Current Developments in Nutrition 8 (2024) 104444

American Society for Nutrition
Excellence in Nutrition Research and Practice

Current Developments in Nutrition CDN

journal homepage: https://cdn.nutrition.org/

Original Research

Metabolomic Profile Alterations Associated with the *SLC16A11* Risk Haplotype Following a Lifestyle Intervention in People With Prediabetes

Magdalena Sevilla-González [1,2,3,*], Maria Fernanda Garibay-Gutiérrez [4,5], Arsenio Vargas-Vázquez [4], Andrea Celeste Medina-García [6,7], Maria Luisa Ordoñez-Sánchez [6], Clary B Clish [8], Paloma Almeda-Valdes [4,9], Teresa Tusie-Luna [6,7]

[1] *Clinical and Translational Epidemiology Unit, Mongan Institute, Massachusetts General Hospital, Boston, MA, United States;* [2] *Department of Medicine, Harvard Medical School, Boston, MA, United States;* [3] *Programs in Metabolism and Medical & Population Genetics, The Broad Institute of MIT and Harvard, Cambridge, MA, United States;* [4] *Unidad de Investigacion en Enfermedades Metabolicas, Instituto Nacional de Ciencias Medicas y Nutricion "Salvador Zubiran," Mexico City, Mexico;* [5] *Departamento de Fisiología. Escuela Nacional de Ciencias Biológicas, Instituto Politecnico Nacional, Mexico City, Mexico;* [6] *Unidad de Biologia Molecular y Medicina Genomica, Insituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico;* [7] *Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico;* [8] *Metabolomics Platform, The Broad Institute of MIT and Harvard, Cambridge, MA, United States;* [9] *Departamento de Endocrinologia y Metabolismo, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico*

A B S T R A C T

Background: A risk haplotype in *SLC16A11* characterized by alterations in fatty acid metabolism emerged as a genetic risk factor associated with increased susceptibility to type 2 diabetes (T2D) in Mexican population. Its role on treatment responses is not well understood.
Objectives: We aimed to determine the impact of the risk haplotype on the metabolomic profile during a lifestyle intervention (LSI).
Methods: We recruited Mexican-mestizo individuals with ≥1 prediabetes criteria according to the American Diabetes Association with a body mass index between 25 and 45 kg/m^2. We conducted a 24-wk quasiexperimental LSI study for diabetes prevention. Here, we compared longitudinal plasma liquid chromatography/mass spectrometry metabolomic changes between carriers and noncarriers. We analyzed the association of risk haplotype with metabolites leveraging repeated assessments using multivariable-adjusted linear mixed models.
Results: Before the intervention, carriers (N = 21) showed higher concentrations of hippurate, C16 carnitine, glycine, and cinnamoylglycine. After 24 wk of LSI, carriers exhibited a deleterious metabolomic profile. This profile was characterized by increased concentrations of hippurate, cinnamoglycine, xanthosine, N-acetylputrescine, L-acetylcarnitine, ceramide (d18:1/24:1), and decreased concentrations of citrulline and phosphatidylethanolamine. These metabolites were associated with higher concentrations of total cholesterol, triglycerides, and low density lipoprotein cholesterol. The effect of LSI on the risk haplotype was notably more pronounced in its impact on 2 metabolites: methylmalonylcarnitine (β: −0.56; P-interaction = 0.014) and betaine (β: −0.64; P-interaction = 0.017). Interestingly, lower consumption across visits of polyunsaturated (β: −0.038; P = 0.017) fatty acids were associated with higher concentrations of methylmalonylcarnitine. Covariates for adjustment across models included age, sex, genetic ancestry principal components, and body mass index.
Conclusions: Our study highlights the persistence of deleterious metabolomic patterns associated with the risk haplotype before and during a 24-wk LSI. We also emphasize the potential regulatory role of polyunsaturated fatty acids on methylmalonylcarnitine concentrations suggesting a route for improving interventions for individuals with high-genetic risk.

Keywords: prediabetes, metabolomics, lifestyle intervention, genetics

Abbreviations: DAG, diacylglycerols; HbA1c, glycated hemoglobin; LSI, lifestyle intervention; OGTT, oral glucose tolerance test.

* Corresponding author. *E-mail address:* msevillagonzalez@mgh.harvard.edu (M. Sevilla-González).

https://doi.org/10.1016/j.cdnut.2024.104444
Received 27 May 2024; Received in revised form 11 August 2024; Accepted 15 August 2024; Available online 22 August 2024

Introduction

Type 2 diabetes mellitus (T2D) is a highly prevalent metabolic disorder that implies significant challenges for health systems worldwide [1]. It is estimated that ~537 million adults were living with diabetes in 2021, and this number is projected to increase to 783 million by 2045 [2]. Prediabetes, a precursor stage to T2D, is characterized by elevated blood glucose concentrations that do not meet the diagnostic criteria for diabetes but indicate increased risk of developing the T2D and cardiovascular morbidity [3–5]. Identifying effective strategies to prevent or delay the progression from prediabetes to diabetes is important to public health owing to its potential to reduce disease burden [4].

Genetic factors play a crucial role in the pathogenesis of T2D and prediabetes. Over the past decade, genome-wide association studies have led to the identification of numerous genetic loci associated with diabetes risk [6,7]. In 2014, a haplotype in SLC*16A11* gene emerged as a genetic risk factor associated with increased susceptibility to T2D in the Mexican population [8,9]. The *SLC16A11* gene, located on chromosome 17, encodes a proton-linked monocarboxylate transporter that plays a role in glucose metabolism and insulin sensitivity [10–12]. The disruption in the function of *SLC16A11* in liver results in alterations in lipid metabolites, including acylcarnitines, diacylglycerols (DAGs), and triacylglycerols [10]. These metabolites are related to fatty acid and lipid metabolism, which are linked to elevated risk of T2D [13]. The liver accumulation of DAGs is key abnormality of lipotoxicity. Furthermore, acylcarnitine's accumulation is associated with hepatic steatosis and changes in AKT, a key regulator of energy utilization [14]. In addition, *SLC16A11* T2D risk variants disrupt oxygen-regulated *SLC16A11* expression in human hepatocytes [15].

Research to date has not yet established the extent to which in vitro associations are observed in human carriers of the *SLC16A11* risk variants. In humans, *SLC16A11* has been associated with in early-onset T2D [16], decreased insulin action, higher acute insulin secretory response to an intravenous glucose bolus, higher alanine aminotransferase concentrations, and increased BMI [8,11,17]. Additionally, an association was found between this variant and the mild obesity diabetes subphenotype, regardless of whether patients were homozygotes or heterozygotes [18]. Mild obesity diabetes is characterized by early onset of the disease, greater adiposity, and selective response to metformin and sulfonylureas [19]. The genetic factors along with unhealthy lifestyles might be contributing to the high prevalence of T2D and metabolic diseases in Hispanic individuals [20,21]. In Mexico, the prevalence of T2D has increased substantially over the past 3 decades [22], and it is significantly higher, estimated at 18%, in comparison with the global average of 10.5% [23]. Lifestyle modifications, including diet and physical activity changes and mobile applications [24], have been shown to improve glycemic control, reduce body weight, and decrease the risk of diabetes [25–27]. Moreover, recent advancements such as high-throughput technologies, including metabolomic analysis—the identification of intermediary molecules and metabolism byproducts [28], have facilitated the recognition of biological pathways potentially impacted by both genetic variations and lifestyle factors [28,29]. This progress opens up new avenues for refining interventions by incorporating individual metabolic profiles [30,31]. To date, to our knowledge, no studies have explored the metabolomic signatures associated with the *SLC16A11* risk haplotype in humans, nor how these signatures might change in response to lifestyle interventions (LSIs) in subjects at risk of T2D. In this study, we aimed to identify the effect of *SLC16A11* risk haplotype on metabolomic signatures before and after a LSI in Mexican individuals with prediabetes.

