Metabolomic Profiling of Fatty Acid and Amino Acid Metabolism in Youth With Obesity and Type 2 Diabetes

Evidence for enhanced mitochondrial oxidation

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OBJECTIVE—We compared acylcarnitine (AcylCN) species, common amino acid and fat oxidation (FOX) byproducts, and plasma amino acids in normal weight (NW; $n = 39$), obese (OB; $n = 64$), and type 2 diabetic ($n = 17$) adolescents.

RESEARCH DESIGN AND METHODS—Fasting plasma was analyzed by tandem mass spectrometry, body composition by dual energy X-ray absorptiometry and computed tomography, and total-body lipolysis and substrate oxidation by [^{2}H$_5$]glycerol and indirect calorimetry, respectively. In vivo insulin sensitivity (IS) was assessed with a 3-h hyperinsulinemic-euglycemic clamp.

RESULTS—Long-chain AcylCNs (C18:2-CN to C14:0-CN) were similar among the three groups. Medium- to short-chain AcylCNs (except C8 and C10) were significantly lower in type 2 diabetes compared with NW, and when compared with OB, C2-, C6-, and C10-CN were lower. Amino acid concentrations were lower in type 2 diabetes compared with NW. Fasting lipolysis and FOX were higher in OB and type 2 diabetes compared with NW, and the negative association of FOX to C10:1 disappeared after controlling for adiposity, Tanner stage, and sex. IS was lower in OB and type 2 diabetes with positive associations between IS and arginine, histidine, and serine after adjusting for adiposity, Tanner stage, and sex.

CONCLUSIONS—These metabolomics results, together with the increased rates of in vivo FOX, are not supportive of defective fatty acid or amino acid metabolism in obesity and type 2 diabetes in youth. Such observations are consistent with early adaptive metabolic plasticity in youth, which over time—with continued obesity and aging—may become dysfunctional, as observed in adults.

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A mong the comorbidities of childhood obesity, insulin resistance and type 2 diabetes have taken a center stage because of their short-term and long-term consequences (1,2). Although the pathophysiology of type 2 diabetes involves insulin resistance and impaired β-cell function, both dysregulated fatty acid and amino acid metabolism are reported in adults with type 2 diabetes or insulin resistance (3–9). We recently demonstrated that plasma long-chain acylcarnitine (AcylCN) species are increased in obese adults with insulin resistance or type 2 diabetes, consequent to an increase in free fatty acid (FFA) flux into the mitochondria (7). Furthermore, adults with type 2 diabetes exhibited greater accumulation of the shorter AcylCN intermediates compared with obese (OB) and normal weight (NW) adults, suggesting that they have a generalized oxidation defect. Others have documented elevations in branched-chain amino acids (BCAAs) and in fatty acid–associated AcylCN species in insulin-resistant adults, independent of BMI (3–5,7–9). In concert with the elevated amino acids are concomitant increases in plasma AcylCN species derived from amino acid oxidation, potentially reflecting increased amino acid flux (3,4).

Despite the increase in the prevalence of obesity, insulin resistance, and type 2 diabetes among youth, macronutrient intermediary metabolism has yet to be investigated. On the basis of adult studies, we hypothesized that adolescents with type 2 diabetes will have higher plasma concentrations of AcylCNs and amino acids compared with their NW peers. Therefore, we used tandem mass spectrometry (MS/MS) to *1*) investigate differences in AcylCN concentrations and plasma amino acid concentrations among NW, OB, and adolescents with type 2 diabetes and *2*) to assess the relationship between AcylCN or amino acid species concentrations, in vivo insulin sensitivity (IS), and in vivo fat oxidation (FOX).

RESEARCH DESIGN AND
METHODS—The study sample consisted of 39 NW (BMI <85th% for age and sex), 64 OB (BMI ≥95th% for age and sex), and 17 obese adolescents with type 2 diabetes, who were participants in our National Institutes of Health–funded K24 grant of "Insulin Resistance in Childhood" and had complete data relevant to

this investigation. Some of these participants were reported before (10). The clinical characteristics of the study participants are summarized in Table 1. NW and OB participants were recruited through newspaper advertisements and fliers posted on bus routes, on the university campus, and in children's recreational areas. Adolescents with type 2 diabetes were recruited from the Diabetes Center at Children's Hospital of Pittsburgh and were negative for glutamic acid decarboxylase and

