Metabolomic Profile Associated With Insulin Resistance and Conversion to Diabetes in the Insulin Resistance Atherosclerosis Study

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Context: Metabolomic profiling of amino acids and acylcarnitines has revealed consistent patterns associated with metabolic disease.

Objective: This study used metabolomic profiling to identify analytes associated with insulin sensitivity (S_I) and conversion to type 2 diabetes (T2D).

Design: A multiethnic cohort from the Insulin Resistance Atherosclerosis Study.

Setting: Community-based.

Patients: A total of 196 subjects (European American, Hispanic, and African American) were selected to represent extremes of the S_I distribution and conversion to T2D between baseline and followup exams.

Main Outcome: Mass spectrometry–based profiling of 69 metabolites. Subjects participated in a frequently sampled iv glucose tolerance test to measure S_I and acute insulin response. T2D status was determined by a 2-hour oral glucose tolerance test.

Results: Logistic regression analysis from 72 high and 75 low S_I subjects revealed significantly decreased glycine and increased valine, leucine, phenylalanine, and combined glutamine and glutamate ($P = .0079–7.7 \times 10^{-6}$) in insulin-resistant subjects. Ethnic-stratified results were strongest in European Americans. Comparing amino acid profiles between subjects that converted to T2D (76 converters; 70 nonconverters) yielded a similar pattern of associations: decreased glycine and increased valine, leucine, and combined glutamine and glutamate ($P = .016–.00010$). Importantly, β-cell function as a covariate revealed a similar pattern of association.

Conclusions: A distinct pattern of differences in amino acids were observed when comparing subjects with high and low levels of S_I. This pattern was associated with conversion to T2D, remaining significant when accounting for β-cell function, emphasizing a link between this metabolic profile and insulin resistance. These results demonstrate a consistent metabolic signature associated with insulin resistance and conversion to T2D, providing potential insight into underlying mechanisms of disease pathogenesis. **(*J Clin Endocrinol Metab* 100: E463–E468, 2015)**

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Abbreviations: AIR, acute insulin response; BMI, body mass index; FSIGTT, frequently sampled intravenous glucose tolerance test IGT, impaired glucose tolerance; IRAS, Insulin Resistance Atherosclerosis Study; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NGT, normal glucose tolerance; S_I, insulin sensitivity; T2D, type 2 diabetes.

Risk for developing type 2 diabetes (T2D) is due to the complex interactions of genetics, lifestyle, and environmental factors. Primary characteristics of T2D are reduced insulin secretion compounded by an attenuated response. Even with recent advances made through genetic studies (1), the underlying metabolic mechanisms of T2D, especially insulin resistance, remain poorly understood. Although high-throughput technologies have accelerated genetic research, the recent advent of technologies for comprehensive metabolic analysis, often termed "metabolomics," have created new capabilities for understanding metabolic diseases such as T2D and its contributors, eg, insulin resistance, β-cell function, and body mass index (BMI). Mass spectrometry (MS) -based profiling of a wide range of metabolic intermediates has revealed not only changes in single metabolites, but comprehensive metabolomics profiles (2).

Recent metabolic profiling of serum or plasma amino acids and acylcarnitines has revealed a strikingly consistent pattern, especially of amino acid profiles, associated with multiple presentations of metabolic disease including obesity (3), coronary artery disease (4, 5), active vs sedentary lifestyle (6), and more recently, development of T2D (7). Additional studies have addressed this amino acid pattern in exercise weight loss (8) and response to bariatric surgery or dietary weight loss (9). A consistent pattern of reduced glycine and increased levels of valine and leucine associated with metabolic dysregulation has been observed with both basal (10) and dynamic measures of insulin resistance (8). Although most studies have been performed in European-derived samples, an analysis in Chinese and Asian-Indian men (10) suggested a conserved metabolic risk profile in other ethnicities. With this foundation, we examined the association of metabolomic profiles in three ethnic groups ascertained and examined using a common protocol that included dynamic measures of glucose homeostasis. These analyses included comparing subjects with high and low insulin sensitivity (S_I) and also assessed metabolomic profile changes when individuals converted to T2D.

