	0.20820304	2.21821584	0.02889904	0.15739536
LPE.22.6	-0.2626752	-2.2121855	0.02932672	0.15739536
Sd18.1.18.1	-0.2218476	-2.2045525	0.02987597	0.15739536

asparagine	0.24727463	2.1610062	0.03318441	0.17066267
Sd18.1.14.0	-0.2116682	-2.150748	0.03400862	0.17070407
trimethylbenzene	-0.2024874	-2.1271242	0.0359746	0.17070407
acetyl.galactosamine	-0.2064157	-2.1269212	0.03599191	0.17070407
urate	-0.2263797	-2.1226986	0.03635364	0.17070407
C4.OH.carnitine	-0.262321	-2.0991318	0.03843087	0.17661845
LPE.18.0	0.24266942	2.03156136	0.04496369	0.1977704
LPC.P.18.0.LPC.O.18.1	0.23261494	2.02494741	0.04565151	0.1977704
Sd18.1.16.1	-0.1787309	-2.0150746	0.04669497	0.1977704
biliverdin	-0.2132152	-2.0150669	0.04669579	0.1977704
N.acetylalanine	-0.2036638	-1.9673194	0.05203456	0.21614355
N1.methyl.2.pyridone.5.carboxamide	-0.2460852	-1.9568056	0.0532774	0.21713055
pseudouridine	-0.1694091	-1.8956703	0.06101356	0.24405426
cinnamoylglycine	0.21596527	1.8665038	0.06502522	0.25537179
LPE.18.1	0.23463696	1.835859	0.06947594	0.26684646
N.methylproline	-0.2495608	-1.8160875	0.07248018	0.26684646
C18.1.carnitine	-0.2507949	-1.8141492	0.07278043	0.26684646
choline	-0.1952357	-1.8134524	0.07288862	0.26684646
methionine.sulfoxide	0.20023337	1.80064219	0.07490154	0.26964554
C14.1.carnitine	-0.2393701	-1.7605009	0.08150971	0.28862454
cytosine	-0.2132408	-1.7170024	0.08920549	0.30756454
Sd18.1.22.0	-0.1836851	-1.6988038	0.09259696	0.30756454
LPC.20.3	0.21075494	1.69379012	0.09354957	0.30756454
arginine	0.18215843	1.69174408	0.0939406	0.30756454
acisoga	-0.1923304	-1.6915485	0.09397806	0.30756454
N4.acetylcytidine	-0.1411256	-1.6492371	0.10236983	0.33002811
LPC.20.5	0.19598025	1.62789971	0.10682597	0.33682481
Sd18.1.20.0	-0.166945	-1.6171259	0.1091346	0.33682481
LPC.18.0	0.1850398	1.61113813	0.1104349	0.33682481
PE.34.0	-0.1959091	-1.6098531	0.11071556	0.33682481
LPE.20.0	0.19708051	1.59568843	0.11384738	0.34154215
GABA	-0.1796392	-1.5645624	0.12097825	0.35405856
alanine	0.20209089	1.55716228	0.12272476	0.35405856
PE.P.38.6.PE.O.38.7	-0.1855823	-1.5562687	0.122937	0.35405856
PC.P.34.2.PC.O.34.3	0.1431997	1.54314051	0.1260889	0.35835794
citrulline	0.16638177	1.51293224	0.13358422	0.37195452
piperine	-0.1931699	-1.4936661	0.13854457	0.37195452
C3.DC.CH3.carnitine	-0.1292942	-1.492119	0.13894908	0.37195452
X4.acetamidobutanoate	-0.1483395	-1.4893247	0.13968201	0.37195452
PC.32.2	-0.1791517	-1.4890094	0.13976489	0.37195452
X1.methylguanosine	-0.1949676	-1.4835548	0.14120496	0.37195452
C12.carnitine	-0.2001572	-1.4406232	0.15294685	0.39803035
N.acetylaspartic.acid	0.20717165	1.42497748	0.15740883	0.40476555

