

Exercise-Induced Changes in Metabolic Intermediates, Hormones, and Inflammatory Markers Associated With Improvements in Insulin Sensitivity

KIM M. HUFFMAN, MD, PHD¹
 CRIS A. SLENTZ, PHD²
 LORI A. BATEMAN, MS²
 DANA THOMPSON, PHD³
 MICHAEL J. MUEHLBAUER, PHD⁴
 JAMES R. BAIN, PHD⁴

ROBERT D. STEVENS, PHD⁴
 BRETT R. WENNER, PHD⁴
 VIRGINIA BYERS KRAUS, MD, PHD¹
 CHRISTOPHER B. NEWGARD, PHD^{4,5}
 WILLIAM E. KRAUS, MD^{2,4}

OBJECTIVE — To understand relationships between exercise training-mediated improvements in insulin sensitivity (S_1) and changes in circulating concentrations of metabolic intermediates, hormones, and inflammatory mediators.

RESEARCH DESIGN AND METHODS — Targeted mass spectrometry and enzyme-linked immunosorbent assays were used to quantify metabolic intermediates, hormones, and inflammatory markers at baseline, after 6 months of exercise training, and 2 weeks after exercise training cessation ($n = 53$). A principal components analysis (PCA) strategy was used to relate changes in these intermediates to changes in S_1 .

RESULTS — PCA reduced the number of intermediates from 90 to 24 factors composed of biologically related components. With exercise training, improvements in S_1 were associated with reductions in by-products of fatty acid oxidation and increases in glycine and proline ($P < 0.05$, $R^2 = 0.59$); these relationships were retained 15 days after cessation of exercise training ($P < 0.05$, $R^2 = 0.34$).

CONCLUSIONS — These observations support prior observations in animal models that exercise training promotes more efficient mitochondrial β -oxidation and challenges current hypotheses regarding exercise training and glycine metabolism.

Diabetes Care 34:174–176, 2011

Targeted mass spectrometry-based measurements of small molecule metabolic intermediates have provided insight into the pathogenesis of chronic metabolic diseases including obesity, type 2 diabetes, and cardiovascular disease (1–5). Our objective was to elucidate mechanisms by which exercise training improves S_1 in a group of middle-aged, sedentary, overweight to obese men and women, at risk for

but without overt type 2 diabetes. In particular, we sought to elucidate relationships between changes in whole-body S_1 and plasma metabolic and inflammatory biomarkers after 6 months of aerobic exercise training.

RESEARCH DESIGN AND METHODS — Study design and inclusion/exclusion criteria have been de-

scribed previously (2). Participants were randomly assigned to 6 months of inactivity or one of three groups of supervised aerobic exercise training (6). Assessments including frequently sampled intravenous glucose tolerance tests and fasting blood sampling were performed at baseline, after 6 months of exercise training, and 2 weeks after cessation of exercise training.

Metabolic, hormone, and inflammatory markers

Methods for measuring circulating metabolic intermediates and S_1 via an intravenous glucose tolerance test have been described previously (2). Adiponectin and leptin were measured at all three time points by ELISAs (adiponectin, ALPCO Diagnostics, Salem, NH, samples diluted 1:1,000; leptin, Millipore: Billerica, MA, samples undiluted). D-Dimer concentrations (measured by ELISA; American Diagnostica, Stamford, CT) and paraoxonase activity (measured by enzyme activity; Invitrogen, Carlsbad, CA) were assayed only at baseline and after 6 months of exercise training. Coefficients of variation for all assays were $<4.5\%$.

Cytokines and inflammatory markers were measured at baseline and after 6 months of training using a Luminex panel (Invitrogen/Biosource, Carlsbad, CA). Values reflect measurement of >100 beads for each analyte.