Materials and Methods

Study subjects

The study design, recruitment, and phenotyping in the Insulin Resistance Atherosclerosis Study (IRAS) have been described (11). Briefly, individuals of European-American, Hispanic, and African-American ethnicity were recruited to reflect an equal representation of glucose tolerance status (normal glucose tolerance [NGT]), impaired glucose tolerance [IGT], and T2D), ethnicity, sex, and age (40–49, 50–59, and 60–69 y). Participants completed an oral glucose tolerance test, a frequently sampled iv glucose tolerance test (FSIGTT), and anthropometric measures. S_I and acute insulin response (AIR) were obtained using the FSIGTT with minimal model analyses (12, 13). AIR was measured 8 minutes following glucose infusion as the mean insulin increment in plasma insulin concentration above basal concentration. Disposition Index, a measure of β-cell compensation for insulin resistance, was calculated as $S_I \times$ AIR. At a 5-year followup examination participants were evaluated for conversion to T2D by oral glucose tolerance test. The 1999 World Health Organization criteria were used to define T2D (fasting glucose concentration $\geq$7.0 mmol/L, 2-hour plasma glucose concentration $\geq$11.1 mmol/L, or treatment with hypoglycemic medications) and IGT (2-hour plasma glucose level between $\geq$7.8 and $<$11.1 mmol/L).

For the initial comparison of high and low S_I, plasma samples from 147 individuals were selected in each ethnic group using the same general criteria. Samples (60% female, 40% male) were chosen from individuals who had diagnoses of NGT or IGT at baseline, and were drawn from the top and bottom 15% of S_I. To minimize extremes of BMI, samples were chosen from individuals whose BMI was $\pm$ 1 SD from the mean BMI of subjects that converted to T2D between exams.

In a second analysis, additional plasma samples were included from each ethnic group to test whether metabolomic profiles were associated with conversion from NGT or IGT to T2D between exams. "Nonconverter" subjects had to be NGT at both exams. A total of 70 nonconverter subjects from the high S_I/low S_I subjects met these criteria and 27 T2D converters met these criteria. An additional 49 plasma samples were chosen from T2D converters for a total of 76 samples.

Analysis

Amino acids and acylcarnitines were analyzed in stored plasma by tandem mass spectometry (MS/MS) as described previously (3, 5, 14–16). All MS/MS analyses employed stable-isotope dilution with internal standards from Isotec, Cambridge Isotope Laboratories and CDN Isotopes. Modest degradation of aromatic amino acids was observed, consistent with extended storage. Relative proportions of other amino acids were consistent with proper preservation.

Logistic regression models were run to evaluate associations between outcomes (high/low S_I and T2D conversion) and measured metabolites adjusting for age, sex, ethnicity, recruitment site, and BMI with and without AIR. Race-stratified logistic regression models were also examined. Odds ratios were calculated based on a 1-SD change in the metabolite and $P < .0018$ was considered significant due to correlation among metabolites (overall multivariate correlation $>$0.80; Supplemental Figure 1).

Results

In the first analysis, subjects with S_I from the extremes of the distribution were profiled in metabolomic analysis (Table 1). High S_I subjects were significantly more insulin sensitive, younger, and had a lower BMI and fasting glucose ($P < 4.5 \times 10^{-6}$). Distribution of sex and AIR did not differ ($P > .20$).

Logistic regression analysis accounting for age, sex, ethnicity, recruitment site, and BMI, revealed a distinct

Table 1. Characteristics (Combined Ethnicities) of High and Low S_I Subjects

Trait	Combined Subjects (n = 147)		High S_I Subjects (n = 72)		Low S_I Subjects (n = 75)		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	*P* Value[a]
Age, y	56 ± 9	56	53 ± 8	52	59 ± 8	62	**4.5E − 06**
Female, %	61%		64%		57%		.42
BMI, kg/m^2	29.0 ± 4.3	28.7	26.8 ± 3.4	26.3	31.2 ± 3.9	30.5	**1.4E − 11**
S_I, $\times 10^{-5}$/pmol/L/min	2.86 ± 3.22	0.90	5.61 ± 2.51	4.87	0.22 ± 0.27	0.15	**1.1E − 28**
AIR, pmol/L	452 ± 455	300	403 ± 395	290	499 ± 504	345	.20
Disposition index ($S_I \times$ AIR), $\times 10^{-5}$/min	1008 ± 1450	335	1936 ± 1604	1404	117 ± 199	9	**1.7E − 14**
Fasting glucose, mg/dl	101 ± 15	97	94 ± 11	92	107 ± 15	104	**1.2E − 08**

[a] Comparison of high and low S_I samples using a two-tailed *t* test with unequal variance.

amino acid profile of decreased glycine and increased valine and phenylalanine ($P < 7.0 \times 10^{-4}$), associated with insulin resistance (Table 2) with nominally significant increases in serine, leucine, tyrosine, and combined glutamine and glutamate ($P < .022$). A 22% lower level of glycine ($P = 7.7 \times 10^{-6}$) with increases of 11–15% in valine and phenylalanine were observed in low S_I subjects and corresponded to odds ratios of 0.17 for glycine to over 2.4 for valine and phenylalanine. Adjustment for AIR to assess the influence of β-cell function had minimal effect (Table 2).