PC.P.36.4.PC.O.36.5	0.15538663	1.41194544	0.16120135	0.40964107
X3.methylxanthine	-0.1441937	-1.3955431	0.16607383	0.4165119
X1.7.dimethyluric.acid	-0.1437281	-1.3899469	0.16776174	0.4165119
PE.34.2	-0.1253309	-1.3779478	0.17142492	0.42077026
C5.1.carnitine	-0.1433486	-1.3598233	0.17707303	0.42682154
X2.aminoisobutyric.acid	-0.1292841	-1.3480638	0.18081223	0.42682154
N6.acetylysine	-0.1349063	-1.3414348	0.18294616	0.42682154
C12.1.carnitine	-0.177118	-1.33837	0.18393909	0.42682154
X1.methylhistamine	0.1425841	1.3357421	0.18479375	0.42682154
diacetylspermine	-0.1365789	-1.3328235	0.18574641	0.42682154
N1.acetylspermidine	-0.1577492	-1.2836652	0.20235092	0.46008209
PE.P.36.4.PE.O.36.5	0.15434071	1.26249791	0.20982988	0.47211723
carnitine	-0.1151537	-1.2377859	0.21881614	0.4851729
N6.N6.N6.trimethyllysine	-0.1529901	-1.2342495	0.22012474	0.4851729
creatine.y	-0.0977873	-1.1937171	0.23553202	0.50622427
linoleoyl.ethanolamide	-0.1397724	-1.1921272	0.23615181	0.50622427
valine	-0.1251492	-1.184878	0.23899257	0.50622427
proline.betaine	-0.1640154	-1.1819584	0.24014356	0.50622427
Sd18.1.16.0	-0.1307243	-1.1787979	0.24139398	0.50622427
PC.34.3	-0.1308011	-1.1408267	0.25678184	0.53331614
phenylalanine	-0.1078506	-1.1279185	0.26216722	0.53745072
PE.36.4	-0.0920375	-1.1241626	0.26374897	0.53745072
glycodeoxycholate.glycochenodeoxycholate	0.11946815	1.10981742	0.26985176	0.54474748
C14.2.carnitine	-0.1442392	-1.0854177	0.2804566	0.55646221
phenylacetylglutamine	0.12447519	1.08039632	0.28267423	0.55646221
PE.P.38.4.PE.O.38.5	0.11412076	1.07879598	0.28338353	0.55646221
LPC.22.6	-0.1192407	-1.0649183	0.28958577	0.56351826
cotinine	-0.0892225	-1.0541132	0.2944786	0.56792301
LPC.22.5	-0.1066787	-1.0378486	0.30194928	0.57717739
C5.carnitine	-0.1204617	-1.0298695	0.30566066	0.57734658
X7.methylxanthine	-0.1203289	-1.0261876	0.3073836	0.57734658
histidine	0.10773818	1.00082301	0.31943001	0.59389126
Sd18.1.22.1	-0.108598	-0.995882	0.32181269	0.59389126
cortisol	-0.1175134	-0.9904605	0.32444406	0.59389126
PC.34.4	-0.103478	-0.9743653	0.3323256	0.60295973
isoleucine	-0.1027244	-0.9598271	0.33955499	0.60295973
proline	0.09017489	0.95376739	0.34259835	0.60295973
hydroxyproline	0.12230937	0.95104704	0.34397031	0.60295973
lysine	-0.1039932	-0.9477849	0.34562022	0.60295973
C20.4.carnitine	0.10023013	0.94143505	0.34884648	0.60295973
ribothymidine	-0.0946853	-0.9383427	0.35042469	0.60295973
adenosine	-0.1251681	-0.9357986	0.35172651	0.60295973
serine	0.09216425	0.92300437	0.35832046	0.60942692