Methods

Study population

Participants were recruited at the Unidad de Investigación de Enfermedades Metabólicas within the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. The quasiexperimental LSI study took place from January 2017 to August 2018. Participants were subjects who responded to social media advertisements. In both cohorts, inclusion criteria for study subjects were as follows—Mexican mestizos with ≥1 prediabetes criteria according to the American Diabetes Association [fasting glucose between 100 and 125 mg/dL, glycosylated hemoglobin (HbA1c) between 5.7 and 6.4, and 2-h blood sugar between 140 and 199 mg/dL after an oral load of 75 g of glucose]; age range between 18 and 65 y; and overweight or obesity (BMI = 25.0–40 kg/m^2). Exclusion criteria included any condition that transiently alter glucose tolerance (i.e., pregnancy, chronic use of medications including medications to reduce lipid and glucose concentrations) and participants who did not attend their follow-up visits in the first 2 mo and with >20% of missing data.

This study was approved by the ethics committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Written informed consent was obtained from each participant. Research was conducted according to the tenets of the Helsinki Declaration of Human Studies principles.

Life style intervention

The LSI of this study has been described previously [32]. In brief, the study included 4 visits: screening, intervention, follow-up, and final. For the screening visit, participants underwent a 2-h oral glucose tolerance test (OGTT) using 75 g of glucose. Anthropometric measures were performed in the screening visit following standardized protocols. The LSI intervention was implemented by individuals holding a bachelor's degree with ≥4 y of training in human nutrition and in a 1-y clinical research rotation at the time of the study. These individuals were trained in dietary and anthropometric evaluation and implementation of the nutritional care process [33]. 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. Additionally, participants received personalized physical activity recommendations to achieve >150 min of moderate-intensity exercise per week. After 12 wk, subjects attended a follow-up visit with the dietitian to reinforce knowledge and goals. The final visit took place 24 wk after the intervention visit, and all measurements were repeated,

including anthropometric measurements, body composition measurements, and OGTT. Adherence to the intervention was assessed via participant self-report on every visit.

Questionnaires and calculations

Daily energy and macronutrient and micronutrient intakes in both studies were assessed through a 24-h food recall at each visit (baseline, intermediate, and final). Data were analyzed using ESHA's Food Processor Nutrition Analysis software. HOMA-IR was computed as fasting glucose × fasting insulin/405 [34]. AUC was calculated using the formula using the OGTT measurements of glucose or insulin: [AUC (mmol/L × min) = 1/2 × (measurement 0 min + measurement 30 min) × 30 min +1/2 × (measurement 30 min+ measurement 60 min) × 30 min +1/2 × (measurement 60 min + measurement 90 min) × 30 min +1/2 × (measurement 90 min+ measurement 120 min) × 30 min]. Incremental AUC was computed using the same formula but subtracting baseline measurements from each time measurement.

Biochemical and genotype analysis

Measurements were derived from serum samples from the OGTT in both studies: glucose, insulin, and lipid profile were analyzed with colorimetric enzymatic methods (Unicel DxC 600 Synchron Clinical System; Beckman Coulter). Insulin was measured with chemiluminescence essay (Access 2; Beckman Coulter). As for HbA1c, a 4-mL peripheral blood sample was taken by venipuncture with standardized technique and measured in the Variant II Turbo, BIORAD using HPLC. Samples were genotyped for the SCL16A11 risk variants rs13342232 A>G and rs75493593 G>C, both as part of the haplotype, including 4 missense and 1 silent variant (V113I, L187L, D127G, G340S, and P443T). Genotyping was performed using the Quant Studio 12K Flex Real-Time PCR platform from ThermoFisher Scientific.

Genetic ancestry principal component calculation and Hardy–Weinberg estimation

In both studies, we estimated global ancestry and corrected for population stratification using a panel of 32 ancestry informative markers, which are highly informative of the major ancestral components of Mexican mestizos. The correlation between the global ancestry proportions calculated using genome-wide data and the panel of 32 ancestry informative markers is $r^2 = 0.972$ [35].

Hardy–Weinberg equilibrium was assessed by computing the allele frequencies of the population sample calculating the expected genotype frequencies using the Hardy–Weinberg equation: $p^2 + 2pq + q^2 = 1$, where p is the frequency of one allele and q is the frequency of the other allele. Compare the observed and expected genotype frequencies using a χ^2 test.

Metabolomic analysis

The plasma metabolomics profiling was performed before and after the intervention in the Metabolomics Platform at the Broad Institute of Massachusetts Institute of Technology and Harvard. We used liquid chromatography tandem mass spectrometry (LC-MS) that couples hydrophilic interaction liquid chromatography with positive ion mode mass spectrometry (HILIC-pos) and that has been described in detail previously [29]. Samples were taken in 3 batches, and we did not detect any batch effect among the metabolomic samples (Supplemental Figure 1). A total of 219 named metabolites were qualified for primary analyses (Supplemental Table 1). To reduce noise in the profiling data, we implemented a quality control (QC) pipeline (https://github.com/broadinstitute/QC_metabolomics) and metabolite signals with noisy trends were removed from the study. Missing data in LC-MS analyses are often attributable to measurements falling below the level of detection. The QC steps included the following: normalization with internal standards and pooled samples, removal of metabolites with >25% of missing values, missing value imputation using half of the minimum value, windsorization to ±5 SD, log normalization, and *z*-score scaling. A total of 15 metabolites (6.8%) showed a missing proportion of >0 and were imputed, and 2 metabolites showed missing values of >10%. The main the missing metabolites primarily reflect external exposures, being associated with substances such as paracetamol, valsartan, and nicotine (Supplemental Table 2). All models were adjusted for age, sex, BMI, and 5 genetic principal components (PCs). For sensitivity analysis, a restricted model using only 2 genetic PCs was also evaluated to ensure 10 individuals per covariate, enhancing the statistical robustness of our findings.

Statistical analysis

The baseline characteristics of the study population were presented with descriptive statistics. Quantitative variables were reported as mean and SD for parametric variables and median and IQR for nonparametric variables, and qualitative variables were presented as frequencies and percentages. The difference preintervention and postintervention was computed using Δs, obtained by subtracting the baseline visit value from the final visit value. Paired *t* tests or Wilcoxon tests were used, according to variable's distribution, for comparing baseline and post-interventional values. To assess statistical distinctions between *SCL16A11* risk carriers and noncarriers, as well as between treatments, *t* tests or Mann–Whitney *U* tests were applied in accordance with the variable distribution. Fold-change was used to visually represent changes preintervention and post-intervention, calculated as follows: (posttreatment value/baseline value) − 1. We used linear models to estimate baseline metabolomic differences between carriers and noncarriers adjusting for covariates: age, sex, BMI, and 5 genetic ancestry PCs. The impact of the *SLC16A11* risk haplotype on longitudinal metabolomic profiles during treatment, as well as its effect modification, was analyzed using linear mixed-effect models. These analyses were performed with the lmer function from the lme4 package in R, which is available at https://cran.r-project.org/web/packages/lme4/index.html. The "visit" variable was treated as a fixed effect, whereas "subject id" served as a random effect within the linear mixed-effect models. These models were adjusted for age, sex, BMI, and 5 genetic ancestry PCs. We also conducted a sensitivity analysis using only 2 PCs, along with age, sex, and BMI, resulting in 5 covariates. This approach was adopted to adhere to avoid exceeding the number of covariates per sample [36]. All analyses were conducted using R package version 4.0.0.

Results

A total of 52 individuals who met the inclusion criteria and had both metabolomic and genomic data were included in the analysis. The subsample was comparable with the original sample [32] in terms of age, sex, BMI, fasting glucose, and HbA1c concentrations.

