Relationships Between Circulating Metabolic Intermediates and Insulin Action in Overweight to Obese, Inactive Men and Women

Kim M. Huffman, MD, PHD[1]
Svati H. Shah, MD, MHS[2]
Robert D. Stevens, PHD[3]
James R. Bain, PHD[3]
Michael Muehlbauer, PHD[3]
Cris A. Slentz, PHD[2]
Charles J. Tanner, MS[4]
Maragatha Kuchibhatla, PHD[5]
Joseph A. Houmard, PHD[4]
Christopher B. Newgard, PHD[3,6]
William E. Kraus, MD[2]

OBJECTIVE — To determine whether circulating metabolic intermediates are related to insulin resistance and β-cell dysfunction in individuals at risk for type 2 diabetes.

RESEARCH DESIGN AND METHODS — In 73 sedentary, overweight to obese, dyslipidemic individuals, insulin action was derived from a frequently sampled intravenous glucose tolerance test. Plasma concentrations of 75 amino acids, acylcarnitines, free fatty acids, and conventional metabolites were measured with a targeted, mass spectrometry–based platform. Principal components analysis followed by backward stepwise linear regression was used to explore relationships between measures of insulin action and metabolic intermediates.

RESULTS — The 75 metabolic intermediates clustered into 19 factors comprising biologically related intermediates. A factor containing large neutral amino acids was inversely related to insulin sensitivity (S_I) ($R^2 = 0.26$). A factor containing fatty acids was inversely related to the acute insulin response to glucose ($R^2 = 0.12$). Both of these factors, age, and a factor containing medium-chain acylcarnitines and glucose were inversely and independently related to the disposition index (DI) ($R^2 = 0.39$). Sex differences were found for metabolic predictors of S_I and DI.

CONCLUSIONS — In addition to the well-recognized risks for insulin resistance, elevated concentrations of large, neutral amino acids were independently associated with insulin resistance. Fatty acids were inversely related to the pancreatic response to glucose. Both large neutral amino acids and fatty acids were related to an appropriate pancreatic response, suggesting that these metabolic intermediates might play a role in the progression to type 2 diabetes, one by contributing to insulin resistance and the other to pancreatic failure. These intermediates might exert sex-specific effects on insulin action.

Diabetes Care 32:1678–1683, 2009

With people in developed countries becoming increasingly less active and more obese, the incidence of type 2 diabetes is growing at an alarming rate. Given the critical role of skeletal muscle in glucose disposal, much work has focused on understanding skeletal muscle insulin resistance. In addition, because progression to overt type 2 diabetes requires defective pancreatic β-cell secretion of insulin (1), efforts are also underway to dissect the mechanisms of pancreatic β-cell failure. Prevailing theories for insulin resistance focus on lipid-mediated mechanisms but also include insulin resistance mediated by elevated concentrations of amino acids and inflammatory signaling molecules as well as combinations of these (2). Similarly, β-cell dysfunction leading to type 2 diabetes has been linked to cellular stress responses triggered by overstimulation of insulin synthesis and secretion, as well as the effects of lipid mediators to cause changes in key metabolic activities of β-cells that impair control of insulin secretion (2).

Most of these hypotheses have revolved around elegant physiological investigations often performed in animal models. However, although these physiological investigations provide great insight into mechanisms, they do not conclusively illustrate that such mechanisms function at the population level in humans. Using a well-characterized sample from a clinical trial, our objective was to determine whether the measurement of metabolic intermediates with a targeted, mass spectrometry–based platform might provide insight into the pathogenesis of insulin resistance, a harbinger and essential component of type 2 diabetes. Our hypothesis was that in this group of men and women at risk for, but without, overt type 2 diabetes, lipid mediators would be related to insulin resistance and hyperglycemia would be related to β-cell dysfunction.