sphingosine	0.12372552	0.91038513	0.36490108	0.61044853
PE.P.38.5.PE.O.38.6	0.11209848	0.90831546	0.36598763	0.61044853
LPE.16.0	0.10984132	0.89859405	0.37111867	0.61044853
C3.carnitine	0.08530714	0.89115574	0.37507514	0.61044853
butyrobetaine	-0.0865815	-0.8883809	0.37655783	0.61044853
C18.2.carnitine	-0.1142673	-0.887757	0.3768917	0.61044853
X4.guanidinobutanoic.acid	0.11337544	0.88437618	0.37870418	0.61044853
tyrosine	-0.085581	-0.8701972	0.38636474	0.61818358
SDMA	0.08567043	0.85901419	0.39247394	0.62334096
C10.2.carnitine	-0.1020992	-0.8454802	0.39994651	0.6305726
PC.P.38.6.PC.O.38.7	-0.0913825	-0.8207387	0.41382981	0.64686416
ectoine	0.12130425	0.81524631	0.41695063	0.64686416
allantoin	-0.102442	-0.8111912	0.41926381	0.64686416
urocanic.acid	-0.1081752	-0.7854602	0.43411931	0.66503383
N.alpha.acetylarginine	0.06118152	0.77177874	0.44214238	0.66800565
C34.1.DAG.or.TAG.NH4.adduct	-0.0758615	-0.7716056	0.44224448	0.66800565
alpha.glycerophosphocholine	0.11608734	0.76389571	0.44680402	0.67003346
PE.P.40.6.PE.O.40.7	-0.0800365	-0.7588696	0.44979098	0.67003346
LPC.16.0	0.09186628	0.75219477	0.45377548	0.67133906
C8.carnitine	-0.0939831	-0.7358159	0.46363795	0.67809207
X1.methylguanine	0.09858147	0.7341981	0.46461864	0.67809207
creatine	-0.0705238	-0.7200237	0.47326095	0.68200651
glutamate	-0.0901319	-0.7194399	0.4736188	0.68200651
Cer.d18.1.24.1	0.07705367	0.71386805	0.47704192	0.68200651
LPE.18.3	0.08960329	0.70918351	0.47993051	0.68200651
pipecolic.acid	-0.0961592	-0.7036381	0.48336242	0.68239401
C10.carnitine	-0.0886473	-0.6858087	0.49448768	0.69356713
dimethylglycine	-0.061462	-0.645835	0.51992803	0.72454486
X2.methylguanosine	-0.0799053	-0.625136	0.53336591	0.73850665
acetylcholine	0.0853802	0.60387447	0.54735236	0.75186043
glycocholate	0.06335514	0.5999229	0.54997198	0.75186043
LPE.18.2	0.07271165	0.5892131	0.55710326	0.75409068
LPE.20.1	0.06612461	0.57136039	0.56909149	0.75409068
xanthosine	0.08026385	0.57026705	0.56982973	0.75409068
guanidinoacetic.acid	0.06644375	0.56717737	0.57191841	0.75409068
methionine	0.06257316	0.56450587	0.57372739	0.75409068
homoarginine	-0.0556162	-0.5639867	0.57407926	0.75409068
X4.aminohippuric.acid	-0.0606687	-0.5610943	0.57604149	0.75409068
LPC.18.3	0.06541013	0.54734084	0.58541563	0.76174564
C26.carnitine	-0.0504314	-0.5344637	0.5942572	0.7686201
PC.36.2	0.05878152	0.52585377	0.60020323	0.76925236
LPC.14.0	-0.0637058	-0.5234491	0.60186875	0.76925236
N.acetyloornithine	0.05399549	0.51301468	0.60912034	0.77253132