Data analysis

Change scores for metabolite concentrations were computed as posttraining minus baseline concentrations. Intervention group differences in metabolite changes were compared with ANOVAs with Dunnett's post hoc testing. Principal components analyses (PCAs) were used to reduce the change scores to a smaller set of variables. Components from PCA were compared with change in S_1 using linear regression (2). Analyses were controlled for age, sex, and baseline waist circumference, determinants of S_1 readily obtained in a clinical setting before initiation of an exercise training program.

From ¹Physical Medicine and Rehabilitation, Veterans Affairs Medical Center, Durham, North Carolina; the ²Division of Cardiovascular Medicine, Department of Medicine, Duke University Medical Center, Durham, North Carolina; the ³Division of Rheumatology, Department of Medicine, Duke University Medical Center, Durham, North Carolina; the ⁴Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, North Carolina; and the ⁵Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina.

Corresponding author: Kim M. Huffman, huffm007@mc.duke.edu.

Received 20 April 2010 and accepted 24 September 2010. Published ahead of print at <http://care.diabetesjournals.org> on 4 October 2010. DOI: 10.2337/dc10-0709. Clinical trial reg. nos. NCT00200993 and NCT00275145, clinicaltrials.gov.

© 2011 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1—Linear regression models for change in insulin sensitivity using backward stepwise variable selection controlling for age, sex, and waist circumference

	Parameter estimate	SE	Partial R ²	F value	P value
Baseline to posttraining S ₁ change model: R ² _{S₁} = 0.59, P < 0.0001					
Age	0.010	0.030	0.00	0.11	0.74
Sex (men = 0; women = 1)	−0.818	0.455	0.04	3.24	0.08
Waist circumference	−0.003	0.023	0.00	0.01	0.91
Factor 1: free fatty acids and by-products of fatty acid oxidation	−0.847	0.188	0.21	20.38	<0.0001
Factor 11: glycine and proline	0.871	0.178	0.24	24.05	<0.0001
Factor 22: C20, acylcarnitine	0.430	0.185	0.06	5.40	0.032
Factor 23: C18:1, hydroxy-acylcarnitine	0.365	0.180	0.04	4.10	0.05
Baseline to 15 days posttraining S ₁ change (sustained change) model: R ² _{S₁} = 0.34, P < 0.003					
Age	0.030	0.035	0.01	0.74	0.39
Sex (men = 0; women = 1)	−2.002	0.573	0.11	12.21	0.0011
Waist circumference	−0.043	0.027	0.04	2.52	0.12
Factor 1: free fatty acids and by-products of fatty acid oxidation	−0.518	0.250	0.06	4.28	0.05
Factor 7: glycine, proline, and alanine	0.776	0.249	0.12	9.75	0.0032

Listed metabolite factors are those that remained significant in multivariable regression models. Models were forced to include age, sex, and waist circumference.

RESULTS

Comparing exercise groups with inactivity

Supplementary Table 1 (available in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc10-0709/DC1>) demonstrates mean baseline to posttraining changes in clinical, metabolic, and inflammatory analytes. Significant changes were noted for arachidoyl carnitine (C20), leptin, and monocyte chemoattractant protein 1 (supplementary Fig. 1, available in an online appendix).

Relationships between changes in metabolic intermediates and S₁

Because we observed a broad range of S₁ changes across exercisers (supplementary Fig. 2, available in an online appendix), when evaluating relationships between changes in concentrations of metabolic intermediates and S₁, we chose to focus only on those individuals (n = 53) randomly assigned to exercise training.

PCA identified 24 factors, consisting of groups of metabolites and other analytes that changed similarly from baseline to posttraining (supplementary Table 2, available in an online appendix). We found four change factors that were independently associated with change in S₁: factor 1 (free fatty acids and by-products

of fatty acid oxidation), factor 11 (glycine and proline), factor 22 (C20, acylcarnitine), and factor 23 (C18:1, hydroxyacylcarnitine) (Table 1, P < 0.05 for all and supplementary Fig. 3, available in an online appendix). This model indicated that improvements in S₁ after 6 months of exercise training were associated with reductions in fatty acids and their by-products, and with increases in glycine, proline, and C20, acylcarnitine and C18:1, hydroxyacylcarnitine. These associations were independent of other known contributors to S₁ (age, sex, and waist circumference).