RESEARCH DESIGN AND METHODS

— This was a cross-sectional evaluation of the relationship between concentrations of circulating metabolic intermediates and insulin action in 73 subjects who enrolled in the first and second Studies of Targeted Risk Reduction Interventions through Defined Exercise (STRRIDE). Inclusion criteria were age between 18 and 70 years, inactivity (not participating in regular exercise), being overweight to mildly obese

From [1]Physical Medicine and Rehabilitation, Veterans Affairs Medical Center, Durham, North Carolina; the [2]Division of Cardiovascular Medicine, Department of Medicine, Duke University Medical Center, Durham, North Carolina; the [3]Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, North Carolina; the [4]Department of Exercise and Sports Science and the Human Performance Laboratory, East Carolina University, Greenville, North Carolina; the [5]Division of Geriatrics, Department of Medicine, Duke University Medical Center, Durham, North Carolina; and the [6]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina.
Corresponding author: Kim M. Huffman, huffm007@mc.duke.edu.
Received 13 January 2009 and accepted 25 May 2009.
Published ahead of print at http://care.diabetesjournals.org on 5 June 2009. DOI: 10.2337/dc08-2075. Clinical trial reg. nos. NCT00200993 and NCT00275145, clinicaltrials.gov.

(BMI 25–35 kg/m^2), dyslipidemia (LDL cholesterol 130–190 mg/dl or HDL cholesterol <40 mg/dl for men and <45 mg/dl for women), and postmenopausal status for women. Exclusion criteria included diagnosed diabetes or fasting glucose >126 mg/dl, medications known to affect carbohydrate or lipid metabolism, a dietary regimen designed to induce weight loss, hypertension (blood pressure >160/90 mmHg), known cardiovascular disease, tobacco use, and musculoskeletal conditions prohibiting exercise training (3).

Circulating metabolite measurements

Fasting EDTA plasma was collected from each participant and stored at −80°C. Circulating metabolites were measured in plasma from 73 subjects at study enrollment. Fifteen amino acids, 45 acylcarnitines, and 9 free fatty acids were measured using a targeted, mass spectrometry–based platform as described previously (4,5). In brief, amino acids and acylcarnitines were methanol precipitated, esterified, and analyzed with tandem mass spectrometry. After methylation with iodomethane and partial purification, free fatty acids were measured with gas chromatography/mass spectrometry. Conventional metabolites including glucose, lactate, uric acid, total ketones, β-hydroxybutyrate, and nonesterified free fatty acids were measured with colorimetric enzymatic assays using a Hitachi 911 analyzer (4). Reagents for Hitachi analyses were obtained from Roche (Indianapolis, IN; glucose, lactate, and uric acid) and Wako USA (Richmond, VA; total ketones, β-hydroxybutyrate, and nonesterified free fatty acids).

Metabolic and cardiovascular risk factor assessments

A frequently sampled intravenous glucose tolerance test was performed in all subjects. Fasting insulin (Access Immunoassay System; Beckman Coulter, Fullerton, CA) and fasting glucose (YSI2300 STAT Plus; YSI, Yellow Springs, OH) were measured. Using these concentrations, insulin sensitivity (S_I) and the acute insulin response to glucose (AIRg) were derived based on Bergman's minimal model (3,6). The disposition index (DI) was calculated as the mathematical product of insulin sensitivity index and AIRg ($S_I \times$ AIRg).

Data analysis

Our main objective was to determine whether metabolic intermediates might be predictive of baseline insulin sensitivity and insulin action. Given the large number of predictors relative to the sample size and collinearity of analytes, principal components analysis (PCA) was used as a means to reduce the complexity of the variables. Each of the metabolic intermediates was logarithmically transformed [log10(metabolite + 1)] to approximate a normal distribution. An orthogonal varimax rotation was used, and factors with an eigenvalue >1 were retained. Individual metabolites with a factor load of $>|0.4|$ for a given PCA-derived factor are reported as composing that factor. Given the presence of certain acylcarnitines having values below the lower limits of quantification, sensitivity analyses of PCA were performed with and without metabolites in which >25% of metabolite values were below the limits of quantification. Similar results were obtained with each (data not shown); hence, models inclusive of all analytes are presented.

For modeling insulin action components (S_I, AIRg, and DI) as outcomes, factors retained in PCA were used in linear regression using backwards stepwise variable selection. S_I, AIRg, and DI were logarithmically transformed before analyses to approximate a normal distribution, and analyses were controlled for age, sex, and waist circumference. To determine whether sex differences exist for those factors found related to insulin action, sex interactions were evaluated. First, full models were fit including each of the independently related factors and each sex-related factor interaction term. If none of the sex interaction terms was found to be significant, these terms were removed, and the main effects model was refit. If any of the sex interactions were found to be significant, then stratified main effects models were evaluated. Statistical analysis was performed using SAS Enterprise Guide (version 8.2; SAS Institute, Cary, NC) with statistical significance established as $P < 0.05$.