C5.DC.carnitine	-0.0456643	-0.5091802	0.61179503	0.77253132
hydroxycotinine	-0.046041	-0.5043614	0.61516383	0.77253132
kynurenic.acid	-0.050361	-0.4847354	0.62896898	0.77834587
valsartan	0.07274486	0.48095411	0.63164417	0.77834587
Cer.d18.1.16.0	-0.0517543	-0.474313	0.6363545	0.77834587
ornithine	0.04769144	0.47399975	0.63657706	0.77834587
methylimidazoleacetic.acid	0.06228132	0.47187761	0.63808563	0.77834587
N6.N6.dimethyllysine	0.03469818	0.45960716	0.64683802	0.77834587
N.acetylhistidine	0.04305797	0.45712419	0.64861521	0.77834587
homocitrulline	0.05300106	0.45711532	0.64862156	0.77834587
PE.P.36.2.PE.O.36.3	0.04746349	0.44291626	0.65882339	0.78532272
cortisone	0.04925823	0.43257862	0.66629177	0.78532272
leucine	-0.0448392	-0.427606	0.66989624	0.78532272
X5.acetylamino.6.amino.3.methyluracil	-0.0417746	-0.4246683	0.67202937	0.78532272
X2.aminooctanoate	-0.0465737	-0.4238633	0.67261436	0.78532272
X3.methylhistidine	-0.0537015	-0.4124189	0.68095255	0.79078361
LPE.20.4	0.04210785	0.38146079	0.70370488	0.81077521
PE.36.2	-0.035875	-0.3759825	0.70776003	0.81077521
trigonelline	-0.0437189	-0.373732	0.70942831	0.81077521
gabapentin	0.04518354	0.34631096	0.72986739	0.82556594
vitamin.A	0.03399565	0.34135834	0.73358035	0.82556594
C18.carnitine	0.04211489	0.34067119	0.734096	0.82556594
thiamine	0.04418653	0.33592837	0.73765845	0.82556594
DMGV	-0.0241689	-0.3148608	0.75355109	0.83900533
X2.aminoheptanoic.acid	-0.035378	-0.2942595	0.76919531	0.84509795
ADMA	0.02969387	0.2925109	0.7705277	0.84509795
betaine	0.03036132	0.2922053	0.77076063	0.84509795
LPC.16.1	-0.0284268	-0.2715111	0.78658137	0.85808877
PE.P.34.2.PE.O.34.3	0.03016	0.26582941	0.79094101	0.85850884
glutamine	0.02319114	0.22054375	0.82591636	0.89198966
N6.methyllysine	-0.0135524	-0.1983676	0.84317717	0.90610084
tryptophan	-0.0186828	-0.1863875	0.85253444	0.9086509
C4.carnitine	-0.0171018	-0.1845602	0.85396357	0.9086509
N.carbamoyl.beta.alanine	0.01797747	0.17707911	0.85981956	0.91039718
PC.P.38.5.PC.O.38.6	-0.0166056	-0.1577176	0.87501077	0.92196257
homocysteine	0.02056582	0.13973735	0.88916046	0.93232359
C9.carnitine	-0.0159486	-0.129694	0.89708007	0.93608355
PE.38.4	0.00945246	0.1222435	0.90296188	0.93769118
X2.hydroxyhippurate	-0.0133732	-0.1137827	0.9096478	0.94011447
cyclohexylamine	-0.0120444	-0.108246	0.91402662	0.94014167
NMMA	-0.0097757	-0.09982	0.92069554	0.94251297
putrescine	0.0047479	0.04046056	0.96781003	0.98515156
threonine	0.00406906	0.0358595	0.97146889	0.98515156

trimethylamine.N.oxide	-0.0032367	-0.0246314	0.98040009	0.98956271
PE.P.34.1.PE.O.34.2	0.0014638	0.01219346	0.99029659	0.99490262
X5.methylcytidine	0.00043544	0.00448954	0.9964272	0.9964272

## tes before and after treatment

B	Metabolite		
3.43537288	cystine		
2.27776651	hypotaurine		
1.50763751	sphinganine		
-0.2647207	C14.carnitine		
-0.3016067	PS.P.36.2.PS.O.36.3		
-0.5287925	inosine		
-0.537967	taurine		
-0.8783534	thyroxine		
-1.0171626	myristoleic.acid		
-1.3150365	X5.hydroxytryptophan		
-1.3396734	serotonin		
-1.8681234	glycine		
-1.8808035	pro.gly		
-1.9194195	niacinamide		
-2.5144285	pantothenate		
-2.6305029	Sd18.1.18.0		
-2.7964721	N.acetylputrescine		
-2.8534929	LPC.P.16.0.LPC.O.16.1		
-2.8675273	PS.P.36.1.PS.O.36.2		
-3.0254459	C2.carnitine		
-3.0822974	C16.carnitine		
-3.3398983	hippurate		
-3.4366179	LPC.20.1		
-3.5633678	PE.40.6		
-3.6367282	X1.methyladenosine		
-3.7493932	LPC.18.2		
-3.8158542	caffeine		
-3.8397836	LPC.18.1		
-3.86028	X1.methylnicotinamide		
-3.9296713	C6.carnitine		
-3.9498758	X7.methylguanine		
-3.9554112	xanthine		
-3.9999932	N2.N2.dimethylguanosine		
-4.0657827	bilirubin		
-4.118207	N.lauroylglycine		
-4.1233432	PC.30.0		
-4.203205	C7.carnitine		
-4.2201957	PE.38.6		
-4.337533	LPC.20.4		
-4.350184	LPE.22.6		
-4.3661527	Sd18.1.18.1		

