Original Research *Highlight article*

Association of plasma acylcarnitines with insulin sensitivity, insulin secretion, and prediabetes in a biracial cohort

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Impact statement

Prediabetes affects ~90 million adults in the US and ~400 million adults worldwide, so the discovery of biomarkers for prediabetes risk would be valuable at the clinical and public health levels. Previous cross-sectional studies have associated alterations in plasma acylcarnitine levels with diabetes and prediabetes risk, but data from prospective studies are lacking. The present report identified one medium-chain and two short-chain acylcarnitines in plasma (out of 45 analyzed) as significant predictors of the risk of progression from normoglycemia to prediabetes during a 5.5-year prospective follow-up period. Based on the strength of the prospective design and the enrollment of a diverse cohort comprising African Americans and European Americans, the present findings support the existence of a signature of acylcarnitines that may serve as a biomarker for prediabetes risk.

Abstract

The ability to predict prediabetes, which affects ~90 million adults in the US and ~400 million adults worldwide, would be valuable to public health. Acylcarnitines, fatty acid metabolites, have been associated with type 2 diabetes risk in cross-sectional studies of mostly Caucasian subjects, but prospective studies on their link to prediabetes in diverse populations are lacking. Here, we determined the association of plasma acylcarnitines with incident prediabetes in African Americans and European Americans enrolled in a prospective study. We analyzed 45 acylcarnitines in baseline plasma samples from 70 adults (35 African-American, 35 European-American) with incident prediabetes (progressors) and 70 matched controls (non-progressors) during 5.5-year (mean 2.6 years) follow-up in the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study. Incident prediabetes (impaired fasting glucose/impaired glucose tolerance) was confirmed with OGTT. We measured acylcarnitines using tandem mass spectrometry, insulin sensitivity by hyperinsulinemic euglycemic clamp, and insulin secretion using intravenous glucose tolerance test. The results showed that progressors and non-progressors during POP-ABC study follow-up were concordant for 36 acylcarnitines and discordant for nine others. In logistic regression models, beta-hydroxy butyryl carnitine (C4-OH), 3-hydroxy-isovaleryl carnitine/malonyl carnitine (C5-OH/C3-DC), and octenoyl carnitine (C8:1) were the only significant predictors of incident prediabetes. The combined cut-off plasma levels of <0.03 micromol/L for C4-OH, <0.03 micromol/L for C5-OH/C3-DC, and >0.25 micromol/L for C8:1 acylcarnitines predicted incident prediabetes with 81.9% sensitivity and 65.2% specificity. Thus, circulating levels of one medium-chain and two short-chain acylcarnitines may be sensitive biomarkers for the risk of incident prediabetes among initially normoglycemic individuals with parental history of type 2 diabetes.

Keywords: Fatty acids, metabolites, octenoyl carnitine, insulin sensitivity, race/ethnicity, impaired fasting glucose, impaired glucose tolerance

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Introduction

Acylcarnitines are fatty acid metabolites that have been associated with insulin resistance and type 2 diabetes (T2D).[1–4] Following absorption, dietary lipids are transported via lymphatic vessels to the systemic circulation as long-chain fatty acyl-CoA. The long-chain fatty acyl-CoA molecules enter the mitochondria via the carnitine shuttle, a process that involves their conversion to acylcarnitines by the outer mitochondrial membrane enzyme carnitine palmitoyltransferase-1 (CPT-1). [5–7] Next, follows the delivery of the acylcarnitines into the mitochondrial matrix by the mitochondrial inner membrane transporter carnitine acylcarnitine translocase. From there the enzyme CPT-2 reconverts the acylcarnitines back into long-chain

acyl-CoA for oxidation.[5–7] Plasma acylcarnitines generally reflect efflux from mitochondria into the circulation, due to mitochondrial overload and incomplete fatty acid oxidation.[8–10]

Cross-sectional studies have reported alterations in plasma levels of short-, medium- or long-chain acylcarnitines in people with T2D or prediabetes versus healthy subjects with normal glucose tolerance (NGT), and in people with insulin resistant states, such as obesity, versus insulin-sensitive people.[1–4] However, these cross-sectional reports have been inconsistent regarding the specific acylcarnitines molecules that are altered when comparing healthy people with those who have T2D or prediabetes.[1–4] Further, the cross-sectional design of these published studies does not permit directional or causal inferences regarding the association between acylcarnitines and states of glucose dysregulation represented by diabetes and prediabetes.