RESULTS — The mean ± SD age of this study population was 51.2 ± 7.7 years. Of the 73 subjects, 35 (48%) were women and 38 (52%) were men. Fifty-eight (79%) were Caucasian, 12 (16%) were African American, 2 (3%) were Asian, and 1 (1%) was Hispanic. The median S_I was 2.88 (interquartile range 1.84–5.61) $\times 10^{-5}$ min^{-1}/[pmol/l]. The median AIRg was 354.9 (183.6–529.1) pmol/l, and median DI was 1,237.3 (583.8–1,732.2) $\times 10^{-5}$ min^{-1}. Supplementary Table 1 (available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc08-2075/DC1) shows median values and ranges for concentrations of metabolic intermediates.

PCA was used to reduce the large number of circulating metabolic intermediates into fewer "factors" containing metabolic intermediates that were highly correlated with one another. Table 1 shows the constituent metabolites for each of the 19 retained factors. As shown in Table 1, the metabolic constituents for each factor were biologically related, such that a biological description for each factor could be applied (Table 1). The first five factors represented a majority of the variance in the dataset and were summarized as factors containing *1*) medium- and long-chain acylcarnitines, *2*) fatty acids and by-products of fatty acid oxidation, *3*) long-chain acylcarnitines and acetylcarnitine, *4*) large neutral amino acids and uric acid, and *5*) medium-chain acylcarnitines and glucose.

After reducing the complexity of the metabolic intermediates into 19 factors of related metabolites, we sought to determine whether these factors could predict S_I. Independent of age, sex, and waist circumference, three of these factors were associated with S_I and accounted for 63% of its variance (Table 2). Of these three factors, the one most associated with S_I was the factor containing large neutral amino acids. Being inversely associated with S_I, this single factor predicted 26% of the variance in insulin sensitivity ($P < 0.0001$) (Fig. 1). These findings are consistent with another recent study from our group in which a branched-chain amino acid (BCAA)–related factor that included leucine/isoleucine, valine, phenylalanine, and tyrosine was the one most strongly associated with a different measure of insulin sensitivity (homeostasis model assessment index) in a cohort of 73 obese and 67 lean subjects (7). Additional independent associations of S_I are shown in Table 2.

When evaluating predictors of pancreatic response, we found that independent of age, sex, and waist circumference, a metabolic factor containing fatty acids and by-products of fatty acid oxidation was inversely related to AIRg. This model accounted for 28% of the variance in pancreatic response (Table 2), and alone this

Table 1—*Results of principal components analysis*

Factor	Metabolites within factor	Description	Eigen value	Variance	Cumulative variance
1	C8 C5-DC C10:1 C10 C12:1 C12 C14:2 C14:1 C14 C16 C8:1-OH/C6:1-DC C8:1-DC C16:2 C16:1 *C18:2*	Medium- and long-chain acylcarnitines	13.1	0.17	0.17
2	FA-C14:0 FA-C16:1 FA-C16:0 FA-α-C18:3 FA-C18:2 FA-C18:1 FA-C18:0 FA-C22:6 Free fatty acids Hydroxybutyrate Ketones *C2*	Free fatty acids and by-products of fatty acid oxidation	9.6	0.13	0.30
3	C2 C6-DC C10-OH/C8-DC C12-OH/C10-DC C14:1-OH C18 C18:1 C18:2 C14-OH/C12-DC C18:1-OH C16:1-OH/C14:1-DC *C16*	Long-chain acylcarnitines and C2-acylcarnitine	6.4	0.09	0.39
4	Proline Valine Leucine/Isoleucine Methionine Phenylalanine Tyrosine Uric acid *Histidine*	Large neutral amino acids and uric acid	4.3	0.06	0.44
5	C8:1 C10:3 Glucose	Medium-chain acylcarnitines and glucose	3.5	0.05	0.49
6	C3 C4/Ci4 C5s	Short-chain acylcarnitines	3.1	0.04	0.53
7	Glutamate/Glutamine *Hydroxybutyrate Ketones Glucose*	Glutamate/Glutamine	2.7	0.05	0.57
8	Glycine Serine	One-carbon metabolism	2.3	0.03	0.60
9	C20 C16-OH/C14-DC C18:1-DC	Long-chain and hydroxy or dicarboxy acylcarnitines	2.1	0.03	0.63
10	Alanine Lactate	Alanine and lactate	2.0	0.03	0.66
11	C6 C5OH/C3-DC	Short-chain and hydroxy or dicarboxy acylcarnitine	1.7	0.02	0.68
12	Citrulline Arginine C10:2 C18-OH/C16-DC	Urea cycle, medium-chain acylcarnitine, and hydroxy or dicarboxy acylcarnitine	1.7	0.02	0.70
13	C20:4	Arachidonoyl carnitine	1.5	0.02	0.72
14	Ornithine FA-C20:4 (neg)	Ornithine and arachidonic acid	1.5	0.02	0.74
15	C5:1 C18:2-OH (neg)	Acylcarnitines	1.6	0.02	0.76
16	C22 C4-OH (neg)	Acylcarnitines	1.3	0.02	0.78
17	Aspartate/Asparagine	Aspartate/Asparagine	1.3	0.02	0.79
18	C18-OH/C16-DC *C22:6*	Hydroxy or dicarboxy acylcarnitine	1.2	0.02	0.81
19	Histidine C7-DC	Histidine and dicarboxy acylcarnitine	1.1	0.01	0.82