Table 1—Subject characteristics and metabolic profile

					Bonferroni P value		
	NW	OB	T2DM	P	NW vs. OB	NW vs. T2DM	OB vs. T2DM
n	39	64	17				
Age (years)	13.0 ± 0.23	13.4 ± 0.23	15.3 ± 1.8	<0.001	NS	<0.001	<0.001
Sex							
Male	20 (51%)	34 (53%)	8 (47%)	NS			
Female	19 (49%)	30 (47%)	9 (53%)				
Race							
White	22 (56%)	33 (52%)	6 (35%)	NS			
Black	17 (44%)	31 (48%)	11 (65%)				
Tanner stage							
II-III	27 (69%)	36 (56%)	1 (6%)	<0.001			
IV-V	12 (31%)	28 (44%)	16 (94%)				
Height (cm)	158.4 ± 1.4	162.7 ± 1.3	169.1 ± 1.9	0.001	NS	0.001	0.05
Weight (kg)	47.8 ± 1.4	92.5 ± 2.7	107.0 ± 5.2	<0.001	<0.001	<0.001	0.01
BMI (kg/m^2)	19.0 ± 0.3	34.6 ± 0.7	37.2 ± 1.4	<0.001	<0.001	<0.001	NS
BMI percentile	50.8 ± 3.9	98.4 ± 0.1	98.9 ± 0.2	<0.001	<0.001	<0.001	NS
Fat mass (kg)	8.2 ± 0.7	36.9 ± 1.1	40.3 ± 2.2	<0.001	<0.001	<0.001	NS
%BF (%)	17.4 ± 1.2	43.5 ± 0.7	42.9 ± 1.2	<0.001	<0.001	<0.001	NS
VAT (cm^2)	15.4 ± 1.6	68.6 ± 5.1	81.0 ± 11.7	<0.001	<0.001	<0.001	NS
SAT (cm^2)	75.4 ± 8.1	520.8 ± 20.2	548.5 ± 31.2	<0.001	<0.001	<0.001	NS
Metabolic profile							
Fasting							
HbA$_{1c}$ (%)	5.3 ± 0.07	5.3 ± 0.06	6.8 ± 0.2	<0.001	NS	<0.001	<0.001
Glucose (mmol/L)	5.3 ± 0.04	5.4 ± 0.04	7.2 ± 0.32	<0.001	NS	<0.001	<0.001
Insulin (pmol/L)	118.3 ± 8.3	237.4 ± 13.2	298.0 ± 36.0	<0.001	<0.001	<0.001	NS
FFA (mmol/L)	0.35 ± 0.04	0.32 ± 0.01	0.28 ± 0.04	NS			
Glycerol Ra (μmol/min)	156.4 ± 17.1	319.0 ± 18.6	293.9 ± 36.7	<0.001	<0.001	0.003	NS
FFA Ra (μmol/min)	469.3 ± 51.3	957.1 ± 55.7	881.8 ± 110.1	<0.001	<0.001	0.003	NS
V$_{O_2}$ (mL/min)	220.0 ± 4.7	288.9 ± 6.5	305.3 ± 14.3	<0.001	<0.001	<0.001	NS
V$_{CO_2}$ (mL/min)	186.6 ± 3.6	239.9 ± 5.2	247.1 ± 11.0	<0.001	<0.001	<0.001	NS
EE (kcal/24 h)	1,507.6 ± 30.9	1,978.4 ± 44.1	2,041.1 ± 90.6	< 0.001	< 0.001	NS	NS
RQ	0.852 ± 0.005	0.836 ± 0.005	0.815 ± 0.007	0.002	NS	0.002	NS
FOX (μmol/min)	161.2 ± 9.3	241.0 ± 10.3	280.4 ± 26.9	<0.001	<0.001	<0.001	NS
GOX (μmol/min)	714.9 ± 29.7	797.5 ± 33.4	707.2 ± 64.2	NS			
FOX/GOX	0.41 ± 0.03	0.60 ± 0.04	0.88 ± 0.15	<0.001	0.023	<0.001	0.017
Clamp							
V$_{O_2}$ (mL/min)	220.0 ± 4.34	289.0 ± 6.24	301.3 ± 14.0	<0.001	<0.001	<0.001	NS
V$_{CO_2}$ (mL/min)	205.6 ± 4.7	263.5 ± 5.4	261.3 ± 11.5	<0.001	<0.001	<0.001	NS
EE (kcal/24 h)	1,538.7 ± 34.5	2,016.6 ± 43.0	2,036.0 ± 86.6	<0.001	<0.001	<0.001	NS
RQ	0.935 ± 0.005	0.911 ± 0.005	0.863 ± 0.01	<0.001	0.005	<0.001	<0.001
FOX (μmol/min)	59.7 ± 6.9	109.5 ± 10.8	152.0 ± 20.4	<0.001	0.004	<0.001	NS
GOX (μmol/min)	898.6 ± 56.9	1,708.4 ± 80.5	1,284.0 ± 166.0	<0.001	<0.001	0.08	0.03
FOX/GOX	0.07 ± 0.01	0.14 ± 0.01	0.30 ± 0.06	<0.001	0.04	<0.001	<0.001
FOX % suppression	70.4 ± 5.5	55.6 ± 3.4	31.4 ± 8.6	<0.001	NS	<0.001	0.017
IS (μmol/min/kg per pmol/L)	8.6 ± 3.3	2.4 ± 1.5	1.2 ± 0.6	<0.001	<0.001	<0.001	NS
IS (μmol/min/kg FFM per pmol/L)	11.5 ± 4.01	4.8 ± 2.9	2.6 ± 1.3	<0.001	<0.001	<0.001	0.040
IS (μmol/min per pmol/L)	416.1 ± 24.2	209.4 ± 13.1	128.0 ± 17.1	<0.001	<0.001	<0.001	0.053