At baseline, we did not observe significant differences in clinical parameters between carriers and noncarriers (Table 1). After 24 wk, LSI was effective in reducing weight, waist circumference, body fat, fasting glucose, and HbA1c and in increasing insulin sensitivity as has been previously described [32]. Additionally, stratification by genotype did not reveal significant differences in changes in clinical parameters resulting from the intervention (Supplemental Table 3).

Metabolomic profile differences before and after the intervention

We studied the metabolomic profile before and after the LSI intervention. LSI was effective in reducing C14 carnitine, PS/P36, inosine, thyroxine, myristoleic acid, and niacinamide abundances, which resulted in changes in the taurine and hypotaurine metabolism pathway. These changes are compatible with biomarkers of protein consumption, particularly, lower red meat and animal fats and higher seafood and vegetables as previously described [32].

Metabolomic profile differences between carriers and noncarriers at baseline

When we contrasted metabolomic profiles of carriers and noncarriers at baseline, carriers demonstrated distinct abundances of carnitines, α-amino acids, and purines than noncarriers (Figure 1). Analysis indicated significant differences in metabolite abundances between carriers and noncarriers (Supplemental Table 4). Specifically, carriers displayed higher concentrations of hippurate (β: 0.59; $P = 0.016$), C16 carnitine (β: 0.60; $P = 0.028$), glycine (β: 0.56; $P = 0.046$), and cinnamoylglycine (β: 0.61; $P = 0.017$). Conversely, they exhibited lower concentrations of glutarylcarnitine (C5-DC carnitine; β: −0.66; $P = 0.028$) and N-α-acetylarginine (β: −0.68; $P = 0.020$). These changes correspond to a 1-SD increment in the genetic variant's effect, compared with noncarriers adjusted for age, sex, BMI, and 5 genetic PCs.

In the full sample, some of these metabolites exhibited associations with a deleterious metabolic profile. Specifically, lower concentrations of baseline glutarylcarnitine were found to correlate with higher concentrations of postprandial insulin at 120 min (β: −0.42; $P = 0.0025$). Additionally, glycine abundances were positively correlated with HbA1c concentrations (β: 0.33; $P = 0.022$), whereas cinnamoylglycine was associated with elevated total cholesterol (β: 0.43; $P = 0.010$) and HDL cholesterol (β: 0.34; $P = 0.030$). Furthermore, the metabolites distinctive between carriers and noncarriers demonstrated significant associations with dietary factors. Specifically, higher

TABLE 1
Baseline characteristics of study participants by genotype: quasiexperimental LSI study.

Variable	All participants, $N = 52$[1]	Carriers, $n = 21$ (40.4%)[1]	Noncarriers, $n = 31$ (59.6%)[1]	P[2]
Genotype, *n* (%)				<0.001
Noncarrier homozygous	31 (59.6)	0 (0.0)	31 (100.0)	
Heterozygous	16 (30.8)	16 (76.2)	0 (0.0)	
Carrier homozygous	5 (9.6)	5 (23.8)	0 (0.0)	
Age (y)	51.94 (11.06)	51.24 (12.32)	52.42 (10.30)	0.719
Gender, *n* (%)				0.556
Female	36 (69.2%)	16 (76.2%)	20 (64.5%)	
Male	16 (30.8%)	5 (23.8%)	11 (35.5%)	
BMI (kg/m^2)	30.02 (4.01)	30.36 (4.21)	29.79 (3.93)	0.627
Fasting glucose (mg/dL)	98.25 (9.05)	99.95 (8.78)	97.10 (9.19)	0.265
Glucose 30 min (mg/dL)	159.24 (28.11)	165.05 (23.68)	155.17 (30.56)	0.200
Glucose 120 min (mg/dL)	128.02 (29.74)	135.33 (26.72)	123.06 (31.06)	0.135
Log fasting insulin	2.15 (0.55)	2.10 (0.56)	2.18 (0.54)	0.620
Log insulin 30 min	4.11 (0.68)	4.11 (0.80)	4.10 (0.61)	0.956
Log insulin 120 min	4.21 (0.76)	4.20 (0.72)	4.21 (0.80)	0.960
Hemoglobin A1c (%)	5.89 (0.25)	5.92 (0.29)	5.86 (0.23)	0.475
Log Matsuda	−0.07 (0.59)	−0.06 (0.58)	−0.08 (0.61)	0.901
HOMA-IR	2.45 (1.50)	2.38 (1.58)	2.50 (1.46)	0.790
Log triglycerides	4.86 (0.62)	4.96 (0.61)	4.81 (0.55)	0.711
Total cholesterol (mg/dL)	192.50 (37.08)	193.19 (39.14)	192.03 (36.28)	0.915
LDL-C (mg/dL)	118.43 (31.10)	116.91 (28.99)	119.45 (32.88)	0.771
ApoB (mg/dL)	113.39 (21.94)	114.02 (18.74)	112.90 (24.55)	0.877
GGT (IU/L)	22.02 (17.26)	20.00 (10.60)	23.43 (20.76)	0.443
AST (IU/L)	27.48 (24.90)	32.55 (38.68)	24.10 (6.15)	0.344
ALT (IU/L)	26.67 (19.28)	31.05 (24.97)	23.71 (13.92)	0.231

Abbreviations: ALT, alanine transaminase; ApoB, apolipoprotein B; AST, aspartate transaminase; GGT, γ-glutamyl transferase; LSI, lifestyle intervention study, HOMA-IR, Homeostasis Model Assestment for Insulin Resistance, LDL-C, low density lipoprotein choleserol.

[1] *n* (%); mean (SD); median (IQR)

[2] Pearson χ^2 test; Welch 2-sample *t* test.