-4.4563001	asparagine		
-4.4772992	Sd18.1.14.0		
-4.5253135	trimethylbenzene		
-4.525724	acetyl.galactosamine		
-4.5342548	urate		
-4.5815828	C4.OH.carnitine		
-4.714606	LPE.18.0		
-4.7274126	LPC.P.18.0.LPC.O.18.1		
-4.7464581	Sd18.1.16.1		
-4.7464728	biliverdin		
-4.8373739	N.acetylalanine		
-4.8571203	N1.methyl.2.pyridone.5.carboxamide		
-4.9700035	pseudouridine		
-5.0226883	cinnamoylglycine		
-5.0772253	LPE.18.1		
-5.1119654	N.methylproline		
-5.1153523	C18.1.carnitine		
-5.116569	choline		
-5.13886	methionine.sulfoxide		
-5.2077529	C14.1.carnitine		
-5.2807641	cytosine		
-5.310801	Sd18.1.22.0		
-5.3190233	LPC.20.3		
-5.3223721	arginine		
-5.3226921	acisoga		
-5.3910821	N4.acetylcytidine		
-5.4249505	LPC.20.5		
-5.441893	Sd18.1.20.0		
-5.4512633	LPC.18.0		
-5.4532699	PE.34.0		
-5.4752883	LPE.20.0		
-5.5230242	GABA		
-5.5342418	alanine		
-5.5355929	PE.P.38.6.PE.O.38.7		
-5.5553586	PC.P.34.2.PC.O.34.3		
-5.6002346	citrulline		
-5.6284139	piperine		
-5.6306618	C3.DC.CH3.carnitine		
-5.6347163	X4.acetamidobutanoate		
-5.6351733	PC.32.2		
-5.6430653	X1.methylguanosine		
-5.7042143	C12.carnitine		
-5.7260718	N.acetylaspartic.acid		

-5.7441034	PC.P.36.4.PC.O.36.5		
-5.7665725	X3.methylxanthine		
-5.7741809	X1.7.dimethyluric.acid		
-5.7903956	PE.34.2		
-5.8146316	C5.1.carnitine		
-5.8301913	X2.aminoisobutyric.acid		
-5.8389052	N6.acetylysine		
-5.8429198	C12.1.carnitine		
-5.8463552	X1.methylhistamine		
-5.8501629	diacetylspermine		
-5.9130907	N1.acetylspermidine		
-5.9394837	PE.P.36.4.PE.O.36.5		
-5.9697596	carnitine		
-5.9740448	N6.N6.N6.trimethyllysine		
-6.0223113	creatinine.y		
-6.0241727	linoleoyl.ethanolamide		
-6.0326294	valine		
-6.0360212	proline.betaine		
-6.0396836	Sd18.1.16.0		
-6.0829404	PC.34.3		
-6.0973318	phenylalanine		
-6.1014893	PE.36.4		
-6.117244	glycodeoxycholate.glycochenodeoxycholate		
-6.1435883	C14.2.carnitine		
-6.148939	phenylacetylglutamine		
-6.1506392	PE.P.38.4.PE.O.38.5		
-6.1652798	LPC.22.6		
-6.1765506	cotinine		
-6.1933048	LPC.22.5		
-6.2014308	C5.carnitine		
-6.2051599	X7.methylxanthine		
-6.2304942	histidine		
-6.2353571	Sd18.1.22.1		
-6.2406658	cortisol		
-6.2562588	PC.34.4		
-6.270128	isoleucine		
-6.2758486	proline		
-6.2784051	hydroxyproline		
-6.2814613	lysine		
-6.2873809	C20.4.carnitine		
-6.2902495	ribothymidine		
-6.2926026	adenosine		
-6.3043412	serine		