Ethnic-stratified analyses were also evaluated in each ethnicity sample independently. Demographic and amino acid data are summarized in Supplemental Table 1. The association with decreased glycine was observed in European Americans and African Americans with comparable evidence for association but the Hispanic regression did not converge (Supplemental Table 2, A–C). Results for other amino acids (eg, branched chain, phenylalanine) that were characteristic of the differences seen between high and low S_I individuals were mirrored in the analysis of European-American samples (Supplemental Table 2A), more weakly in African Americans (Supplemental Table 2B), and with little evidence of association in Hispanics (Supplemental Table 2C).

In parallel we evaluated association of 45 acylcarnitines (Supplemental Table 3) between the high and low S_I samples. The associations were nominal and largely insignificant with the most significant being 3-hydroxy-butyryl carnitine/β-hydroxy butyryl carni-

Table 2. Logistic Regression Analysis Comparing Amino Acids of High and Low S_I Samples

Metabolite, μmol/L	High S_I Subjects (n = 72)		Low S_I Subjects (n = 75)		High S_I (n = 72): Low S_I (n = 75)[a]			High S_I (n = 72): Low S_I (n = 75)[b]		
	Mean ± SD	Median	Mean ± SD	Median	OR	95% CI	*P* Value[c]	OR	95% CI	*P* Value[c]
Glycine	258 ± 68	249	202 ± 50	194	0.17	0.076–0.36	**7.7×10^{-6}**	0.17	0.079–0.38	**1.3×10^{-5}**
Alanine	341 ± 69	332	386 ± 80	382	1.48	0.90–2.42	.12	1.47	0.90–2.40	.13
Serine	102 ± 23	102	91 ± 19	90	0.51	0.30–0.88	.015	0.52	0.30–0.90	.021
Proline	163 ± 54	157	173 ± 42	166	1.2	0.75–1.93	.44	1.17	0.74–1.88	.49
Valine	192 ± 32	186	225 ± 36	225	2.42	1.46–4.40	**.00070**	2.81	1.61–4.89	**.00030**
Leucine or isoleucine	134 ± 26	135	155 ± 29	153	2	1.12–3.33	.0079	2.4	1.14–4.41	.0023
Methionine	19 ± 5	17	20 ± 6	18	1.58	0.75–3.33	.22	1.83	0.84–3.99	.13
Histidine	77 ± 13	77	76 ± 13	74	0.88	0.57–1.36	.55	0.94	0.60–1.46	.78
Phenylalanine	64 ± 12	64	72 ± 14	71	3.77	1.76–8.06	**.00060**	3.8	1.77–8.16	**.00060**
Tyrosine	68 ± 13	68	79 ± 18	76	1.93	1.10–3.39	.022	2.07	1.14–3.76	.016
Asparagine and aspartate	55 ± 45	46	54 ± 42	44	0.6	0.27–1.35	.23	0.6	0.27–1.33	.2
Glutamine and glutamate	91 ± 31	86	111 ± 37	101	2.47	1.38–4.39	.0023	2.57	1.14–4.38	**.0018**
Ornithine	54 ± 13	52	53 ± 14	52	0.76	0.48–1.21	.25	0.81	0.50–1.31	.4
Citrulline	32 ± 9	32	32 ± 9	32	0.9	0.58–1.41	.66	0.9	0.56–1.43	.65
Arginine	79 ± 16	75	81 ± 17	80	1.23	0.80–1.90	.34	1.31	0.83–2.07	.24

Abbreviations: CI, confidence interval, OR, odds ratio.

[a] Covariates: age, sex, BMI, and ethnicity.

[b] Covariates: age, sex, BMI, ethnicity, and AIR.

[c] Values in bold indicate statistical significance corrected for multiple comparisons ($P < 0.0018$).