Data are mean ± SEM or n (%). Three subjects did not have IS, 13 subjects did not have fat mass and % BF, and 7 subjects did not have VAT and subcutaneous adipose tissue. EE, energy expenditure; FFM, fat-free mass; GOX, glucose oxidation; NS, not significant; RQ, respiratory quotient; SAT, subcutaneous adipose tissue; T2DM, type 2 diabetes; V$_{O_2}$, oxygen consumption.

insulinoma-associated protein-2 auto-antibody as reported before (11). Subjects with type 2 diabetes were treated with lifestyle intervention alone (*n* = 4), metformin with lifestyle intervention (*n* = 10), metformin + insulin (*n* = 1), or insulin alone (*n* = 2). In diabetic subjects, metformin and long-acting insulin were discontinued 48 h before metabolic studies. None of the other participants, some of whom were reported previously (12), took any other medications that affect glucose, fat, or protein metabolism. Studies took place at the Children's Hospital of Pittsburgh National Institutes of Health–funded Pediatric Clinical and Translational Research Center (PCTRC) after institutional review board approval. Parental consent and participant assent were obtained prior to participation. All participants were documented to be in good health by history, physical examination, and routine hematological and biochemical tests. Pubertal development was assessed using Tanner criteria (13).

Metabolic studies
The afternoon prior to testing, all subjects were admitted to the PCTRC for testing the next morning after a 10–12 h overnight fast. Total-body lipolysis was measured at baseline for 2-h using a primed (1.2 μmol/kg) constant rate (0.08 μmol/kg·min) infusion of [^{2}H$_5$]glycerol, as described (14,15). In vivo IS was evaluated with a 3-h hyperinsulinemic-euglycemic clamp begun immediately after the baseline period. Briefly, intravenous crystalline insulin (Humulin; Lilly, Indianapolis, IN) was infused at a constant rate as reported (16,17), and plasma glucose was clamped at 100 mg/dL (5.5 mmol/L) with a variable-rate infusion of 20% dextrose based on arterialized plasma glucose determinations every 5 min. Continuous indirect calorimetry by a ventilated hood system (Deltatrac metabolic monitor; SensorMedics, Anaheim, CA) was used to evaluate substrate oxidation during the last 30 min of the baseline period on two separate occasions within a 2- to 3-week period, and the last 30 min of the 3-h clamp on one occasion, as described (14,15).

AcylCN and amino acid profiling
Archived fasting plasma samples (−80°C) were analyzed for AcylCN and amino acid profiles by MS/MS of butyl derivatives as described previously (7). AcylCN species were quantified by calculating the concentrations of metabolites relative to the deuterated internal standard closest in mass (eight different masses; Cambridge Isotope Laboratories, Andover, MA). Each AcylCN species presented in the tables and figures was detected in each plasma sample. Amino acids were quantified using internal standards as previously described (18).