In the Pathobiology of Prediabetes in a Biracial Cohort (POP-ABC) study, we screened African American (AA) and European American (EA) adult offspring of parents with T2D, enrolled those with normoglycemia, followed them for 5.5 years for the primary outcome of incident prediabetes (impaired fasting glucose [IFG] or impaired glucose tolerance [IGT]}. [11–13] Prediabetes affects one-third of adults in the US and is associated with the risks of progression to T2D and development of vascular complications.[14] The POP-ABC study has identified numerous baseline behavioral, clinical, and biochemical factors associated with the risk of progression from normoglycemia to prediabetes in our diverse study population.[13,15–19] Moreover, the POP-ABC data showed that incident prediabetes occurred at similar rates among offspring with either one parent or both parents with T2D.[13] In the present report, we measured a panel of 45 acylcarnitines in baseline plasma specimens to test the hypothesis that a signature of acylcarnitines would be predictive of incident prediabetes among initially normoglycemic offspring of parents with T2D.

Materials and methods

Study subjects

We selected 70 POP-ABC study participants (35 AA, 35 EA) who progressed from normoglycemia to prediabetes (progressors) and 70 participants who maintained normoglycemia (non-progressors) during 5.5 years of follow-up (mean 2.62 years).[11–13] The POP-ABC distributions of age, sex, and ethnicity were used in matching progressors and non-progressors in the nested cohort. As previously reported in the design of the overall POP-ABC study, inclusion criteria were age 18–65 years, White (EA) or Black (AA) race/ethnicity status, parental history of T2D, normal fasting plasma glucose (FPG; <100 mg/dL[5.6 mmol/L]) and/or NGT (2-h plasma glucose [2hrPG] <140 mg/dL [7.8 mmol/L]) during a 75-g oral glucose tolerance test (OGTT).[11–13,20] By design, the POP-ABC study enrolled 75% of participants who had both normal FPG and normal 2hrPG and 25% with either normal FPG or normal 2hrPG.[11–13] The exclusion criteria were a history of diabetes (including gestational diabetes), use of medications that affect body weight or blood glucose, and enrollment in medical or surgical weight-loss interventions.[11–13] The University of Tennessee Institutional Review Board approved the POP-ABC study protocol; all participants gave written informed consent before undergoing study procedures at the University of Tennessee General Clinical Research Center (GCRC).

Assessments

Study subjects fasted overnight before arriving at the GCRC. During the baseline visit, a medical history and physical examination were performed; the height, weight, and waist circumference were measured; and a standard 75-g OGTT was completed.[11–13] We derived the body-mass index (BMI) as the weight in kilogram divided by the height in meter squared. The Food Habits Questionnaire was used to capture nutritional data, as described previously.[14,21] The HbA1c and fasting lipid profile were also measured at baseline and repository blood specimens were obtained. Participants were then followed quarterly at the GCRC for 5.5 years (mean 2.62 years) for scheduled procedures. During those visits, the FPG was measured quarterly; OGTT, insulin secretion, and body fat analysis (dual energy x-ray absorptiometry (DEXA)) were assessed annually; and insulin sensitivity was measured in years 1, 3, and 5.[11–13] The primary outcome of the POP-ABC study was progression to prediabetes, as defined by IFG (FPG 100–125 mg/dL or 5.6–6.9 mmol/L) and/or IGT (2hrPG 140–199 mg/dL or 7.8–11.0 mmol/L). [11–13] Participants who met these endpoints were classified as progressors and those who remained normoglycemic during follow-up were non-progressors. The Institutional Data and Safety Officer (Murray Heimberg, MD, PhD) independently adjudicated the endpoints.

Measurement of insulin sensitivity and insulin secretion

We measured insulin sensitivity with the hyperinsulinemic euglycemic clamp and insulin secretion with the frequently sampled intravenous glucose tolerance test (IVGTT), as previously described.[11–13,22,23] In brief, overnight fasted participants received continuous i.v. infusion of regular human insulin (2 mU kg^{-1} min^{-1};12 pmol kg^{-1} min^{-1}) for 3 h along with dextrose (20%) infused at a variable rate to maintain target normoglycemia (~100 mg/dL of 5.6 mmol/L). Arterialized blood samples were drawn every 10 min for measurement of glucose and insulin. Insulin sensitivity index (Si-clamp) was derived from the steady-state (final 60 min) glucose disposal rate (*M*) corrected for the steady-state plasma insulin levels.[22] The mean steady-state plasma insulin level during the clamp procedure was 857 ± 316 pmol/L.

We measured insulin secretion with the IVGTT after an overnight fast. Arterialized blood samples for glucose and insulin levels were obtained 30 min before and at 2, 3, 4, 5, 7, and 10 min following the i.v. dextrose (25 g) bolus.[11–13] The acute insulin response to glucose (AIRg) was calculated as the mean incremental insulin level at 3, 4 and 5 min after the dextrose bolus. [11–13]

Biochemical measurements

We measured plasma glucose and insulin in-house, using the YSI glucose analyzer (Yellow Spring Instruments Co., Inc., Yellow Spring, OH) and a chemiluminescent insulin assay (Immulite, Siemens Ltd., Llanberis, Gwynedd, UK), and hemoglobin A1c (HbA1c) in a contract clinical laboratory. The insulin assay had a sensitivity of 2 μIU/mL and within-batch and between-batch variation coefficients of 4.7% and 8%, respectively.