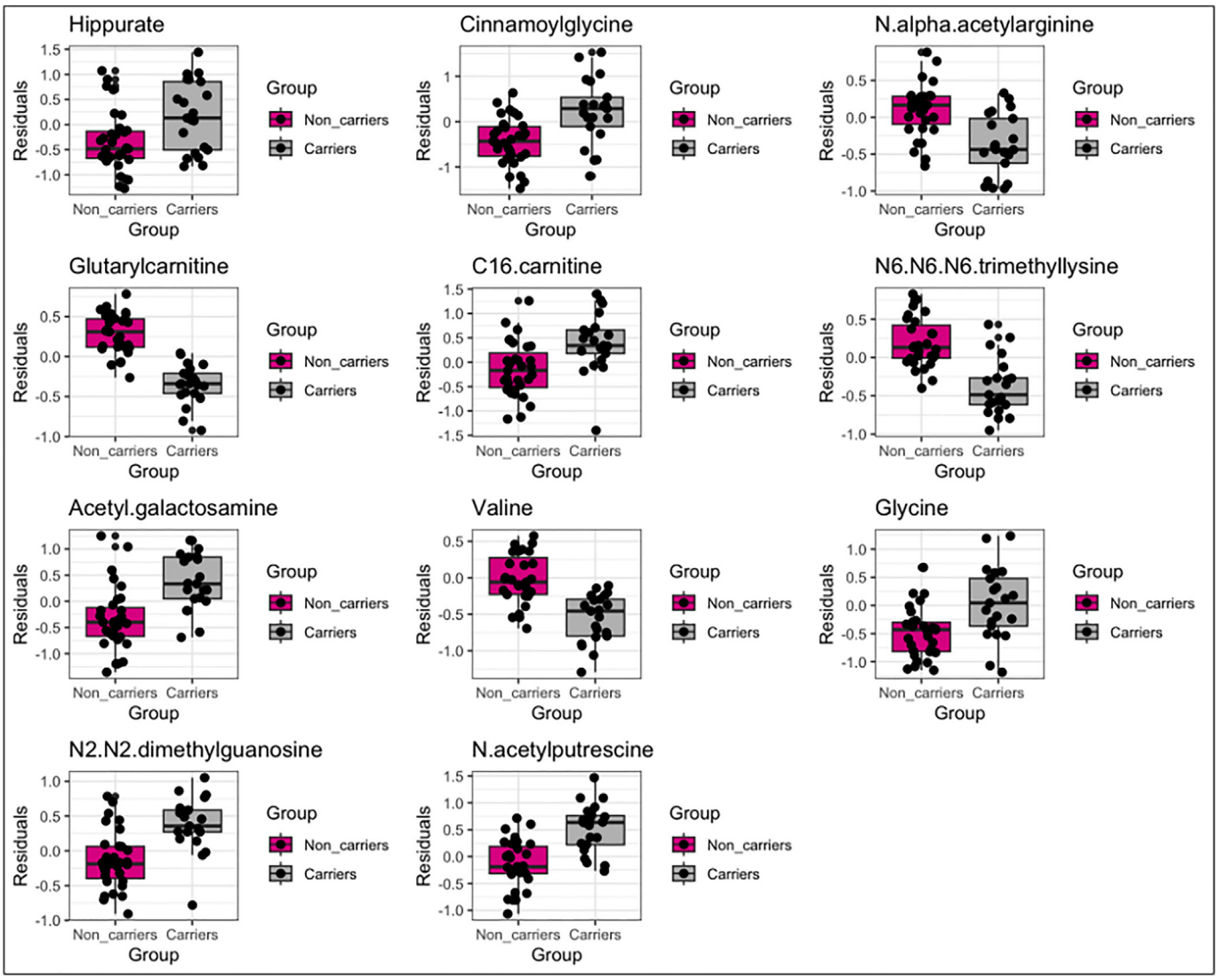

FIGURE 1. Significant metabolomic differences at baseline between *SLC16A11* carriers and noncarriers. The plot displays the metabolites that show significant differences in abundance ($P < 0.05$) between the 2 groups. Each point on the plot represents the mean and the bars the SE of the residuals from a linear regression model. The model was adjusted for age, sex, BMI, and 5 principal components.

intake of PUFAs exhibited correlations with lower abundances of glutarylcarnitine (β: −0.15; $P = 0.038$) and higher abundances of acetylgalactosamine (β: 0.18; $P = 0.004$). Additionally, lower total lipid consumption (%) (β: −0.02; $P = 0.008$) and SFA intake (β: −0.35; $P = 0.018$) showed correlations with N-α-acetylarginine concentrations.

Longitudinal metabolomic differences between carriers and noncarriers

Next, we tested whether metabolomic differences by genotype were consistent across the 2 visits. We identified, 8 metabolites for which differences between carriers and noncarriers were consistent across visits (Figure 2; Supplemental Table 5). Hippurate and cinnamoylglycine consistently exhibited higher abundances among carriers. In contrast, a group of lipids demonstrated varying abundances: ceramide (d18:1/24:1) and L-acetylcarnitine (C2 carnitine) were found in higher concentrations in carriers, whereas phosphatidylethanolamine (PE) (P-36:4)/PE(O-36:5) showed lower abundances in carriers compared with noncarriers. These lipids are known for their role in regulating metabolic health, specifically in the transportation of fatty acids [13]. In our sample, these metabolites demonstrated longitudinal associations with glucose and lipid traits. For example, ceramide (d18:1/24:1) exhibited a strong correlation with elevated concentrations of total cholesterol (β: 0.42; $P = 7.8 \times e^{-10}$), LDL cholesterol (β: 0.34; $P = 0.003$), apolipoprotein B (β: 0.29; $P = 0.003$), and triglycerides (β: 0.19; $P = 0.02$).

Moreover, the pattern observed in carriers of lower concentrations of PE(P-36:4)/PE(O-36:5) also correlated with higher concentrations of triglycerides (β: −0.18; $P = 0.017$), apolipoprotein B (β: −0.22; $P = 0.042$), and postprandial glucose concentrations at 120 min (β: −0.17; $P = 0.043$). Interestingly, we observed protein consumption might be correlated with PE(P-36:4)/PE(O-36:5) concentrations (β: −0.17; $P = 0.043$). Additionally, for citrulline, the lower profile observed in carriers correlated with a deleterious glucose profile, as evidenced by its

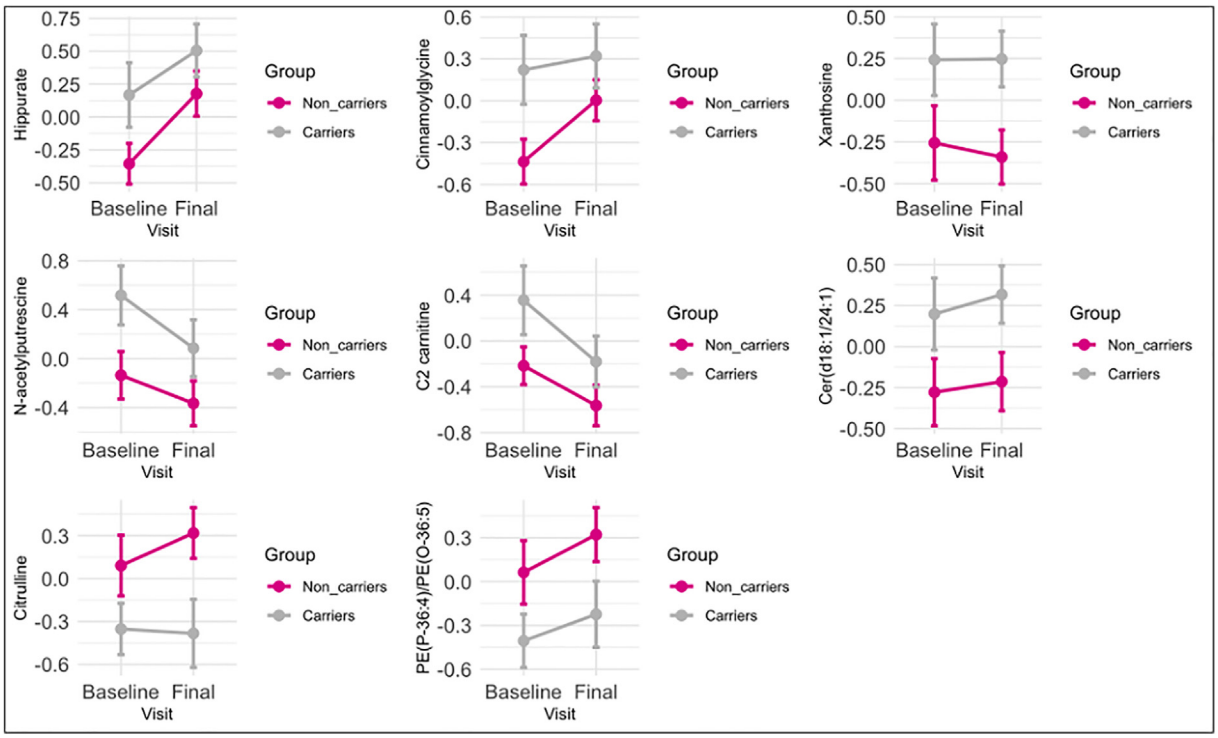

FIGURE 2. The plot displays longitudinal metabolite abundances differences between *SLC16A11* carriers and noncarriers ($P < 0.05$). The plot shows the mean values and SEs for each group across visits (baseline and final).

association with higher fasting glucose (β: −0.23; $P = 0.016$) and postprandial glucose concentrations at 120 min (β: −0.25; $P = 0.026$) (Figure 3).