Body composition and abdominal adiposity
Body composition was assessed by dual-energy X-ray absorptiometry and subcutaneous and visceral adiposity by a single slice computed tomography scan at L4-L5 as reported previously (19,20). Data are not available for subjects whose weight exceeded the limit for dual-energy X-ray absorptiometry scan (*n* = 13; 9 males/4 females; 6 black/7 white; 8 OB/5 type 2 diabetes), and for computed tomography imaging (*n* = 7; 2 males/5 females; 3 black/4 white; 2 NW, 4 OB, and 1 type 2 diabetes). Including or excluding these 13 individuals did not influence the results, and therefore they were kept in the analyses.

Biochemical measurements
Plasma glucose was determined with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH), and insulin by radioimmunoassay as before (21). HbA$_{1c}$ was measured by high-performance liquid chromatography (Tosoh Medics, Inc. 1998). Pancreatic autoantibodies were determined in the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington (Seattle, WA) using the National Institute of Diabetes and Digestive and Kidney Diseases–sponsored standardization assay (11). FFAs were determined by an enzymatic colorimetric assay (Wako nonesterified fatty acid C test kit; Wako, Osaka, Japan). Deuterium enrichment of glycerol in plasma was determined on a 5971 mass spectrometer coupled to a Hewlett-Packard Co. 5890 Series II gas chromatograph according to our previously described method (22).

Calculations
Total-body lipolysis was calculated from the rate of appearance (Ra) of glycerol in plasma according to steady-state tracer dilution equations (14,15). Plasma FFA Ra was calculated by multiplying glycerol Ra by three, because when a triglyceride molecule is hydrolyzed, one glycerol molecule and three fatty acid molecules are produced (14). Basal and insulin stimulated glucose oxidation and lipid oxidation rates were the calculated mean from

indirect calorimetry measurements according to the equation developed by Frayn (23). This equation (23) was developed using a triacylglycerol very similar to the average composition of human adipose tissue and is more reflective of human obesity.

Statistical analysis
ANOVA with Bonferroni post hoc correction for quantitative variables and χ^2 test for categorical variables were used to examine subject characteristics among the three groups. ANCOVA models were used to assess differences in amino acid and AcylCN values among the three groups, adjusting for age, Tanner stage, and race. To assess the relationships between fatty acid– and amino acid–derived AcylCN species, plasma amino acids, IS, and FOX, bivariate Pearson or Spearman correlations were applied according to data distribution. Partial correlation analyses between IS or FOX and AcylCN or amino acids were used to adjust for Tanner stage because data suggest that increased lipid oxidation during puberty may contribute to the transient insulin resistance of puberty (14), adiposity measures (BMI, percent body fat [%BF], and visceral adipose tissue [VAT]), and sex. All statistical assumptions were met. Data are presented as mean ± SEM unless otherwise indicated. Statistical significance was set at $P < 0.05$. The statistical analyses were performed using PASW Statistics (version 18; SPSS Inc., Chicago, IL).

RESULTS

Participants' characteristics
As expected, OB and diabetic subjects had body composition indices that were all significantly higher ($P < 0.001$) than their NW peers but were not different from each other (Table 1). Adolescents with type 2 diabetes were older with more advanced pubertal stages than the other two groups.

Metabolic profile
As expected, HbA$_{1c}$ and fasting glucose were higher in the subjects with type 2 diabetes compared with the other two groups (Table 1). Fasting plasma insulin was higher in the OB and diabetic subjects compared with the NW subjects. Fasting glycerol Ra, FFA Ra, and FOX were significantly higher in OB and adolescents with type 2 diabetes compared with NW adolescents, but were not different from each other. The significant differences among the groups in glycerol Ra and

FFA Ra disappeared after adjusting for BMI and Tanner stage with no change in the other variables. The difference in FOX between NW and OB subjects disappeared after adjusting for BMI and Tanner stage. Fasting FFA levels did not differ among the three groups despite rates of whole body lipolysis in OB and adolescents with type 2 diabetes that were double that of NW youth. During the hyperinsulinemic-euglycemic clamp, IS was significantly lower in OB and diabetic youth compared with NW and in adolescents with type 2 diabetes compared with OB when expressed per fat-free mass.