Key metabolites within each retained factor (i.e., metabolites with factor load ≥ |0.4|) and an overall description of each factor are presented. Italicized metabolites represent those with a factor load ≥|0.4| on a second factor. DC, dicarboxylic; FA, fatty acid.

factor accounted for 12% of the variance in AIRg ($P < 0.002$) (Fig. 1). Because an appropriate pancreatic response should vary based on tissue insulin sensitivity, we also sought to better understand predictors of an appropriate pancreatic response, as represented by DI. We were able to explain 39% of the variance in the DI with a model containing age, sex, waist circumference, and three metabolic factors (Table 2). Inverse associations were seen for DI and factors containing free fatty acids (Fig. 1), large neutral amino acids (Fig. 1), and medium-chain acylcarnitines and glucose. In addition, age was independently and inversely associated with DI. Body composition, as measured by waist circumference, was an independent predictor of both S_I and AIRg, but not of DI.

We also explored sex differences in predictors of insulin action because of previous observations of sex-specific effects of metabolic factors on measures of insulin action. For S_I, we observed a significant interaction between sex and the factor composed of glutamine and glutamate, factor 7 ($P < 0.03$). Using sex-stratified analyses to further elucidate this sex interaction (supplementary Table 2, available in the online appendix), we observed that, for men, waist circumference and factors 4, 7, and 8 remained significant independent predictors of insulin sensitivity and accounted for 64% of the variance in S_I (model $P < 0.0001$) (supplementary Table 2). However, for women, factor 7 was not predictive of S_I, whereas waist circumference and factors 4 and 8 remained independent predictors of S_I (model $P < 0.0001$, model $R^2 = 0.61$) (supplementary Table 2).

There was no significant interaction between sex and factor 2 for the relationship with AIRg ($P = 0.35$), and, thus, stratified analyses were not performed for this measure of insulin action. When evaluating sex differences in metabolic relationships for DI, we observed a significant interaction between sex and factor 5 ($P < 0.01$). In sex-stratified analyses, factor 4 was the only significant predictor of DI for men (model $P < 0.0001$) (supplementary Table 2). In contrast, in women, factor 4 was not independently related to DI, but factors 5 and 2 remained significant independent predictors of DI (model $P < 0.0001$) (supplementary Table 2).

CONCLUSIONS — Our objective was to determine whether circulating metabolic intermediates, as measured with a mass spectrometry–based platform, are associated with insulin action in a mixed-sex population at risk for, but without, overt type 2 diabetes. Using a

Table 2—*Linear regression models for measures of insulin action using backward stepwise variable selection controlling for age, sex, and waist circumference*

	Parameter estimate	SEM	*F*	*P*
S_I model: $R^2_{SI} = 0.63$, $P < 0.0001$				
Factor 4: Large neutral amino acids and uric acid	−0.123	0.027	21.41	<0.0001
Waist circumference (cm)	−0.014	0.003	16.28	0.0001
Factor 8: Glycine and serine	0.091	0.025	13.57	0.0005
Factor 7: Glutamate/Glutamine (ketones and glucose)	−0.093	0.027	11.95	0.001
AIRg model: $R^2_{AIRg} = 0.28$, $P < 0.0002$				
Factor 2: Fatty acids and by-products of fatty acid oxidation	−0.088	0.043	4.07	0.048
DI model: $R^2_{DI} = 0.39$, $P < 0.0001$				
Factor 4: Large neutral amino acids and uric acid	−0.145	0.044	10.56	0.002
Factor 2: Free fatty acids and by-products of fatty acid oxidation	−0.131	0.046	8.06	0.006
Age (years)	−0.014	0.006	5.92	0.018
Factor 5: Medium-chain acylcarnitines and glucose	−0.092	0.042	4.83	0.032

Metabolite factors are those that remained significant in multivariable regression models. R^2 values reflect values for models presented and include effects of age, sex, and waist circumference. Only significant predictors are presented and individual R^2 values are discussed in text.