Finally, we assessed whether the effect of the risk genotype influenced significant changes on metabolite concentrations across visits. We observed drastic changes in betaine (N,N,N-trimethylglycine) concentrations between carriers and noncarriers across visits. Carriers exhibited higher abundances at baseline, which significantly decreased by the final visit, whereas noncarriers displayed opposite trajectories (β-interaction: −0.46; *P*-interaction = 0.034). Moreover, changes in methylmalonylcarnitine (C3-DC-CH3-carnitine) were also observed across visits. Although both carriers and noncarriers exhibited a reduction in methylmalonylcarnitine concentrations after 24 wk of LSI, carriers displayed a more pronounced change (β-interaction: −0.38; *P*-interaction = 0.035), highlighting the distinctive impact of the intervention in carriers (Figure 4; Supplemental Table 6). Changes in betaine and methylmalonylcarnitine concentrations may also correlate with changes in glucose profile. This is supported by the association observed between higher concentrations of betaine and reductions in fasting glucose (β: −0.27; $P = 0.009$), and fasting (β: −0.18; $P = 0.04$) and postprandial (β: −0.24; $P = 0.01$) insulin. Similarly, higher concentrations of methylmalonylcarnitine also displayed associations with lower concentrations of glucose at 120 min (β: −0.23; $P = 0.01$) (Figure 5).

The changes in LSI that resulted in metabolic changes might be correlated with modifications in lipid profile consumption. Carriers who increased fat consumption experienced a decrease in postprandial glucose at 120 min (β-interaction = −0.54; *P*-interaction = 0.009). To further explore the sources of these changes, we analyzed the associations with various types of fatty acid intake. We initially observed that higher baseline concentrations of MUFA (β: −0.022; $P = 0.005$), PUFA (β: −0.016; $P = 0.0006$), and total lipids (β: −0.005; $P = 0.007$) were associated with lower concentrations of methylmalonylcarnitine at the final visit. However, these associations became nonsignificant after the exclusion of 3 outlier individuals. Nonetheless, a consistent relationship was noted with the average intake of PUFAs, as measured across three 24-h recalls (at baseline, intermediate, and final visits), which was still associated with lower concentrations of methylmalonylcarnitine (β: −0.038; $P = 0.017$) (Figure 6). These findings indicate a consistent inverse relationship between the intake of PUFAs and lipid concentrations and methylmalonylcarnitine concentrations. This suggests that unsaturated fatty acids could serve as potential therapeutic targets in carriers of the *SLC16A11* risk haplotype to regulate methylmalonylcarnitine concentrations.

Discussion

In this study, we investigated the effect of the *SCL16A11* risk haplotype on the metabolomic profile before and after 24 wk of LSI intervention, the most cost-effective treatment for T2D prevention. In this report, we found that carriers displayed a deleterious metabolomic profile across the 2 visits, characterized by the higher concentrations of hippurate, cinnamoglycine, xanthosine, N-acetlyputrescine, L-acetylcarnitine, and ceramide (d18:1/24:1) and lower concentrations of citrulline and pPE(P-36:4)/PE(O-36:5). The effect of LSI on the risk haplotype was notably more pronounced in its impact on 2 metabolites: methylmalonylcarnitine and betaine.

In our study, the metabolomic profile found in carriers was largely concordant to the one described previously by Rusu et al.

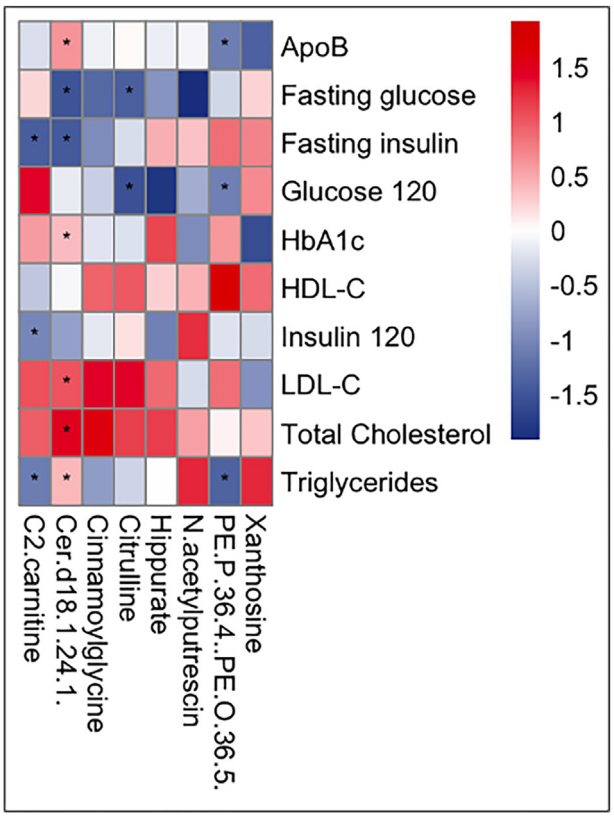

FIGURE 3. Metabolites associated with differences in abundance across visits in individuals with the *SLC16A11* risk haplotype compared with noncarriers and their relationship with glucose and lipid traits. *Metabolites with statistically significant differences ($P < 0.05$). The plot displays scaled estimates from a linear mixed model regression that has been adjusted for age, sex, and BMI.

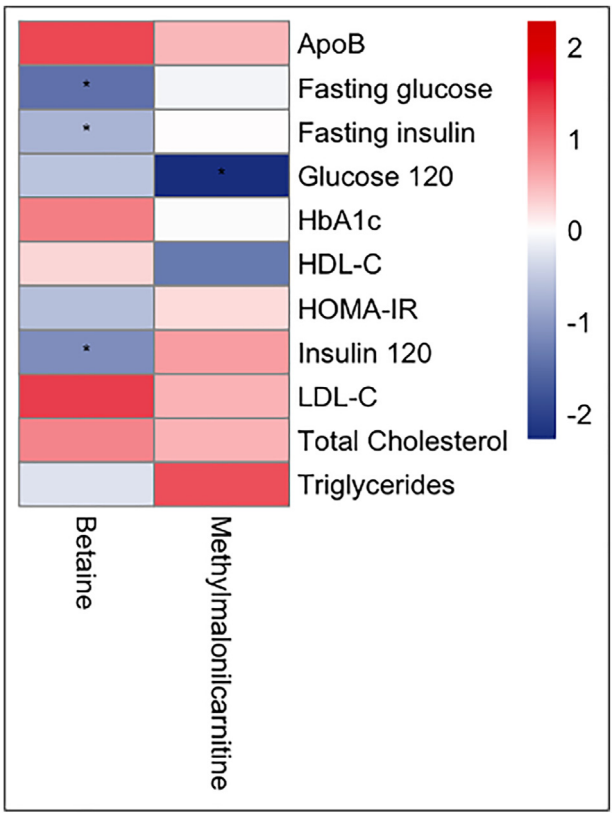

FIGURE 5. Associations of metabolites with significant change across visits and their association with metabolic traits. The plot shows scaled estimates from a regression analysis, adjusted for age, sex, BMI, and 5 genetic principal components.