AcylCN intermediates

Plasma very long- and long-chain AcylCN (C18:2-CN to C14:0-CN), derived primarily from the entry of fatty acids into β-oxidation, were similar in all three groups (Supplementary Table 1). Plasma short- and medium-chain AcylCN species were lower in youth with type 2 diabetes compared with NW, with a trend toward lower values in the OB subjects. Specifically, C10:1-, C10-, and C6-CN, fatty acid–derived intermediates, and C2-CN, the last product of fatty acid oxidation, were lower in youth with type 2 diabetes compared with NW and OB subjects (Fig. 1). In addition, both C3-CN and C5-CN, intermediates derived from BCAA oxidation, were significantly lower in the diabetic group compared with NW. Similarly, C4-CN, a short chain species that derives from both the latter stages of fatty acid oxidation and from the BCAA valine, was lower in the group with type 2 diabetes relative to NW. Lastly, free carnitine (freeCN) was lower in adolescents with type 2 diabetes compared with NW, with a similar tendency ($P < 0.083$) in OB.

Plasma amino acid profiles

Plasma amino acids were lowest in adolescents with type 2 diabetes compared with both OB and NW adolescents (Supplementary Table 2). Fasting plasma concentrations of neutral BCAA including leucine and isoleucine (which in MS/MS appear together at the same mass) and valine were lower in adolescents with type 2 diabetes compared with both the NW and OB subjects (Fig. 2). The other neutrally transported amino acids, phenylalanine and methionine, exhibited similar patterns, with the last member, tyrosine, trending in that direction (this method does not report tryptophan values). Similarly, the diabetic subjects had lower
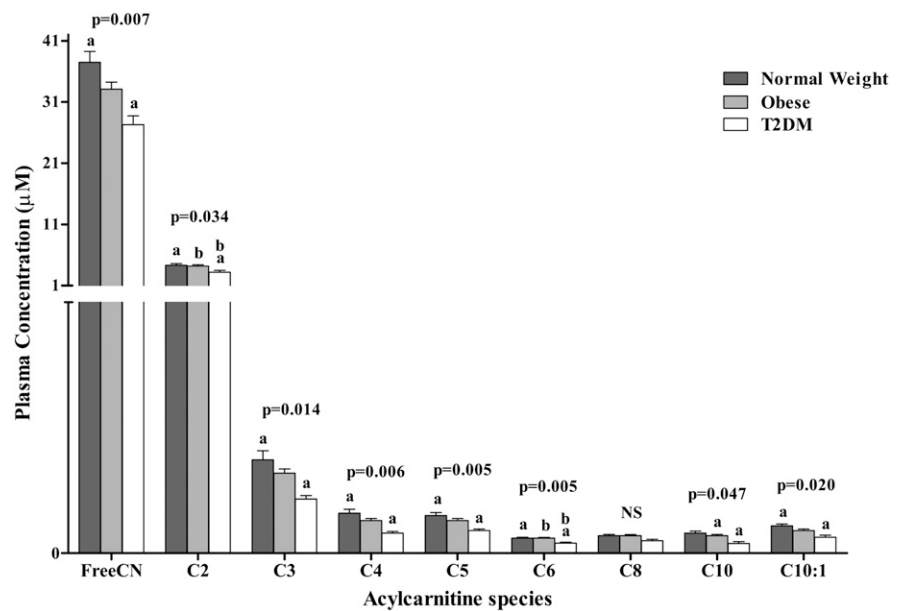

Figure 1—*Plasma free carnitine and AcylCN concentrations in NW, OB, and type 2 diabetic (T2DM) subjects.* P *ANOVA among the three groups is shown above each species. Bonferroni post hoc analyses for significant* (P < 0.05) *differences between any two groups are indicated with the same letter.*