-6.3157638	sphingosine		
-6.3176224	PE.P.38.5.PE.O.38.6		
-6.3262971	LPE.16.0		
-6.3328725	C3.carnitine		
-6.3353116	butyrobetaine		
-6.335859	C18.2.carnitine		
-6.3388187	X4.guanidinobutanoic.acid		
-6.3511105	tyrosine		
-6.3606672	SDMA		
-6.3720701	C10.2.carnitine		
-6.3924546	PC.P.38.6.PC.O.38.7		
-6.3968988	ectoine		
-6.4001612	allantoin		
-6.4204879	urocanic.acid		
-6.4310325	N.alpha.acetylarginine		
-6.4311648	C34.1.DAG.or.TAG.NH4.adduct		
-6.437025	alpha.glycerophosphocholine		
-6.4408141	PE.P.40.6.PE.O.40.7		
-6.4458078	LPC.16.0		
-6.4578769	C8.carnitine		
-6.4590547	X1.methylguanine		
-6.4692649	creatine		
-6.4696812	glutamate		
-6.4736376	Cer.d18.1.24.1		
-6.4769404	LPE.18.3		
-6.4808224	pipecolic.acid		
-6.4930992	C10.carnitine		
-6.5194897	dimethylglycine		
-6.5325379	X2.methylguanosine		
-6.5455019	acetylcholine		
-6.5478622	glycocholate		
-6.5541821	LPE.18.2		
-6.5644659	LPE.20.1		
-6.5650855	xanthosine		
-6.56683	guanidinoacetic.acid		
-6.5683308	methionine		
-6.5686217	homoarginine		
-6.5702372	X4.aminohippuric.acid		
-6.577806	LPC.18.3		
-6.5847235	C26.carnitine		
-6.5892573	PC.36.2		
-6.5905104	LPC.14.0		
-6.5958821	N.acetyloornithine		

-6.5978291	C5.DC.carnitine		
-6.6002552	hydroxycotinine		
-6.6098995	kynurenic.acid		
-6.6117138	valsartan		
-6.6148662	Cer.d18.1.16.0		
-6.6150138	ornithine		
-6.6160113	methylimidazoleacetic.acid		
-6.6216914	N6.N6.dimethyllysine		
-6.6228227	N.acetylhistidine		
-6.6228267	homocitrulline		
-6.6291786	PE.P.36.2.PE.O.36.3		
-6.6336776	cortisone		
-6.635804	leucine		
-6.6370487	X5.acetylamino.6.amino.3.methyluracil		
-6.6373883	X2.aminooctanoate		
-6.6421467	X3.methylhistidine		
-6.6543682	LPE.20.4		
-6.6564319	PE.36.2		
-6.6572711	trigonelline		
-6.6670923	gabapentin		
-6.6687866	vitamin.A		
-6.6690197	C18.carnitine		
-6.6706162	thiamine		
-6.6774378	DMGV		
-6.6836821	X2.aminoheptanoic.acid		
-6.6841927	ADMA		
-6.6842817	betaine		
-6.6900875	LPC.16.1		
-6.691607	PE.P.34.2.PE.O.34.3		
-6.7025711	glutamine		
-6.7071962	N6.methyllysine		
-6.7094913	tryptophan		
-6.7098288	C4.carnitine		
-6.7111759	N.carbamoyl.beta.alanine		
-6.7144037	PC.P.38.5.PC.O.38.6		
-6.7170671	homocysteine		
-6.7184147	C9.carnitine		
-6.7193495	PE.38.4		
-6.7203441	X2.hydroxyhippurate		
-6.7209563	cyclohexylamine		
-6.7218295	NMMA		
-6.7259769	putrescine		
-6.7261518	threonine		

-6.7264901	trimethylamine.N.oxide		
-6.7267182	PE.P.34.1.PE.O.34.2		
-6.7267823	X5.methylcytidine		