[10]. These in vitro results demonstrated that disrupting *SLC16A11* expression in human hepatocytes using small-interfering RNAs induced significant metabolic changes. Specifically, there were elevated concentrations of intracellular acylcarnitines, DAGs, and triacylglycerols compared with cells treated with negative-control small-interfering RNAs. These findings indicate a deleterious metabolomic profile in *SLC16A11* carriers, marked by altered cellular fatty acid and lipid metabolism. Among the 8 metabolites identified in our study as differing between carriers and noncarriers, 5 were also found to be among the 350 metabolites analyzed by Rusu et al. [10]: notably, higher concentrations of hippurate, citrulline, L-acetylcarnitine, ceramide (d18:1/24:1), and pPE(P-36:4)/PE(O-36:5). The accumulation of acylcarnitines is indicative of decreased β-oxidation of fatty acids within the mitochondria. Such an accumulation has been associated with hepatic steatosis and alterations in AKT, a crucial regulator of energy utilization. Furthermore, large epidemiologic studies have established acylcarnitines as markers for the onset of T2D [13,37,38]. Additionally, the tissue accumulation of DAGs is a central abnormality contributing to lipotoxicity, the predominant theory explaining insulin resistance in liver and muscle tissues. DAGs facilitate the PKCε-mediated phosphorylation of Thr1160 on the insulin receptor, which subsequently inhibits its tyrosine kinase activity, disrupting insulin signaling. These biochemical

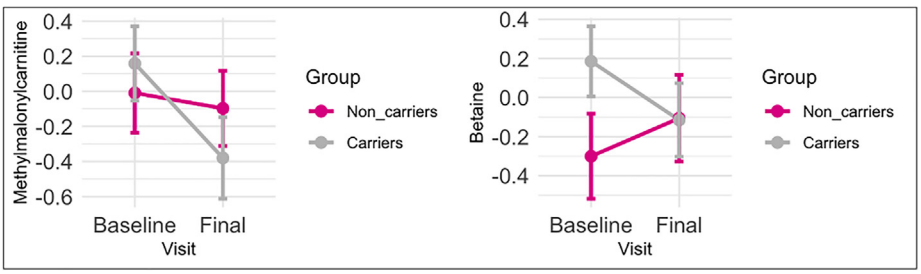

FIGURE 4. Significant interactions ($P < 0.05$) between the *SLC16A11* risk haplotype and the timing of the visits (baseline and final) on metabolite levels. The plot presents the mean values for each group at both the baseline and final visits, providing a clear comparison of how metabolite levels change over time in relation to the genetic risk haplotype.

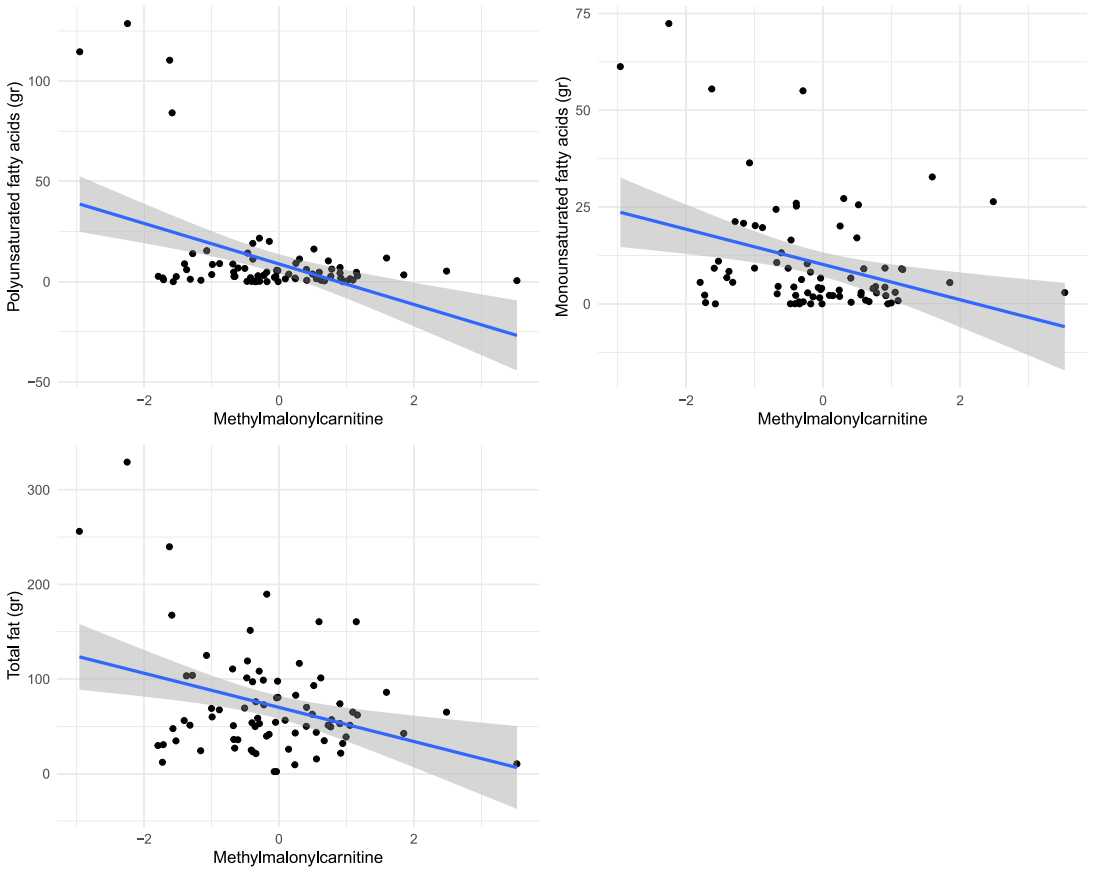

FIGURE 6. Associations between the consumption of polyunsaturated, monounsaturated, and total fats at baseline and the concentrations of methylmalonylcarnitine measured at the final visit. The plot shows regression estimates, depicted with their corresponding CIs (indicated by gray shading), adjusted for age, sex, BMI, and 5 genetic principal components.

pathways may underpin the heightened T2D risk observed in *SLC16A11* carriers.

The concentrations of betaine and methylmalonylcarnitine, which changed over visits, have been found to increase in vitro in hepatocytes. Betaine is a gut microbiome derivate osmolytes, and its concentration in plasma is in part dependent on dietary intake [39]. Betaine plays a key role in the synthesis of choline, an essential metabolite that has been associated with the risk of T2D [38,39]. In the liver, betaine serves as a methyl donor in a reaction catalyzed by betaine homocysteine methyltransferase, which converts homocysteine to methionine, a precursor of the universal methyl donor S-adenosylmethionine, with an essential role in several methylation processes [39,40]. By decreasing S-adenosylmethionine availability, betaine deficiency may decrease phosphatidylcholine synthesis, promote hepatic steatosis, and modify VLDL synthesis and secretion [40,41]. Betaine was shown to be inversely associated with triglycerides and phospholipid transfer protein activity, suggesting that low betaine concentrations may alter liver fat accumulation and lipid/lipoprotein metabolism [42]. In our study, we observed a trend of negative correlation between betaine concentrations and fasting triglycerides (β: 0.018; $P = 0.06$). Moreover, lower plasma betaine concentrations have been reported in subjects with metabolic syndrome, T2D, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis and may also predict the future development of T2D [39–42]. Our results showed a decrease in betaine concentrations in carriers over time. This behavior might suggest an additional aspect of the deleterious metabolic profile of *SLC16A11*, as lower betaine concentrations may alter metabolic traits and lead to the accumulation of lipids, consistent with the phenotype observed in the in vitro hepatocytes.

Methylmalonylcarnitine exhibited varying concentrations between carriers and noncarriers across the 2 visits. Previous studies have observed an association between acylcarnitines concentrations and T2D [38,43], cardiovascular disease, and stroke alone in participants at high risk of cardiovascular disease [44]. Elevated concentrations of long-chain acylcarnitines in serum or plasma serve as markers of incomplete fatty acid oxidation and disruptions in carbohydrate and lipid metabolism [13], as evidenced by their association with postprandial glucose concentrations in our study. It is hypothesized that the increased intracellular presence of long-chain acylcarnitines acts as a feedback mechanism for insulin action [37], potentially indicating enhanced postprandial fatty acid utilization. Moreover, carnitine is endogenously synthesized: the majority is obtained from the diet [45]. Our results show that higher consumption of unsaturated fatty acids was associated with lower concentrations of methylmalonylcarnitine. Serum carnitine concentration is lower in vegetarians and in patients on parenteral diet than that in omnivores, suggesting that serum carnitine is a potential marker of meat intake [46]. The dietary changes related to the reduction of red meat consumption and an increment of protein

sources and sea food observed in our study sample [32] can be leading to the reduction of methylmalonylcarnitine in *SLC16A11* gene carriers. Additionally, the apparent specificity of our findings for this particular carnitine compound could be attributed to insufficient statistical power, as other carnitines exhibited similar trends but did not achieve statistical significance.