Measurement of acylcarnitines

Plasma acylcarnitine levels were analyzed using stable isotope dilution techniques, under contract, at the Duke University Metabolomics Laboratory (Durham, NC). The blood specimens had been collected during the baseline POP-ABC study visit in EDTA tubes and, after separation, plasma samples were stored at −80°C prior to assay. Acylcarnitines were measured using flow injection tandem mass spectrometry, as described previously.[24,25] Plasma specimens were injected directly into the mass spectrometer, without prior chromatographic separation. Thus, the concentrations of isomers and isobars (compounds with the same mass-to-charge ratio) were not reported separately. The limit of quantitation for the acylcarnitines assay was ∼0.01 μM.

Statistical analysis

Data are means ± SD. Unpaired *t* test and chi-squared test were used to analyze continuous and categorical variables, respectively; linear regression models and Pearson correlation coefficients were used to analyze the associations between plasma acylcarnitine levels and insulin sensitivity and insulin secretion. Acylcarnitines with significant differences in progressors versus non-progressors at the $P < 0.15$ level in univariate analyses were further analyzed using stepwise logistic regression models, adjusted for age, sex, race/ethnicity, and BMI. The analyses were conducted using SAS (version 9.4, Cary, NC, USA). $P < 0.05$ was deemed significant.

Results

Cohort characteristics

The baseline characteristics of progressors to prediabetes and non-progressors are summarized in Table 1. The mean enrollment age, 2hrPG, HbA1c, insulin sensitivity, insulin secretion, and self-reported scores for food habits were similar in progressors and non-progressors. Both groups had equal representation of AA and EA subjects, and the female:male ratios did not differ significantly. The baseline BMI and FPG values were higher in progressors than non-progressors (Table 1).

Total acylcarnitine levels

The total plasma acylcarnitine level (the sum of all 45 acylcarnitines assayed) was 9.09 ± 2.17 μM for the participants in the present study. Values were similar in AA vs. EA participants (8.79 ± 2.36 micromol/L vs. 9.39 ± 1.93 micromol/L, $P = 0.10$); men vs. women (8.99 ± 2.04 micromol/L vs. 9.21 ± 2.24 micromol/L, $P = 0.37$); and progressors vs. non-progressors (9.00 ± 2.12 micromol/L vs. 9.18 ± 2.24 micromol/L, $P = 0.63$). Analyzed within each sex, total plasma acylcarnitines were similar in AA vs. EA women (8.81 ± 2.33 micromol/L vs. 9.64 ± 2.03 micromol/L, $P = 0.079$) and in AA men vs. EA men (8.74 ± 2.48 micromol/L vs. 8.94 ± 2.57 micromol/L, $P = 0.67$). The total plasma acylcarnitine level correlated significantly with age ($r = 0.22$, $P = 0.01$) and insulin sensitivity ($r = 0.20$, $P = 0.05$) but not weight, BMI, total body fat mass, trunk fat mass, 2hrPG or total cholesterol (Supplemental Table S1). Further, there was no significant association between total plasma acylcarnitine level and components of the metabolic syndrome, including waist circumference, FPG, systolic blood pressure, HDL cholesterol or triglycerides (Supplemental Table S1).

Acylcarnitine levels and incident prediabetes

Of the 45 acylcarnitines analyzed in baseline specimens, progressors to prediabetes and non-progressors had similar values for 36 and significantly different values for 9 acylcarnitines. Table 2 shows plasma levels of the nine discordant acylcarnitines. Compared with values in non-progressors,

Table 1. Baseline characteristics of study participants.

Characteristics	All	Non-progressors	Progressors	*P* value
Number (AA/EA)	140 (70/70)	70 (35/35)	70 (35/35)	
Female/Male	90/50	48/22	42/28	0.29
Age (yrs)	48.1 ± 8.69	47.6 ± 9.16	48.5 ± 8.23	0.55
BMI (kg/m^2)	30.1 ± 5.78	29.03 ± 5.22	31.3 ± 6.12	0.02
FPG (mg/dL)	92.7 ± 5.84	91.3 ± 6.18	94.03 ± 5.17	0.006
2hrPG (mg/dL)	121 ± 23.3	119 ± 25.02	123 ± 21.4	0.37
HbA1c (%)	5.56 ± 0.47	5.51 ± 0.42	5.62 ± 0.51	0.17
Si-clamp	0.127 ± 0.067	0.138 ± 0.068	0.118 ± 0.065	0.14
AIRg (μU/mL)	84.1 ± 75.4	88.8 ± 89.7	79.6 ± 59.6	0.49
FHQ score	2.56 ± 0.49	2.51 ± 0.49	2.56 ± 0.50	0.52