Table 3. Characteristics (Combined Ethnicities) of T2D Converter and Nonconverter Subjects

	Combined Subjects (n = 146)		Nonconverter (n = 70)		T2D Converter (n = 76)		
Trait	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	***P* Value[a]**
Age, y	56 ± 8	56	54 ± 9	54	57 ± 7	57	.058
Female, %	63%		64%		62%		.76
BMI, kg/m^2	29.6 ± 5.4	28.5	28.3 ± 4.3	27.6	30.9 ± 6.0	29.8	**.0023**
S_I, $\times 10^{-5}$/pmol/L/min	2.44 ± 2.78	1.43	3.64 ± 3.10	4.18	1.34 ± 1.87	1.02	**4.4E − 07**
AIR, pmol/L	404 ± 399	286	546 ± 474	397	273 ± 256	179	**4.2E − 05**
Disposition index ($S_I \times$ AIR), $\times 10^{-5}$/min	868 ± 1350	283	1495 ± 1696	1038	290 ± 414	119	**1.5E − 07**
Fasting glucose, mg/dl	101 ± 13	99	95 ± 10	94	108 ± 13	106	**1.8E − 10**

[a] Comparison of high and low S_I samples using a two-tailed *t* test with unequal variance. Bold indicates statistical significance ($P < 0.05$).

tine (C4-OH; *P* .0074). Thus, the focus of this report is on the amino acid analysis.

In the second analysis, the amino acid measures of participants that converted to T2D between the baseline and 5-year followup examination were compared with samples from participants that remained NGT. This analysis included additional T2D converter samples (n = 49) to increase power. Participants that converted to T2D did not differ by age or sex ($P > .058$) but were obese, less insulin sensitive, had lower AIR and Disposition Index, and higher fasting glucose ($P < .0023$) (Table 3). Table 4 shows that a broadly similar pattern of association with amino acids was observed with conversion to T2D: increased valine ($P = .00010$) characterized T2D converters with nominal association and consistent direction of effect for glycine, leucine, phenylalanine, and combined glutamine and glutamate. There were nominal differences with the high/low S_I comparisons; alanine increased and aspartate/asparagine decreased in the T2D converters, but not in the high/low S_I comparison. Additional adjustment for AIR, measure of β-cell insulin secretory capacity, or lipid and blood pressure medication usage did not strongly affect the evidence of association. In comparison, adjustment for S_I diminished significance for all amino acids evaluated (Table 4). AIR was the strongest predictor of conversion to T2D in earlier epidemiological studies in IRAS (17, 18). Odds ratios for the T2D conversion analysis were broadly in the same range, but modestly lower for significant associations compared with the high/low S_I analysis.

Discussion

A common metabolomic profile observed in a wide variety of studies investigating different aspects of metabolic dysregulation (3–6, 8–10, 15, 19) is replicated in analyses comparing subjects with high and low insulin sensitivity

Table 4. Logistic Regression Analysis Comparing Amino Acids of T2D Converters and Nonconverters

	Nonconverter (*n* = 70)		T2D Converter (n = 76)		T2D Converter (n = 76): Nonconverter (n = 70)[a]			T2D Converter (n = 76): Nonconverter (n = 70)[b]			T2D Converter (n = 76): Nonconverter (n = 70)[c]		
Metabolite, μmol/L	**Mean ± SD**	**Median**	**Mean ± SD**	**Median**	**OR**	**95% CI**	***P* Value[d]**	**Odds Ratio**	**95% CI**	***P* Value[d]**	**OR**	**95% CI**	***P* Value[d]**
Glycine	238 ± 71	227	204 ± 45	197	0.58	0.39–0.88	.0098	0.56	0.36–0.88	.011	0.8026	0.52–1.25	.33
Alanine	359 ± 78	365	405 ± 91	390	1.64	1.10–2.44	.016	1.79	1.15–2.70	.0089	1.4918	0.97–2.30	.069
Serine	98 ± 20	99	92 ± 20	91	0.79	0.53–1.20	.28	0.76	0.49–1.19	.24	0.9165	0.58–1.44	.71
Proline	168 ± 51	162	175 ± 43	166	1.1	0.76–1.59	.61	1.19	0.80–1.79	.39	1.0241	0.69–1.52	.91
Valine	201 ± 36	195	232 ± 36	235	2.22	1.47–3.33	**.00010**	2.08	1.37–3.23	**.00080**	1.7731	1.15–2.74	.0099
Leucine or isoleucine	140 ± 30	141	155 ± 30	157	1.59	1.09–2.33	.016	1.47	0.98–2.22	*.065*	1.3681	0.92–2.03	.12
Methionine	19 ± 6	17	18 ± 5	17	0.7	0.45–1.11	.14	0.67	0.40–1.11	.12	0.6478	0.39–1.06	.087
Histidine	77 ± 13	77	73 ± 14	72	0.78	0.55–1.10	.15	0.75	0.52–1.10	.14	0.8165	0.56–1.18	.28
Phenylalanine	67 ± 13	66	72 ± 12	72	1.56	1.01–2.44	.045	1.72	1.06–2.86	.027	1.1473	0.71–1.85	.57
Tyrosine	72 ± 17	69	78 ± 17	78	1.23	0.85–1.79	.26	1.41	0.93–2.13	.11	0.9307	0.61–1.41	.74
Asparagine and aspartate	55 ± 44	44	35 ± 32	15	0.42	0.24–0.73	.0022	0.41	0.23–0.75	.0036	0.4617	0.27–0.80	.0063
Glutamine and glutamate	97 ± 32	92	112 ± 35	109	1.82	1.16–2.78	.0080	1.75	1.12–2.78	.015	1.4813	0.93–2.37	.10
Ornithine	54 ± 14	52	58 ± 15	59	1.18	0.83–1.69	.36	1.09	0.74–1.59	.67	1.1976	0.83–1.74	.34
Citrulline	33 ± 9	32	33 ± 9	32	1.1	0.77–1.54	.62	1.02	0.70–1.49	.9	1.2642	0.86–1.87	.24
Arginine	80 ± 17	78	86 ± 18	87	1.3	0.90–1.85	.17	1.22	0.83–1.79	.31	1.3363	0.90–1.98	.15