PCA strategy to reduce the dimensionality of the large number of metabolic variables, we observed several components of insulin action to be independently operative in this population. A single factor containing large neutral amino acids was inversely related to both S_I and DI. In addition, a factor containing free fatty acids and by-products of fatty acid oxidation was inversely related to both AIRg and DI. Thus, both large neutral amino acids and fatty acids might contribute to progression to type 2 diabetes and might exhibit differential contributions for insulin action for each sex.

The potential for amino acids to confer insulin resistance has been recognized for some time (8). As with many obesity-related derangements, amino acid–induced insulin resistance probably results from mechanisms that have evolved to operate in a low-calorie, high-activity environment now functioning in a high-calorie, low-activity environment

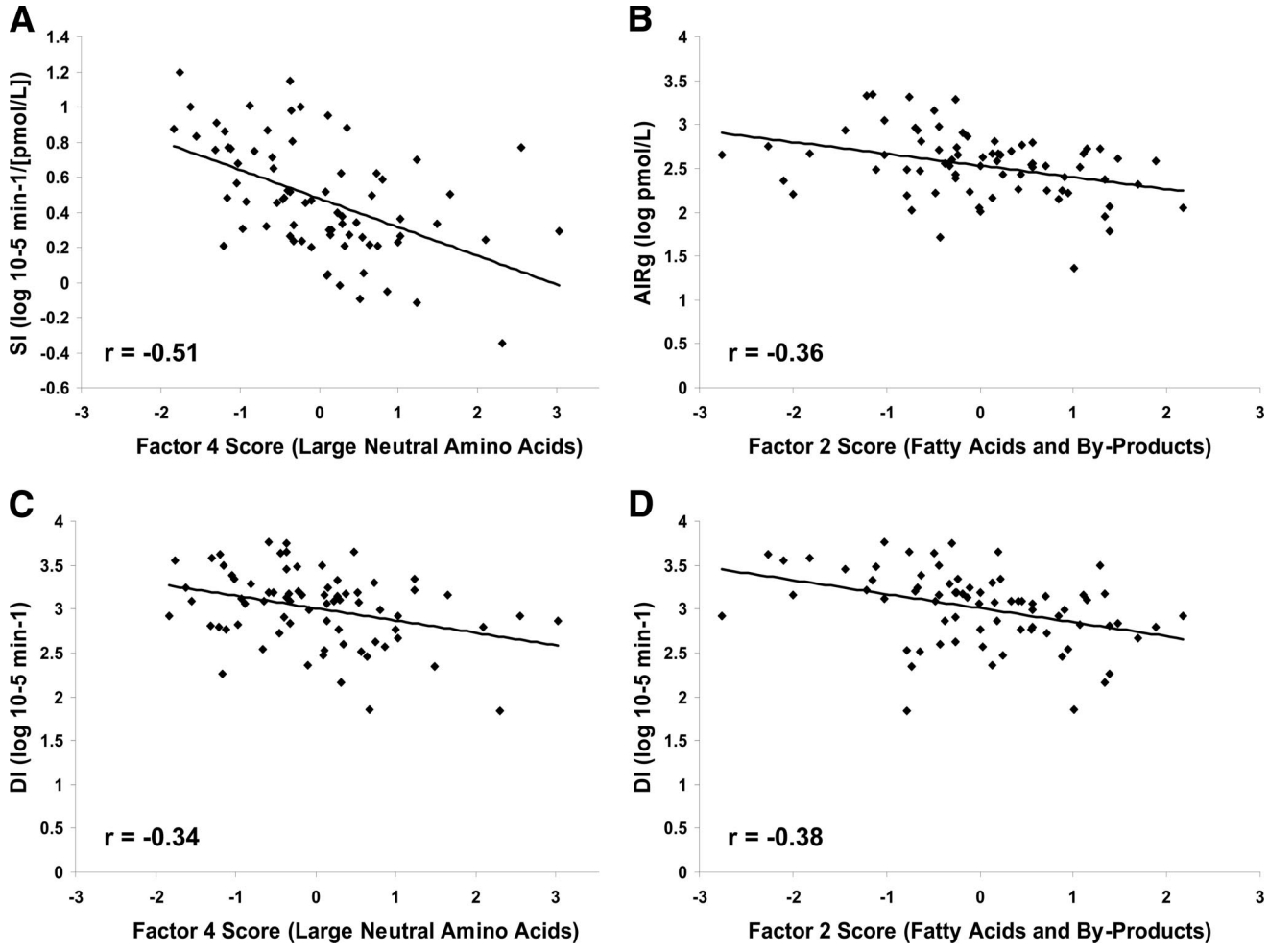

**Figure 1—*Relationships between factor scores and measures of insulin action.* A: *Relationship between factor 4 score and* S_I. B: *Relationship between factor 2 score and AIRg.* C: *Relationship between factor 4 score and DI.* D: *Relationship between factor 2 score and DI.*

(9). In a low-calorie environment in which high-protein meals are infrequent, it is not surprising that large neutral amino acids would promote an anabolic state by inhibiting proteolysis and directly stimulating protein synthesis (10). Similarly, elevated concentrations of amino acids produce insulin resistance by disrupting insulin-mediated glucose uptake pathways, resulting in reduced glucose uptake and glycogen synthesis (11). Both stimulation of protein synthesis and inhibition of insulin-stimulated glucose uptake occur at the level of the mammalian target of rapamycin (12), a key nodal point in signaling for protein synthesis (13) and insulin-stimulated glucose uptake (10,12). This coordinated promotion of protein synthesis and disruption of glucose uptake pathways would function well with occasionally elevated amino acid concentrations. However, in obesity, elevated concentrations of amino acids are sustained even in the fasting state (8), promoting sustained insulin resistance and, in the presence of other pathological aberrations, progression to diabetes. Recently, compelling support for the idea that these elevations could be related to pathophysiology was provided by findings that BCAA and several related amino acids and metabolites were elevated in obese, insulin-resistant subjects compared with lean control subjects, and these