AA, African-American; EA: European-American; AIRg: acute insulin response to i.v. glucose; BMI: body-mass index; FHQ: food habits questionnaire; FPG: fasting plasma glucose; 2hrPG: 2-h plasma glucose during 75-g oral glucose tolerance test; Si-clamp: insulin sensitivity by euglycemic clamp; to convert the values for glucose to millimoles per liter, multiply by 0.0555; to convert the values for insulin (AIRg) to picomoles per liter, multiply by 6.0.
Data are means ± SD.

progressors had lower baseline plasma levels, lower levels of beta-hydroxy butyryl carnitine (C4-OH), C5-DC (glutaryl carnitine), 3-hydroxy-isovaleryl carnitine/malonyl carnitine (C5-OH/C3-DC), myristoyl carnitine (C14), palmitoyl carnitine (C16), stearoyl carnitine (C18), and linoleoyl carnitine (C18:2; $P = 0.02$–<0.0001) and higher levels of octenoyl carnitine (C8:1; $P = 0.02$). The levels of methylmalonyl carnitine/succinyl carnitine (C4-DC/Ci4-DC) were borderline lower in progressors versus non-progressors ($P = 0.0689$). Using stepwise logistic regression models that included all nine acylcarnitines, we identified C4-OH ($P = 0.0025$) and C8:1 ($P = 0.01$) as significant predictors of progression to prediabetes. In the fully adjusted model (controlling for age, sex, race/ethnicity, BMI, and plasma glucose), the acylcarnitines significantly associated with incident prediabetes were C4-OH (odds ratio 0.656 [95% CI 0.449–0.956], $P = 0.0284$) and C5-OH/C3-DC (odds ratio 0.516 [95% CI 0.290–0.919], $P = 0.0246$), with C8:1 showing a trend (Table 3).

Furthermore, we performed receiver operating characteristic (ROC) curve analyses to determine the sensitivity and specificity of the three acylcarnitines identified as significant predictors of incident prediabetes (Figure 1 and Table 4). A plasma C4-OH level of 0.03 micromol/L or lower predicted incident prediabetes with 69% sensitivity and 64% specificity, the same plasma level cut-off point for C5-OH/C3-DC had a sensitivity of 63% and a specificity of 57%. A plasma C8:1 level of 0.25 micromol/L or higher predicted incident prediabetes with 37% sensitivity and 73% specificity. The combination of all three acylcarnitines showed the greatest area under the curve (AUC) for prediabetes prediction compared with individual acylcarnitines or dual combinations (Figure 1 and Table 4). The combined cut-off plasma levels of 0.03 micromol/L or lower for C4-OH and C5-OH/C3-DC, respectively, and 0.25 micromol/L or higher for C8:1 predicted incident prediabetes with 81.9% sensitivity and 65.2% specificity (Table 4).

Table 2. Acylcarnitines with discordant baseline levels in progressors versus non-progressors to prediabetes.

		Plasma level (micromol/L)		
Acylcarnitine	**Common name**	**Progressors**	**Non-progressors**	***P* value**
C4-OH	Beta-hydroxy butyryl carnitine	0.027 ± 0.014	0.039 ± 0.016	<0.0001
C4-DC/Ci4-DC	Methylmalonyl carnitine/ Succinyl carnitine	0.037 ± 0.014	0.041 ± 0.013	0.0689
C5-OH/C3-DC	3-Hydroxy-isovaleryl carnitine/ Malonyl carnitine	0.029 ± 0.008	0.034 ± 0.012	0.0025
C8:1	Octenoyl carnitine	0.250 ± 0.135	0.204 ± 0.100	0.0240
C5-DC	Glutaryl carnitine	0.043 ± 0.013	0.049 ± 0.017	0.0244
C14	Myristoyl carnitine	0.019 ± 0.006	0.022 ± 0.007	0.0191
C16	Palmitoyl carnitine	0.069 ± 0.018	0.078 ± 0.019	0.0041
C18:2	Linoleoyl carnitine	0.041 ± 0.011	0.046 ± 0.012	0.0077
C18	Stearoyl carnitine	0.027 ± 0.008	0.031 ± 0.007	0.0026

Table 3. Logistic regression of acylcarnitines as predictors of incident prediabetes.