levels of the gluconeogenic amino acid alanine relative to the other two groups. In the OB and diabetic youth, the basic amino acids, histidine and arginine, were lower relative to the NW subjects, as was plasma serine (Fig. 2). Plasma citrulline was also reduced in diabetic adolescents, paralleling the decrease in arginine and refuting any possibility that the arginine reduction results from enhanced nitric oxide synthesis. Lastly, glycine was lower in adolescents with type 2 diabetes compared with NW. When data were analyzed for AcylCN and amino acids for each sex separately, the trends were similar with variable statistical significance (data not shown) because of the limited number of subjects in each group.
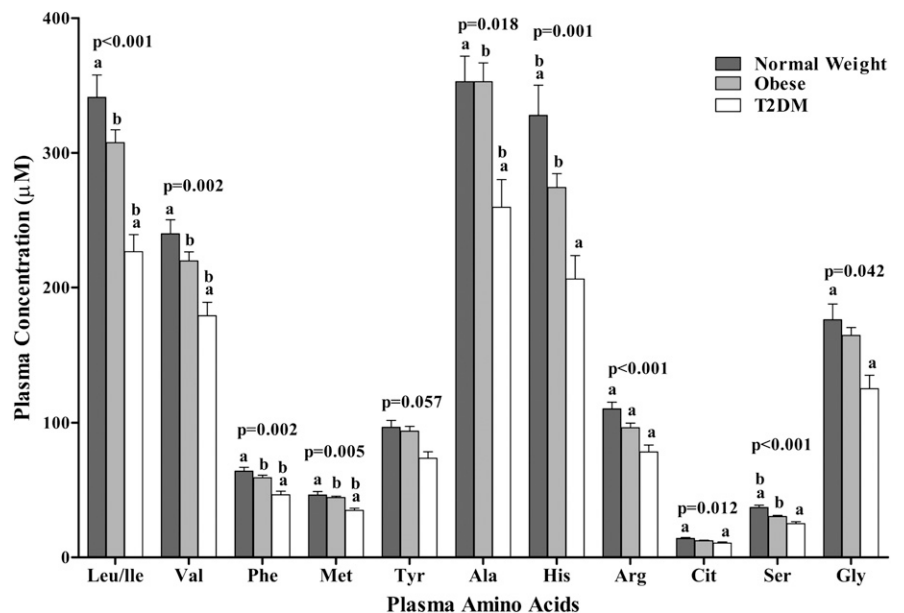

Figure 2—*Plasma amino acid concentrations in NW, OB, and type 2 diabetic (T2DM) subjects.* P *ANOVA among the three groups is shown above each species. Bonferroni post hoc analyses for significant* (P < 0.05) *differences between any two groups are indicated with the same letter.*

Association of FOX with AcylCN species and plasma amino acids

Fasting FOX was negatively associated with freeCN, C5-CN, C6-CN, and C10:1-CN. However, these relationships disappeared after adjusting for BMI, Tanner stage, and sex. Amino acids valine, phenylalanine, methionine, tyrosine, alanine, histidine, and serine showed a negative association with rates of FOX (Table 2). However, after adjusting for BMI, Tanner stage, and sex these relationships disappeared. Similar results were observed upon adjustment for %BF or VAT differences (data not shown).

Associations of in vivo IS with AcylCN species and plasma amino acids

IS was positively associated with freeCN, C6-CN, and C10:1-CN (Table 2). The relationships between IS and AcylCN species disappeared after adjustment for adiposity indices (BMI, %BF, or VAT), Tanner stage, and sex. All amino acids, except for phenylalanine, methionine, tyrosine, and alanine, were positively associated with IS. The relationship between IS and arginine, histidine, serine, and glycine remained significant after adjustment for adiposity measures, BMI or %BF, or VAT, Tanner stage, and sex.

CONCLUSIONS—The primary focus of this study was to determine whether or not obese youth with or without type 2 diabetes show the fasting plasma metabolic signatures of elevated amino acid and medium- to short-chain AcylCN species reported in adults. Our findings demonstrate that the general trend, in both the OB and the diabetic youth, is for lower plasma short- and medium-chain AcylCN with unchanged long-chain AcylCN, in addition to lower plasma concentrations of most amino acids. Thus, both the OB and the diabetic youth showed no evidence of defects in fatty acid or amino acid metabolism compared with their NW peers, despite being insulin resistant.

Obese adults with or without type 2 diabetes are characterized by dysregulated fatty acid and amino acid metabolism. Recent investigations have applied comprehensive metabolomic profiling to gain a broader understanding of the metabolic differences between lean, obese, and diabetic adults. We and others recently demonstrated elevations in AcylCN and amino acid concentrations in obese compared with lean (3,9), in adults with diabetes compared with nondiabetic individuals (6–8), and in lean insulin-resistant compared with lean insulin-sensitive adults