concentrations were linearly related to the homeostasis model assessment index (7). Further, in rats, a high-fat diet supplemented with BCAA produced insulin resistance, whereas supplementation of normal chow did not, and the insulin resistance provoked by high-fat/BCAA feeding was reversed with administration of the mammalian target of rapamycin inhibitor, rapamycin (7). Although we recognize that other mechanisms of insulin resistance are also operative with similar teleological explanations, our work clearly supports older as well as new data that relate insulin resistance and concentrations of large neutral amino acids at the population level and in a mixed-sex population.

In addition to insulin resistance, a defective β-cell compensatory response is critical to the development of type 2 diabetes. Similar to the detrimental effects of chronically elevated amino acids on skeletal muscle, emerging evidence suggests that chronic lipid exposure plays a key role in molecular events culminating in β-cell failure (2). In β-cells, lipid oxidation to acetyl-CoA activates pyruvate carboxylase and initiates a pyruvate cycle, which results in an increase in concentrations of molecules that amplify insulin secretion. Thus, in healthy, fasting humans, acutely elevated free fatty acids promote β-cell secretion of insulin to suppress rampant adipocyte lipolysis and prevent subsequent ketoacidosis (2). As insulin resistance develops, elevated concentrations of free fatty acids promote greater insulin secretion to compensate for insulin resistance, contributing to the well-recognized consequence of hyperinsulinemia. However, sustained exposure to elevated concentrations of fatty acids and chronic activation of pyruvate carboxylase results in a dampening of the response of this pathway to glucose (2,14) and deficits in β-cell insulin secretion (15–17). In addition, this qualitative defect is probably compounded by β-cell apoptosis and a quantitative reduction in β-cells driven by increased oxidative and endoplasmic reticulum stress that occurs as a result of an overload of β-cell lipid metabolism (2,15). We observed that a factor containing free fatty acids and byproducts of fatty-acid oxidation was inversely associated with the acute pancreatic response and the pancreatic response when insulin sensitivity is accounted for (DI). Our findings show that a poorer compensatory response in insulin production is associated with higher concentrations of circulating fatty acids, potentially supporting a toxic effect of lipids on β-cells in pre-diabetic subjects such as those included in the current study.