Acylcarnitine (μM)	**Common name**	**Point estimate**	**95% Confidence interval**		***P* value**
Model 1					
C4-OH	Beta-hydroxy butyryl carnitine	0.588	0.417	0.830	0.0025
C5-OH/C3-DC	3-Hydroxy-isovaleryl carnitine/Malonyl carnitine	0.612	0.374	1.002	0.0509
C4-DC/Ci4-DC	Methylmalonyl carnitine/Succinyl carnitine	0.969	0.662	1.419	0.8720
C8:1	Octenoyl carnitine	1.065	1.015	1.117	0.0109
C5-DC	Glutaryl carnitine	0.840	0.612	1.153	0.2801
C14	Myristoyl carnitine	0.725	0.310	1.698	0.4592
C16	Palmitoyl carnitine	1.078	0.786	1.479	0.6401
C18:2	Linoleoyl carnitine	0.777	0.507	1.192	0.2481
C18	Stearoyl carnitine	0.903	0.477	1.707	0.7532
Model 2					
C4_OH	Beta-hydroxy butyryl carnitine	0.656	0.449	0.956	0.0284
C5-OH/C3-DC	3-Hydroxy-isovaleryl carnitine/Malonyl carnitine	0.516	0.290	0.919	0.0246
C4-DC/Ci4-DC	Methylmalonyl carnitine/Succinyl carnitine	0.780	0.509	1.195	0.2534
C8:1	Octenoyl carnitine	1.054	0.994	1.118	0.0769
C5-DC	Glutaryl carnitine	0.744	0.509	1.088	0.1272
C14	Myristoyl carnitine	0.958	0.365	2.512	0.9305
C16	Palmitoyl carnitine	0.895	0.618	1.297	0.5577
C18:2	Linoleoyl carnitine	0.778	0.477	1.271	0.3165
C18	Stearoyl carnitine	0.779	0.383	1.586	0.4913

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, race/ethnicity, BMI, and fasting plasma glucose.

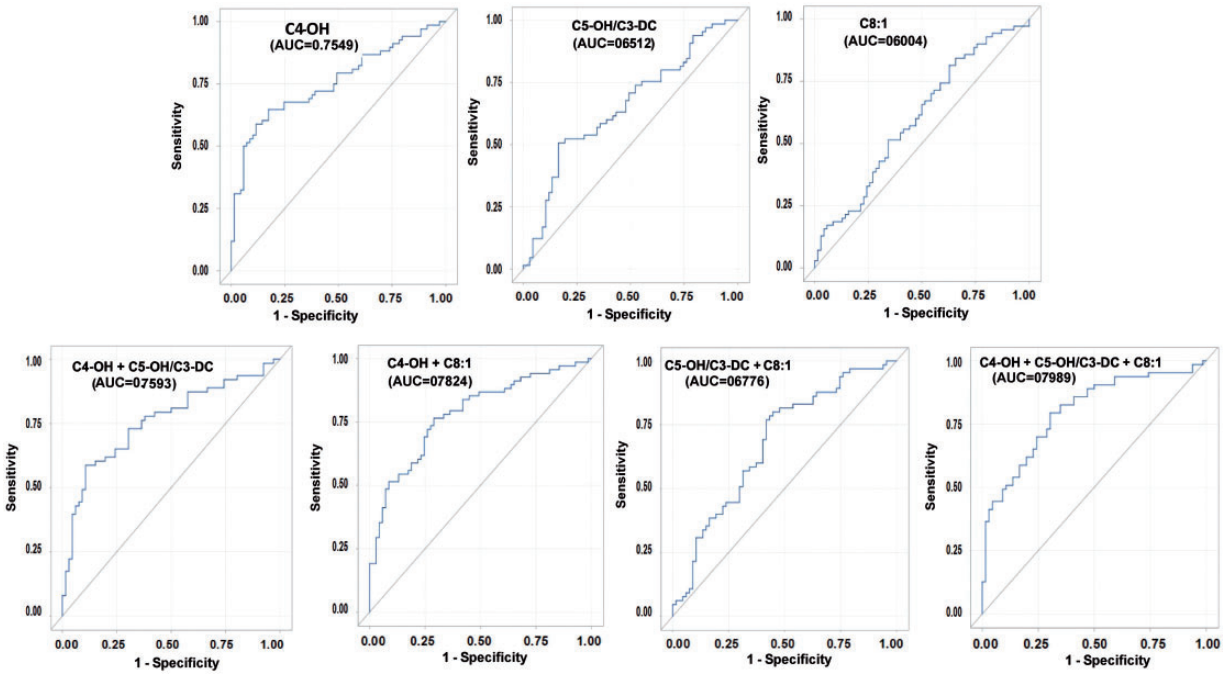

Figure 1. Receiver operating characteristics plots for individual acylcarnitines (upper panel) and their combinations (lower panel) as predictors in incident prediabetes. AUC: area under the curve.