(5). On the basis of these observations of altered fatty acid and amino acid metabolism in adults, we hypothesized that adolescents with type 2 diabetes would have higher concentrations of specific AcylCN species and of specific plasma amino acids when compared with their OB and NW peers. In contrast to adults, our results show that adolescents with type 2 diabetes have lower concentrations of specific fatty acid– and amino acid–derived AcylCN species along with lower plasma amino acid concentrations. The lower concentrations of fatty acid–derived AcylCN species observed in our diabetic adolescents are unlikely to be attributable to greater fatty acid reesterification because fasting FFAs and the percentage of FFAs reesterified were comparable among all three groups (data not shown). Also, the concentrations of long-chain AcylCN species (C18:2-CN to C14:0-CN) were comparable among groups, suggestive of similar inputs into β-oxidation. Additionally, the ratio between freeCN and C16-CN, which is increased when carnitine palmitoyltransferase-1 function is blocked, did not differ among groups (NW, 761.0 ± 48.9; OB, 820.0 ± 64.2; and type 2 diabetes, 735.4 ± 91.5; $P = 0.69$). Yet, the presence of lower concentrations of the later β-oxidation intermediates (C10:1-CN to C2-CN) in the diabetic subjects is consistent with enhanced rather than reduced utilization of these intermediates. These observations from metabolomics are consistent with the in vivo indirect calorimetry findings of increased FOX and V_{O_2} in youth with type 2 diabetes compared with NW, whether expressed in absolute terms or controlling for fat-free mass (data not shown). Additionally, the lower amino acid concentration with the concomitant decreases in amino acid–associated AcylCN species (C3-CN, C4-CN, and C5-CN) derived from the catabolism of BCAA also suggests enhanced utilization through β-oxidation. On the other hand, the lower plasma BCAA may be due to increased gluconeogenic drive typically seen in diabetes, with the BCAA being channeled to fuel gluconeogenesis through conversion to glutamate, pyruvate, and alanine. Additionally, increased fat mass and higher rates of lipolysis in obesity and diabetes contribute increased glycerol which fuels gluconeogenesis (24) and may contribute to the lower BCAA concentrations being utilized as gluconeogenic substrates. The observed lower alanine concentrations in type 2 diabetic youth is in favor of increased gluconeogenic drive. Lastly, a reduction in

Table 2—Significant* correlations of AcylCN and amino acids with fasting FOX, BMI, and IS from the hyperinsulinemic-euglycemic clamp

	FOX	Adjusted P	IS	Adjusted P	BMI	Adjusted P†
	r	FOX	r	IS	r	BMI
AcylCN						
FreeCN	−0.23		0.24		−0.31	0.001
C3-CN					−0.18	
C4-CN					−0.28	0.004
C5-CN	−0.25				−0.26	0.003
C6-CN	−0.19		0.25		−0.23	0.018
C10-CN					−0.18	
C10:1-CN	−0.21		0.29		−0.19	0.049
Amino acids						
Leucine/isoleucine			0.25		−0.26	0.004
Valine	−0.27		0.23		−0.27	0.003
Phenalalanine	−0.27				−0.23	0.012
Methionine	−0.23					
Alanine	−0.19			0.041		
Arginine			0.39	0.006	−0.29	<0.001
Histidine	−0.21		0.38	0.010	−0.32	<0.001
Serine	−0.23		0.52	<0.001	−0.37	<0.001
Glycine			0.24			
Citrulline					−0.27	0.001
BMI	0.44		−0.75			

FOX and IS adjustments are for BMI, Tanner stage, and sex. Similar results were obtained when adjustments were made for %BF or VAT. *$P < 0.05$. †BMI adjustments are for Tanner stage and sex.

amino acids might also reflect decreased catabolism or increased anabolism in growing adolescents. We had shown that proteolysis and protein oxidation are lower during puberty compared with prepuberty in normal weight youth (25). Even though data are lacking in obese youth, it is possible that obesity-associated hyperinsulinemia may further augment this together with their higher dietary intakes. On the other hand, there are data suggesting that BCAA uptake by adipose tissue is reduced in an adult transgenic moderately obese mouse model (26). It remains to be determined if such is the case in human obesity and whether or not there are differences in adipose tissue BCAA uptake between growing adolescents versus adults. This may be yet another area where adolescents and adults may differ.