Abbreviations: CI, confidence interval, OR, odds ratio.

[a] Covariates: age, sex, BMI, and ethnicity.

[b] Covariates: age, sex, BMI, ethnicity, and AIR.

[c] Covariates: age, sex, BMI, ethnicity, and S_I.

[d] Values in bold indicate statistical significance corrected for multiple comparisons ($P < 0.0018$).

measured by FSIGTT despite reduced power in the current study due to sample size. Although the evidence for association and relative magnitude of effects may differ between studies, the pattern of reduced plasma glycine and increased branched chain and aromatic amino acids in metabolically less healthy subjects holds true. Largely the same pattern is observed in an analysis of subjects that converted to T2D during a 5-year followup period in IRAS. This latter observation is consistent with the results from a recent metabolomic assessment of incidence (20) and conversion to T2D (7) in European Americans.

There are several novel features of this study: metabolomic profiles have been analyzed in a multiethnic sample in which each ethnicity was ascertained and examined in a uniform way including dynamically measured insulin sensitivity (S_I) and β-cell secretory capacity (AIR). In this study the differences in amino acid concentrations were more striking in the European American sample than in African Americans and Hispanics (Supplemental Table 2) although overall patterns of decreased or increased concentrations were preserved in each ethnic sample. With valine, for example, the sample mean concentrations were 206, 209, and 211 μmol/L for European American, African American, and Hispanic, respectively. The difference between the means of valine for the high S_I and low S_I samples were 39.2, 28.7, and 32.2 μmol/L, respectively. Thus, there was no obvious difference between ethnicities. We hypothesize that metabolomic analysis of all IRAS samples (>1200 subjects) would reveal consistent evidence of association with the same pattern of amino acids in each individual ethnic group.

Measurement of insulin sensitivity (S_I) and β-cell secretory capacity (AIR) has enabled us to perform a comparison of metabolomic profiles of insulin sensitive and insulin resistant participants, confirming the metabolomic pattern of amino acids associated with insulin sensitivity is not limited to basal measures of insulin sensitivity. It is important to note that these analyses accounted for major contributors to S_I: age, sex, and BMI. Importantly, the FSIGTT protocol also included measurement of AIR. Adjusting for AIR in the analysis of metabolic data led to negligible changes in the inferences (Table 4). Thus, accounting for β-cell function validates the link between this metabolic profile and insulin resistance. In addition, these data demonstrate the central contribution that insulin resistance leads to T2D susceptibility. Recent genetic studies have identified primarily genes associated with β-cell defects, leading some to speculate that the genetic basis of T2D susceptibility is more strongly affected by β-cell function. It is noteworthy that the metabolomic pattern of branched chain and aromatic amino acids, which we observed has been demonstrated to be highly heritable in other studies (5). Our observations add more evidence that a distinct metabolomic profile is associated with metabolic disease and reemphasizes the importance of understanding the biochemistry and physiology which lead to these associations. In addition, these results suggest the potential utility for metabolomic analysis in dissecting the genetic contributions to insulin resistance and T2D susceptibility.

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