This study has several strengths, first, the utilization of a longitudinal design with a robust statistical approach enabled us to evaluate causal associations in changes in metabolomic profile and treatment responses under the most cost-effective intervention to prevent T2D. Additionally, the adjustment for important covariates, such as population substructure, enabled us to draw more reliable conclusions. We acknowledge several limitations in our study. First, the sample size may have constrained our statistical power to detect significant differences in various outcomes. Second, the absence of replication in an external sample limits the generalizability of our findings. Finally, our metabolomic analysis used a single LC-MS method targeting ~200 metabolites, potentially restricting the identification of additional metabolites. Future investigations should prioritize cohorts with larger sample sizes and extended follow-up durations to explore additional outcomes, such as the incidence of T2D.

Our results sheds light on potential pathways of individual's response to common and cost-effective treatments such as LSI to reduce T2D risk, highlighting a promising application of personalized nutrition approaches anchored in genetics to prevent T2D. By identifying metabolomic biomarkers, particularly carnitines and their modulators, this study elucidates a targeted mechanism of action of LSIs. Future studies are needed to replicate these associations and expand to longer follow-ups. If replicated and expanded, the implementation of these results may significantly contribute to alleviating the disease burden of T2D within the Mexican population and contribute to the efforts in improving the effectiveness of the interventions in individuals with prediabetes.

Acknowledgments

We express our gratitude to dietitians involved in the study recruitment: Pedro Cisneros-Guzman and Laura Lazaro-Carrera; and the following from the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán: Carmen Moreno, Lucia Guillen, and Rosario Rodríguez-Guillen.

Author contributions

The authors' responsibilities were as follows – MS-G, MS-G: implemented the study, M-SG & MFG-G: drafted the manuscript, MS-G & TT-L: conceived the idea, MS-G: designed the study designed, MS-G, AV-V performed the statistical analysis, MLO-S, TT-L: performed the genetic analysis, CBC: performed the metabolomic sample analysis, MS-G, PA-V, ACM-G: interpreted the results ; and all authors: have read and approved the final manuscript.

Conflicts of interest

The authors report no conflicts of interest.

Funding

MSG is supported by the American Diabetes Association grant 9-22-PDFPM-04 and by the NIH NIDDK grant 5U24DK132733-02.

Data availability

The summary statistics for the genetic variant-metabolite associations will be accessible upon the publication of this study. Detailed data can be provided upon reasonable request to the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cdnut.2024.104444.

References

[1] M.A.B. Khan, M.J. Hashim, J.K. King, R.D. Govender, H. Mustafa, J. Al Kaabi, Epidemiology of type 2 diabetes—global burden of disease and forecasted trends, J. Epidemiol. Glob. Health. 10 (1) (2020) 107–111.
[2] N.H. Cho, J.E. Shaw, S. Karuranga, Y. Huang, J.D. da Rocha Fernandes, A.W. Ohlrogge, et al., IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045, Diabetes Res. Clin. Pract. 138 (2018) 271–281.
[3] American Diabetes Association Professional Practice Committee, 2, Classification and diagnosis of diabetes: standards of medical care in diabetes—2022, Diabetes Care 45 (Suppl 1) (2022) S17–S38.
[4] N. Bansal, Prediabetes diagnosis and treatment: a review, World J Diabetes 6 (2) (2015) 296–303.
[5] X. Cai, Y. Zhang, M. Li, J.H.Y. Wu, L. Mai, J. Li, et al., Association between prediabetes and risk of all cause mortality and cardiovascular disease: updated meta-analysis, BMJ 370 (2020) m2297.
[6] A. Mahajan, C.N. Spracklen, W. Zhang, M.C.Y. Ng, L.E. Petty, H. Kitajima, et al., Multi-ancestry genetic study of type 2 diabetes highlights the power of diverse populations for discovery and translation, Nat. Genet. 54 (5) (2022) 560–572.
[7] K. Suzuki, K. Hatzikotoulas, L. Southam, H.J. Taylor, X. Yin, K.M. Lorenz, et al., Multi-ancestry genome-wide study in >2.5 million individuals reveals heterogeneity in mechanistic pathways of type 2 diabetes and complications [Preprint], medRxiv (2023), Mar 31: 2023.03.31.23287839, https://doi.org/10.1101/2023.03.31.23287839.
[8] A.L. Williams Amy, S.B.R. Jacobs Suzanne, H. Moreno-Macías, A. Huerta-Chagoya, C. Churchhouse, C. Márquez-Luna, et al., Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico, Nature 506 (7486) (2014) 97–101.
[9] B.A. Hidalgo, T. Sofer, Q. Qi, N. Schneiderman, Y.I. Chen, R.C. Kaplan, et al., Associations between SLC16A11 variants and diabetes in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), Sci. Rep. 9 (1) (2019) 1–7.
[10] V. Rusu, E. Hoch, J.M. Mercader, M. Gymrek, M. von Grotthuss, P. Fontanillas, et al., Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms, Cell 170 (1) (2017) 199–212.e20.
[11] P. Almeda-Valdes, D.V.G. Velasco, O.A. Campos, O.Y. Bello-Chavolla, M.D.R. Sevilla-González, T.V. Ruiz, et al., The SLC16A11 risk haplotype is associated with decreased insulin action, higher transaminases and large-size adipocytes, Eur. J. Endocrinol. 180 (2) (2019) 99–107.
[12] C.A. Aguilar-Salinas, M.T. Tusie Luna, The role of SLC16A11 variations in diabetes mellitus, Curr. Opin. Nephrol. Hypertens. 32 (5) (2023) 445–450.