Table 4. ROC analysis of acylcarnitines predicting progression to prediabetes.

Acylcarnitine	AUC	Cut-off point (μM)	Sensitivity	Specificity
C4-OH	0.7549	0.03	0.69	0.64
C5-OH/C3-DC	0.6512	0.03	0.63	0.57
C8:1	0.6004	0.25	0.37	0.73
C4-OH+ C5-OH/ C3-DC+ C8:1	0.7989	0.03/0.03/0.25	0.81	0.65

C4-OH: beta-hydroxy butyryl carnitine; C5-OH/C3-DC: 3-hydroxy-isovaleryl carnitine/malonyl carnitine; C8:1: octenoyl carnitine.

Association of acylcarnitines with insulin sensitivity and insulin secretion

We examined the associations between the levels of individual acylcarnitines and insulin sensitivity and insulin secretion. Among the eight acylcarnitines, whose levels were lower in progressors than non-progressors to prediabetes, there were significant direct correlations between insulin sensitivity and levels of the long-chain acylcarnitines C16 ($r = 0.28$, $P = 0.011$) and C18:2 ($r = 0.36$, $P = 0.0003$) and a similar trend for C14 and C18 (Supplemental Table S2 and Figure 2). In contrast, plasma levels of the medium-chain C8:1 (the only acylcarnitine with higher levels in progressors to prediabetes vs. non-progressors) were inversely correlated with insulin sensitivity ($r = -0.19$, $P = 0.046$; Supplemental Table S2 and Figure 2). No significant association was observed between insulin secretion and any of the discordant acylcarnitines, except for C5-DC (glutaryl carnitine; $r = -0.24$, $P = 0.005$; Supplemental Table S2).

We expanded our analysis to include all 45 acylcarnitines that were measured in the present study (Supplemental Table S2). As already noted in Supplemental Table S1, the total plasma level of all 45 acylcarnitines correlated with insulin sensitivity ($r = 0.2$, $P = 0.05$) but not with insulin secretion. Significant correlations were observed between the levels of each of 16 individual acylcarnitines and insulin sensitivity ($r = 0.15$–0.37; $P = 0.06$–0.0003). Further, plasma levels of each of the following three acylcarnitines showed significant inverse correlation with insulin secretion: C5-DC (glutaryl carnitine, $r = -0.24$, $P = 0.005$), C6-DC/C8-OH (methylglutaryl carnitine/3-hydroxy-octanoyl carnitine, $r = -0.25$, $P = 0.0038$ and C14:1-OH (3-hydroxy-tetradecenoyl carnitine, $r = -0.17$, $P = 0.047$). The levels of two acylcarnitines, C6-DC/C8-OH (methylglutaryl carnitine/3-hydroxy-octanoyl carnitine) and C14:1-OH (3-hydroxy-tetradecenoyl carnitine), were significantly correlated with both insulin sensitivity and insulin secretion. The levels of several other acylcarnitines showed nominal correlations with insulin sensitivity and insulin secretion (Supplemental Table S2).

Discussion

Based on the known association of obesity, insulin resistance and T2D with impaired fatty acid oxidation, and increased plasma acylcarnitine levels, we had hypothesized that elevated acylcarnitines at baseline would predict progression to prediabetes among initially normoglycemic POP-ABC study participants. The total