In addition to evaluating relationships between circulating metabolites and measures of insulin action, we also explored sex differences in relationships between circulating metabolites and insulin action. We observed that for men, insulin sensitivity was most related to the factor containing large neutral amino acids and another containing glutamine and glutamate. For women, concentrations of these two sets of metabolites were much less strongly related to insulin sensitivity, but waist circumference and a factor containing glycine and serine were most related. In addition, the inverse relationship between concentrations of fatty acids and the appropriate pancreatic response (DI) was more robust for women than for men. We find these sex differences intriguing in light of emerging evidence that supports sex differences in development of insulin resistance and diabetes (18). Before adolescence, girls are more insulin resistant than boys, but during adolescence, a shift occurs such that in adulthood, women are more insulin sensitive than men (19). Whereas several studies have shown that both male rats (20) and men (21) are more susceptible than female rats and women to fatty acid–induced insulin resistance, less work has been focused on examining sex differences in relationships between amino acids and insulin resistance.

It is notable that most of the work examining BCAAs in the context of obesity and insulin resistance has been performed predominantly in men (8,11,13). In addition, prior investigations have shown that men are more likely to develop insulin resistance as a consequence of low birth weight (22) and gestational protein availability (23) and exhibit stronger relationships between the insulin-like growth factor axis and insulin sensitivity (24). Such reports led us to hypothesize that in men, gestational programming, which is designed to meet greater anabolic demands and that operates under abundant amino acid exposure later in life, drives our findings of a stronger relationship between amino acid concentrations and insulin resistance. Our findings that women might be more susceptible to β-cell lipotoxicity could be related to the observed sex differences in hormone-sensitive lipase-mediated regulation of lipolysis in pancreatic β-cells (25). Most important, the observed sex differences in these relationships emphasize the critical nature of careful attention to sex differences and hormonal influences on insulin action in our efforts to uncover the pathogenesis of type 2 diabetes.

We recognize that this investigation has several limitations. The moderate sample size and cross-sectional nature of this investigation may limit the applicability of our findings. Although this cross-sectional design allows only inferences about causality, we have taken advantage of this design to confirm the findings of prior physiological studies in humans at a population level. In addition, our moderate sample size did not allow for exploration of race or ethnic variations in predictors of insulin action. Similarly, we were unable to account for other obvious influences on insulin action such as genetic ones. However, we were able to explore sex differences in both insulin resistance and β-cell dysfunction. Also, we were able to demonstrate the applicability of a targeted, mass spectrometry–based platform to a clinical sample of

modest size. Rather than presenting a large number of correlations between biological assays and clinical measurements, we have illustrated that PCA is a means of data complexity reduction with biological plausibility.

Thus, in a mixed-sex population at risk for, but without, overt type 2 diabetes, concentrations of large neutral amino acids were related to insulin resistance, and concentrations of free fatty acids and by-products of fatty acid oxidation were related to an impaired pancreatic response. There was also a sex difference in the strength of these relationships, such that men seemed to be more susceptible to amino acid-induced insulin resistance and women were more vulnerable to lipid-mediated β-cell toxicity. Although these findings merit validation in other cohorts, they support the recognition that metabolic processes that have evolved to sustain demands in a low-calorie environment produce many of the aberrations characteristic of type 2 diabetes when metabolic fuels are in excess.

Acknowledgments— This work was supported by the National Heart, Lung, and Blood Institute, National Institutes of Health (NIH) (Grant R01HL-57354, W.E.K., principal investigator [PI]), the National Institute on Aging (Grant P30 AGO28716-01, H.J. Cohen, PI, and AG028930-01, D.M. Muoio, PI), and the Atkins Foundation. K.M.H. was supported by National Institute of Arthritis & Musculoskeletal & Skin Diseases, NIH (K23AR054904).

No potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the 2008 American Physiological Society Intersociety Meeting, The Integrative Biology of Exercise V, Hilton Head, South Carolina, 24–27 September 2008.

We thank the rest of the STRRIDE research team at East Carolina University and Duke University. We appreciate technical support from Brett Wenner, Lauren C. Naliboff, and Erin Chu.