Although further studies are required to clarify the underlying mechanism(s) for these metabolic differences in youth, mitochondrial adaptation and metabolic plasticity early in the course of obesity in youth may be a likely explanation. Similar adaptive mitochondrial responses were identified in a serial study of Zucker diabetic fatty rats where the animals initially upregulated their oxidative capacity in the face of progressive insulin resistance (27). Yet, the upregulated oxidative capacity was unable to stop the diabetic progression, and with age, the mitochondrial adaptive response was lost. Consistent with our data, a recent investigation in young adults (mean age 22 years) demonstrated comparable long-chain AcylCN species but lower medium-chain AcylCN species in obese compared with lean individuals (28). These results support the hypothesis that adolescents and young adults with obesity and type 2 diabetes have not yet developed the mitochondrial defects that are documented in older adults (29,30), and they may even have enhanced mitochondrial activity as an adaptation. In support of the latter are previous reports of increased FOX in obese children, proposed to be a metabolic defense against further weight gain (31,32).

Thus, with the present findings of enhanced rates of β-oxidation in OB and diabetic youth, we propose that their mitochondrial function is not impaired. However, over time and with continued progressive obesity from childhood to adulthood, chronic exposure to excessive β-oxidation results in mitochondrial overload (33) and oxidative stress, culminating

in a reduced overall oxidative capacity, similar to the changes found in diet-induced insulin-resistant mice (34). Our diabetic youth compared with NW were unable to suppress their FOX during hyperinsulinemic conditions (30 vs. 70%), resulting in continued exposure and demand for excessive β-oxidation. Longitudinal studies of metabolomics from youth to adulthood are needed to test our hypothesis. Moreover, it remains to be determined if the progression to abnormal mitochondrial function could be prevented or aborted. We further propose, based on the inverse relationship between IS and FOX ($r = -0.502$, $P < 0.001$), that the increased rates of FOX in youth with obesity and diabetes may play a role in their insulin resistance through the Randle cycle of the competition between fatty acid and glucose oxidation (35). We previously demonstrated that the Randle cycle is operative in pubertal adolescents, potentially explaining the physiology of pubertal insulin resistance (14,36).

Lastly, a cluster of obesity-related amino acid and AcylCN metabolites have demonstrated an inverse relationship with IS among adults. Specifically, Newgard et al. (3) observed associations between BCAA and insulin resistance. Likewise, Tai et al. (5) reported that insulin resistance was associated with leucine/isoleucine, phenylalanine, tyrosine, and alanine, and a cluster of BCAA and related amino acids identified by principal components analysis. Thus, our second aim was to assess the relationship between IS or FOX with AcylCN species or amino acid concentrations. We found that once we accounted for the level of adiposity (BMI, %BF, or VAT), Tanner stage, and sex, the relationship between AcylCN species and IS or FOX disappeared. Furthermore, the observed correlations between BMI and IS and FOX (Table 2), suggest that the AcylCN associations are mediated by obesity in our pediatric population. In contrast, the relationship between IS and arginine, histidine, serine, and glycine concentrations remained significant after controlling for adiposity, Tanner stage, and sex.

The strengths of the current study are *1*) the number of otherwise healthy NW and OB adolescents and youth with type 2 diabetes; *2*) the extensive investigations that included the state-of-the-art use of stable isotopes together with indirect calorimetry and the hyperinsulinemic-euglycemic clamp to assess in vivo lipolysis, lipid oxidation, and IS; *3*) the comprehensive

evaluation of whole body and abdominal adiposity; and *4*) a first-time metabolomics analysis in youth. A potential weakness is the absence of in vivo protein turnover examination. Another perceived weakness is that youth with type 2 diabetes were on treatment. However, ethically, patients cannot be evaluated without the provision of proper therapy. Moreover, the differing treatment modalities were driven by standard clinical recommendations and not by a research protocol.

In summary, our results show that in contrast to adults, OB youth with or without type 2 diabetes do not demonstrate impaired fatty acid or amino acid metabolism. In fact, the metabolomics and the in vivo data favor enhanced mitochondrial function, as obese adolescents with type 2 diabetes demonstrate lower concentrations of the later β-oxidation intermediates along with higher rates of FOX. It is our theory that these contrasting results between adolescents and adults is a reflection of the duration of obesity and the consequent and gradual evolution of failure of mitochondrial adaptive mechanisms as the obese individual transitions from youth to adulthood and forward with continued obesity. Future longitudinal studies of metabolomics will be required to test this theory and to determine if this early adaptive metabolic plasticity disintegrates over time and if intervention(s) could prevent such maladaptive progression into adulthood.

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