[13] Q. Yang, A. Vijayakumar, B.B. Kahn, Metabolites as regulators of insulin sensitivity and metabolism, Nat. Rev. Mol. Cell Biol. 19 (10) (2018) 654–672.
[14] B. Lair, C. Laurens, B. Van Den Bosch, C. Moro, Novel insights and mechanisms of lipotoxicity-driven insulin resistance, Int. J. Mol. Sci. 21 (17) (2020) 1–27.
[15] A. Ainbinder, L. Zhao, P. Glover, K. Gelinas-Roa, V. Rusu, A. Harney, et al., Mct11 deficiency alters hepatic glucose metabolism and energy homeostasis [Preprint], bioRxiv (2021), Sep 8:2021.09.08.459307, https://doi.org/10.1101/2021.09.08.459307.
[16] S. Srinivasan, L. Chen, J. Todd, J. Divers, S. Gidding, S. Chernausek, et al., The first genome-wide association study for type 2 diabetes in youth: the Progress in Diabetes Genetics in Youth (ProDiGY) consortium, Diabetes 70 (4) (2021) 996–1005.
[17] L. Mardones, F. Petermann-Rocha, M.A. Martinez-Sanguinetti, A.M. Leiva, C. Troncoso-Pantoja, M. Martorell, et al., Genetic variants in the SLC16A11 gene are associated with increased BMI and insulin levels in nondiabetic Chilean population, Arch. Endocrinol. Metab. 65 (3) (2021) 305–314.
[18] O.Y. Bello-Chavolla, J.P. Bahena-López, A. Vargas-Vázquez, N.E. Antonio-Villa, A. Márquez-Salinas, C.A. Fermín-Martínez, et al., Clinical characterization of data-driven diabetes subgroups in Mexicans using a reproducible machine learning approach, BMJ Open Diabetes Res. Care 8 (1) (2020) e001550.
[19] A.J. Deutsch, E. Ahlqvist, M.S. Udler, Phenotypic and genetic classification of diabetes, Diabetologia 65 (11) (2022) 1758–1769.
[20] M. Del Rocío Sevilla-González, J. Merino, H. Moreno-Macias, R. Rojas-Martínez, D.V. Gómez-Velasco, A.K. Manning, Clinical and metabolomic predictors of regression to normoglycemia in a population at intermediate cardiometabolic risk, Cardiovasc. Diabetol. 20 (1) (2021) 56.
[21] C.A. Aguilar-Salinas, T. Tusie-Luna, P. Pajukanta, Genetic and environmental determinants of the susceptibility of Amerindian derived populations for having hypertriglyceridemia, Metabolism 63 (7) (2014) 887–894.
[22] J.A. Seiglie, R.R. Franco, V.J. Wirtz, J.B. Meigs, M.A. Mendoza, J.J. Miranda, et al., Regional and state-level patterns of type 2 diabetes prevalence in Mexico over the last three decades, Diabetes Res. Clin. Pract. 177 (2021) 108927.
[23] IDF Diabetes Atlas, IDF Diabetes Atlas [Internet] [cited 2023 May 5]. Available from: https://diabetesatlas.org/atlas/tenth-edition/, 2021.
[24] M. Del Rocio Sevilla-Gonzalez, B. Bourguet-Ramirez, L.S. Lazaro-Carrera, A.J. Martagon-Rosado, D.V. Gomez-Velasco, T.L. Viveros-Ruiz, Evaluation of a web platform to record lifestyle habits in subjects at risk of developing type 2 diabetes in a middle-income population: prospective interventional study, JMIR Diabetes 7 (1) (2022) e25105.
[25] M. Uusitupa, T.A. Khan, E. Viguiliouk, H. Kahleova, A.A. Rivellese, K. Hermansen, et al., Prevention of type 2 diabetes by lifestyle changes: a systematic review and meta-analysis, Nutrients 11 (11) (2019) 2611.
[26] P.L. Toi, T. Anothaisintawee, U. Chaikledkaew, J.R. Briones, S. Reutrakul, A. Thakkinstian, Preventive role of diet interventions and dietary factors in type 2 diabetes mellitus: an umbrella review, Nutrients 12 (9) (2020) 1–17.
[27] W.C. Knowler, E. Barrett-Connor, S.E. Fowler, R.F. Hamman, J.M. Lachin, E.A. Walker, et al., Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin, N. Engl. J. Med. 346 (6) (2002) 393–403.
[28] M. Delgado-Velandia, V. Gonzalez-Marrachelli, A. Domingo-Relloso, M. Galvez-Fernandez, M. Grau-Perez, P. Olmedo, et al., Healthy lifestyle, metabolomics and incident type 2 diabetes in a population-based cohort from Spain, Int. J. Behav. Nutr. Phys. Act. 19 (1) (2022) 8.
[29] K. Watanabe, T. Wilmanski, C. Diener, J.C. Earls, A. Zimmer, B. Lincoln, et al., Multiomic signatures of body mass index identify heterogeneous health phenotypes and responses to a lifestyle intervention, Nat. Med. 29 (4) (2023) 996–1008.
[30] D.D. Wang, F.B. Hu, Precision nutrition for prevention and management of type 2 diabetes, Lancet Diabetes Endocrinol 6 (5) (2018) 416–426.
[31] D.K. Tobias, J. Merino, A. Ahmad, C. Aiken, J.L. Benham, D. Bodhini, et al., Second international consensus report on gaps and opportunities for the clinical translation of precision diabetes medicine, Nat. Med. 29 (10) (2023) 2438–2457.
[32] M.D.R. Sevilla-Gonzalez, A.K. Manning, K.E. Westerman, C.A. Aguilar-Salinas, A. Deik, C.B. Clish, Metabolomic markers of glucose regulation after a lifestyle intervention in prediabetes, BMJ Open Diabetes Res. Care 10 (5) (2022) e003010.
[33] N. Hakel-Smith, N.M. Lewis, A standardized nutrition care process and language are essential components of a conceptual model to guide and document nutrition care and patient outcomes, J. Am. Diet. Assoc. 104 (12) (2004) 1878–1884.
[34] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (7) (1985) 412–419.
[35] A. Huerta-Chagoya, H. Moreno-Macías, J.C. Fernández-López, M.L. Ordóñez-Sánchez, R. Rodríguez-Guillén, A. Contreras, et al., A panel of 32 AIMs suitable for population stratification correction and global ancestry estimation in Mexican mestizos, BMC Genet 20 (1) (2019) 5.
[36] P.C. Austin, E.W. Steyerberg, The number of subjects per variable required in linear regression analyses, J. Clin. Epidemiol. 68 (6) (2015) 627–636.
[37] M.G. Schooneman, F.M. Vaz, S.M. Houten, M.R. Soeters, Acylcarnitines: reflecting or inflicting insulin resistance? Diabetes 62 (1) (2013) 1–8.
[38] Y. Sun, H.Y. Gao, Z.Y. Fan, Y. He, Y.X. Yan, Metabolomics signatures in type 2 diabetes: a systematic review and integrative analysis, J. Clin. Endocrinol. Metab. 105 (4) (2020) 1000–1008.
[39] C.R. Day, S.A. Kempson, Betaine chemistry, roles, and potential use in liver disease, Biochim. Biophys. Acta. 1860 (6) (2016) 1098–1106.
[40] E. Garcia, M.C.J. Osté, D.W. Bennett, E.J. Jeyarajah, I. Shalaurova, E.G. Gruppen, et al., High betaine, a trimethylamine N-oxide related metabolite, is prospectively associated with low future risk of type 2 diabetes mellitus in the PREVEND study, J. Clin. Med. 8 (11) (2019) 1813.
[41] S. Sookoian, P. Puri, G.O. Castaño, R. Scian, F. Mirshahi, A.J. Sanyal, et al., Nonalcoholic steatohepatitis is associated with a state of betaine-insufficiency, Liver Int 37 (4) (2017) 611–619.
[42] R.P.F. Dullaart, E. Garcia, E. Jeyarajah, E.G. Gruppen, M.A. Connelly, Plasma phospholipid transfer protein activity is inversely associated with betaine in diabetic and non-diabetic subjects, Lipids Health Dis 15 (1) (2016) 143.
[43] J. Morze, C. Wittenbecher, L. Schwingshackl, A. Danielewicz, A. Rynkiewicz, F.B. Hu, et al., Metabolomics and type 2 diabetes risk: an updated systematic review and meta-analysis of prospective cohort studies, Diabetes Care 45 (4) (2022) 1013–1024.
[44] M. Guasch-Ferré, Y. Zheng, M. Ruiz-Canela, A. Hruby, M.A. Martínez-González, C.B. Clish, et al., Plasma acylcarnitines and risk of cardiovascular disease: effect of Mediterranean diet interventions, Am. J. Clin. Nutr. 103 (6) (2016) 1408–1416.
[45] A.J. Kuszak, M.G. Espey, M.J. Falk, M.A. Holmbeck, G. Manfredi, G.S. Shadel, et al., Nutritional interventions for mitochondrial OXPHOS deficiencies: mechanisms and model systems, Annu. Rev. Pathol. 13 (2018) 163–191.
[46] S.S. Patel, M.Z. Molnar, J.A. Tayek, J.H. Ix, N. Noori, D. Benner, et al., Serum creatinine as a marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature, J. Cachexia Sarcopenia Muscle 4 (1) (2013) 19–29.