References

1. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. 1922. Can Med Assoc J 1991;145:1281–1286
2. Muoio DM, Newgard CB. Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:193–205
3. Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE. Effect of the volume and intensity of exercise training on insulin sensitivity. J Appl Physiol 2004;96:101–106
4. Ha AM, Lien LF, Boan J, Arlotto M, Slentz CA, Muehlbauer MJ, Rochon J, Gallup D, McMahon RL, Bain JR, Stevens R, Millington D, Butler MD, Newgard CB, Svetkey LP. The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) weight loss project: rationale and design. Contemp Clin Trials 2005;26:616–625
5. Lien LF, Ha AM, Arlotto M, Slentz CA, Muehlbauer MJ, McMahon RL, Rochon J, Gallup D, Bain JR, Ilkayeva O, Wenner BR, Stevens RD, Millington DS, Muoio DM, Butler MD, Newgard CB, Svetkey LP. The STEDMAN project: biophysical, biochemical and metabolic effects of a behavioral weight loss intervention during weight loss, maintenance, and regain. Omics 2009;13:21–35
6. Bergman RN. Orchestration of glucose homeostasis: from a small acorn to the California oak. Diabetes 2007;56:1489–1501
7. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Ha AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS Jr, Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD, Svetkey LP. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9:311–326
8. Felig P, Marliss E, Cahill GF Jr: Plasma amino acid levels and insulin secretion in obesity. N Engl J Med 1969;281:811–816
9. Neel JV. The "thrifty genotype" in 1998. Nutr Rev 1999;57:S2–S9
10. Patti ME, Brambilla E, Luzi L, Landaker EJ, Kahn CR. Bidirectional modulation of insulin action by amino acids. J Clin Invest 1998;101:1519–1529
11. Krebs M, Krssak M, Bernroider E, Anderwald C, Brehm A, Meyerspeer M, Nowotny P, Roth E, Waldhausl W, Roden M. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes 2002;51:599–605
12. Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85α: the two sides of a coin. Diabetes 2006;55:2392–2397
13. Drummond MJ, Bell JA, Fujita S, Dreyer HC, Glynn EL, Volpi E, Rasmussen BB: Amino acids are necessary for the insulin-induced activation of mTOR/S6K1 signaling and protein synthesis in healthy and insulin resistant human skeletal muscle. Clin Nutr 2008;27:447–456
14. Boucher A, Lu D, Burgess SC, Telemaque-Potts S, Jensen MV, Mulder H, Wang MY, Unger RH, Sherry AD, Newgard CB. Biochemical mechanism of lipid-induced impairment of glucose-stimulated insulin secretion and reversal with a malate analogue. J Biol Chem 2004;279:27263–27271
15. Cnop M, Welsh N, Jonas JC, Jorns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes 2005;54(Suppl. 2);S97–S107
16. Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF. Prolonged elevation of plasma free fatty acids impairs pancreatic β-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. Diabetes 2000;49:399–408
17. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R, Cusi K. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes 2003;52:2461–2474
18. Franconi F, Seghieri G, Canu S, Straface E, Campesi I, Malorni W. Are the available experimental models of type 2 diabetes appropriate for a gender perspective? Pharm Res 2008;57:6–18
19. Moran A, Jacobs DR Jr, Steinberger J, Steffen LM, Pankow JS, Hong CP, Sinaiko AR. Changes in insulin resistance and cardiovascular risk during adolescence: establishment of differential risk in males and females. Circulation 2008;117:2361–2368
20. Hevener A, Reichart D, Janez A, Olefsky J. Female rats do not exhibit free fatty acid-induced insulin resistance. Diabetes 2002;51:1907–1912
21. Frias JP, Macaraeg GB, Ofrecio J, Yu JG, Olefsky JM, Kruszynska YT. Decreased susceptibility to fatty acid-induced peripheral tissue insulin resistance in women. Diabetes 2001;50:1344–1350
22. Flanagan DE, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. Am J Physiol Endocrinol Metab 2000;278:E700–E706
23. Sugden MC, Holness MJ. Gender-specific programming of insulin secretion and action. J Endocrinol 2002;175:757–767
24. Flanagan DE, Holt RI, Owens PC, Cockington RJ, Moore VM, Robinson JS, Godsland IF, Phillips DI. Gender differences in the insulin-like growth factor axis response to a glucose load. Acta Physiol (Oxf) 2006;187:371–378
25. Peyot ML, Nolan CJ, Soni K, Joly E, Lussier R, Corkey BE, Wang SP, Mitchell GA, Prentki M. Hormone-sensitive lipase has a role in lipid signaling for insulin secretion but is nonessential for the incretin action of glucagon-like peptide 1. Diabetes 2004;53:1733–1742