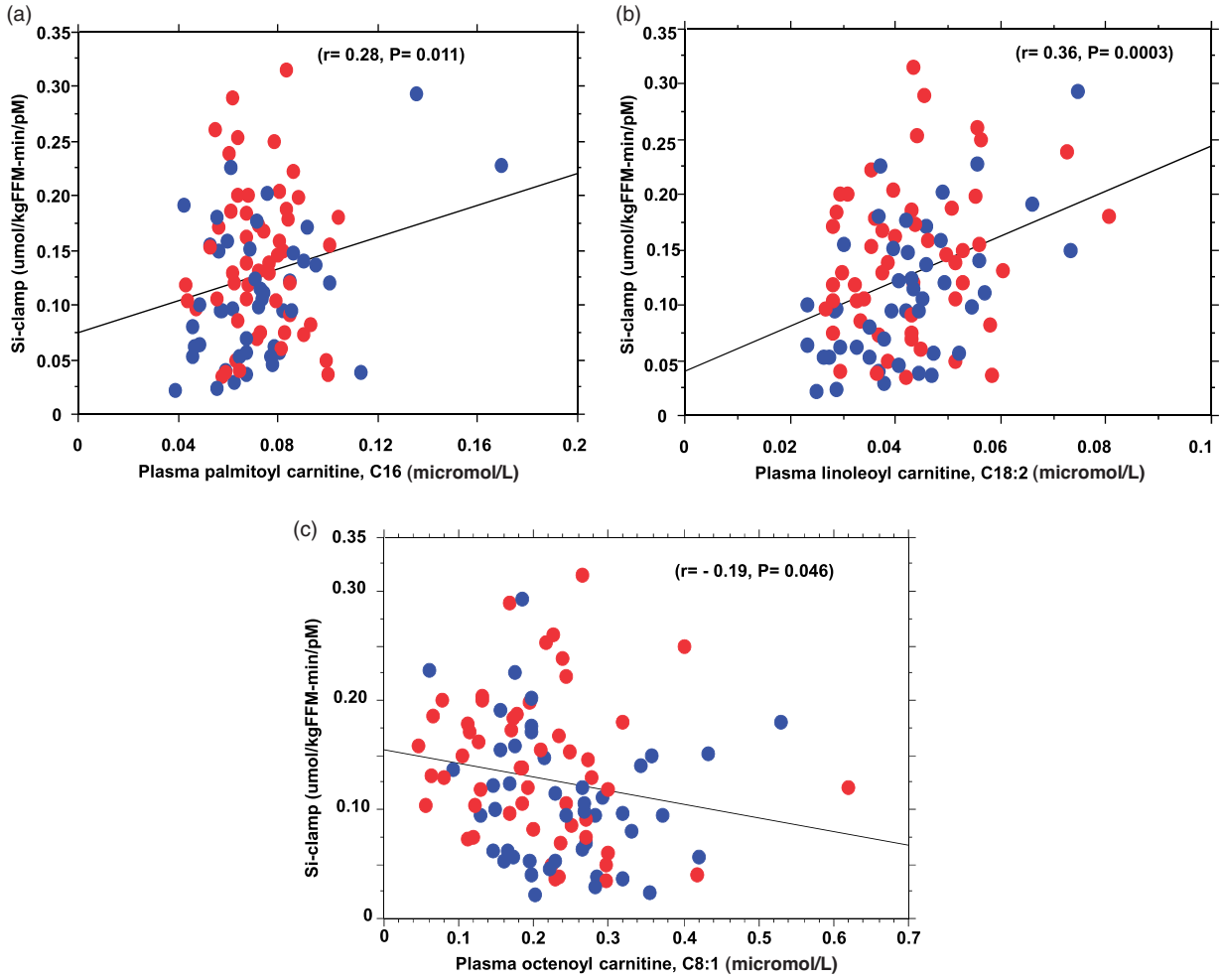

Figure 2. Direct correlation of insulin sensitivity (Si-clamp) with two long-chain (a, b) and inverse correlation with one medium-chain (c) plasma acylcarnitine levels in African-American (blue) and European-American (red) adults with parental type 2 diabetes. (A color version of this figure is available in the online journal.)

plasma acylcarnitine levels (sum of all 45 acylcarnitines assayed) were similar in progressors to prediabetes versus non-progressors. The levels of most of the individual acylcarnitines were also similar in progressors and non-progressors. However, logistic regression models and ROC analyses identified three acylcarnitines, whose baseline levels significantly predicted the risk of progression from normoglycemia to prediabetes during 5.5 years of follow-up of participants in the diverse POP-ABC study.

Although increased acylcarnitine levels, indicating incomplete fatty acid oxidation, is the general finding among people with insulin resistance and T2D, decreased levels have also been observed.[1–4] In a study of obese African-American women with or without T2D, the presence of diabetes was associated with higher levels of most of the 42 acylcarnitines studied.[4] Notably, the investigators reported that plasma levels of propionylcarnitine (C3) were lower in women with diabetes compared with BMI-matched control women.[4] In the present study, we observed that the combination of lower plasma levels of C4-OH and C5-OH/C3-DC, and higher levels of C8:1 acylcarnitines, predicted incident prediabetes with 81.9% sensitivity and 65.2% specificity. Compared with non-progressors, participants who progressed to prediabetes had higher BMI and FPG at baseline. However, the predictive value of the acylcarnitines identified in the present study remained significant after adjustment for BMI, age, sex, race/ethnicity, and FPG levels.

The absence of a generalized elevation of acylcarnitines at baseline in progressors versus non-progressors indicates that fatty acid oxidation was largely comparable in the two groups at enrollment, when all subjects were normoglycemic. The higher plasma acylcarnitine levels observed in people with obesity, insulin resistance or T2D can be decreased by insulin infusion.[1] Thus ambient insulinemia modulates circulating acylcarnitine levels, even in insulin-resistant states. Our POP-ABC study participants are all offspring of parents with T2D who were required to have normoglycemia at enrollment.[11–13] Given their high-risk parental history, obesity (mean BMI of ~30 kg/m^2) and insulin resistance, individuals who qualified for enrollment had sufficiently preserved insulin secretory capacity to maintain normoglycemia. In a previous report, we demonstrated that POP-ABC study participants had robust insulin secretion comparable to that of normoglycemic individuals without a family history of T2D.[26] Thus, our observation of relatively modest perturbations in baseline acylcarnitines in participants who subsequently progressed to T2D

probably reflects their normoglycemic and intact insulin secretory status at enrollment in the POP-ABC study. Indeed, there is support for a gradation toward higher acylcarnitine levels across the spectrum from IFG to IGT to T2D.[3]

The lower baseline levels of short-chain acylcarnitine observed in progressors could be due to impaired generation from branched-chain amino acids or increased oxidation of short-chain fatty acids. The latter would indicate a shift in fuel preference from glucose to short-chain fatty acids in the years preceding progression from normoglycemia to prediabetes. We do not have a clear physiological explanation for why C8:1 was the only acylcarnitine whose baseline level was higher in progressors than non-progressors. However, increased plasma levels of a closely related molecule, octanoyl carnitine (C8), are used clinically for the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency, an inborn error of metabolism and the most common disorder of fatty acid oxidation.[27]

Comparative data from prospective studies of initially normoglycemic individuals, similar to ours, are lacking in the literature. Cross-sectional studies have yielded conflicting reports. One such study reported higher levels of long-chain acylcarnitines, but similar levels of short- and medium-chain acylcarnitines, in subjects with prediabetes ($N = 20$) or T2D ($N = 21$) compared with healthy control ($N = 20$).[2] Another cross-sectional study comparing plasma levels of 24 acylcarnitines in 1019 subjects (635 with NGT, 271 with prediabetes and 112 with T2D) reported differences in short-, medium- and long-chain acylcarnitines across the glycemic groups.[3] Within the prediabetes group, the levels of C14:1, C14:2, and C18:1 were reported to be significantly higher in persons with IGT than those with IFG.[3] Because carbon atoms from glucose can serve as substrates for acylcarnitines, and hyperglycemia is associated with increased plasma acylcarnitines, it is difficult to interpret these cross-sectional data.[1,4,8,28,29] Our findings showing a signature of one medium-chain and two short-chain acylcarnitines predictive of incident prediabetes emanated from a milieu free of the confounding effects of hyperglycemia. In that regard, and along with the prospective design, our study does extend the findings of previous cross-sectional reports.[1–4]

Acylcarnitines are measurable in plasma under physiological conditions, with levels that fluctuate according to fasting or fed states.[8,30] In people with insulin resistance and T2D, elevated acylcarnitines indicate impaired fatty acid oxidation.[4,8–10] However, the metabolic significance of circulating acylcarnitines in healthy or less perturbed metabolic states is unclear, given the many sources of acylcarnitines besides fatty acid oxidation (such as Coenzyme A esters, branched-chain amino acids, and carbon molecules from glucose).[8,28,29,31–33] In mechanistic protocols, we demonstrated significant associations between plasma levels of individual acylcarnitines and measures of insulin sensitivity and insulin secretion. Most notably, plasma levels of C8:1 (the only acylcarnitine with higher baseline level in progressors than non-progressors) correlated inversely with insulin sensitivity. Thus the higher baseline levels in progressors connoted decreased insulin sensitivity. This finding suggests that C8:1 level might be a biomarker of insulin resistance and prediabetes risk.

The strengths of our study include the prospective design, the demographic diversity of the cohort, the rigorous ascertainment of prediabetes endpoints, and the use of robust measures of insulin sensitivity and insulin secretion. One limitation is that POP-ABC study participants were all offspring of parents with T2D, which might affect the generalizability of our findings. Another limitation is the requirement that our participants have normoglycemia at enrollment. As already noted, criterion of normoglycemia in the setting of obesity and insulin resistance favored individuals with robust beta-cell function. Thus, the generalizability of our observations could also be further limited by the unique metabolic features of our study cohort. Despite these limitations, our findings identify a novel signature of acylcarnitines that significantly predicted the risk of prediabetes, a condition that affects ~90 million adults in the US and ~400 million worldwide. The ability to predict individuals at risk for progression from normoglycemia to prediabetes could advance clinical and public health initiatives aimed at early preventive measures.[34–36]

AUTHORS' CONTRIBUTIONS

All authors materially participated in the research and article preparation and gave final approval for the version submitted. SD-J conceived of and designed the study, analyzed data, wrote manuscript; IO, NU, FS collected data, reviewed, and revised manuscript; JW performed statistical analysis, reviewed, and revised manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

The POP-ABC study protocol was approved by the University of Tennessee Institutional Review Board. All participants gave written informed consent before initiation of the study.

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SUPPLEMENTAL MATERIAL

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