To understand how glycine and proline concentrations were related to S₁ changes, we examined relations for these two variables alone. A model containing glycine change explained 62% of the variance in S₁ change, whereas a model containing proline change explained a smaller amount (44%) of variance in S₁ change (supplementary Table 3, available in an online appendix).

Relations between sustained changes in metabolic intermediates and S₁ after cessation of training

PCA also identified factors of naturally grouped metabolic and inflammatory markers that changed similarly from baseline to the end of the 2-week training

cessation period (supplementary Table 4, available in an online appendix). Modeling indicated that improvements in S₁ retained 15 days after cessation of exercise training were associated with sex (men demonstrated greater sustained improvements than women), reductions in fatty acids and by-products of fatty acid oxidation, and increases in glycine, proline, and alanine (Table 1).

Similar to the models for S₁ change, we repeated modeling for these three variables alone and in combinations of two to better understand how glycine, alanine, and proline concentrations were related to sustained S₁ change. A model containing glycine change explained a comparable 39% of variance in sustained S₁ change from baseline, whereas a model containing alanine, proline, or a combination of the two changes explained smaller percentages (27, 28, and 30%, respectively) of variance in sustained (before 15 days) S₁ change (supplementary Table 3).

CONCLUSIONS— After 6 months of exercise training in a group of 53 middle-aged, overweight and moderately obese, inactive men and women with a significant burden of metabolic syndrome, improvements in S₁ with exercise training were associated with reductions in concentrations of circulating free fatty acids and fatty acid by-products and with increased plasma levels of the amino acid glycine and, to a lesser significant extent, proline. Of importance, these relationships were sustained despite 2 weeks of training cessation. These findings suggest that by providing an increased energy demand, exercise training either promotes more efficient mitochondrial function and β -oxidation (7) or reduces lipolysis via enhanced insulin action.

Consistent with the former (improved mitochondrial efficiency) is the strong effect on glycine, for which improvements in insulin sensitivity were associated with recovery of glycine concentrations. Glycine conjugates, specifically acylglycines, are used as a means to purge excess metabolic fuels via the urine (8). Thus, one might expect that in an attempt to relieve overloaded, inefficient mitochondria, glycine-adduct formation depletes the glycine pool as evidenced by our prior reports of cross-sectional associations between lower glycine concentrations and poorer S₁ in this population (2). The recovery of glycine in exercising subjects may therefore serve as an index of a return of metabolic effi-

ciency and clearing of incompletely oxidized substrates from the mitochondria.

Acknowledgments—This work was supported by the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (grant R01-HL-57354 [W.E.K., principal investigator]) and National Institute on Aging (grants P30-AG-028716-01 [Harvey J. Cohen, principal investigator] and AG-028930-01 [Deborah M. Muoio, principal investigator]) and by the Atkins Foundation. K.M.H. was supported by the NIH National Institute of Arthritis & Musculoskeletal & Skin Diseases (grant K23-AR-054904) and by an American College of Rheumatology Research and Education Foundation (ACR-REF)/Association of Specialty Professors (ASP) Junior Career Development Award in Geriatric Medicine funded via Atlantic Philanthropies, ACR-REF, the John A. Hartford Foundation, and ASP.

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

K.M.H. performed data analysis, participated in conceptual design, participated in key discussions, and wrote the manuscript. C.A.S. and L.A.B. participated in primary data collection, participated in key discussions, and revised/edited the manuscript. D.T., M.J.M., J.R.B., R.D.S., and B.R.W. performed laboratory analyses, participated in key discussions, and revised/edited the manuscript. V.B.K., C.B.N., and W.E.K. participated in conceptual

design, participated in key discussions, and revised/edited the manuscript.

We thank the rest of the STRRIDE research team at East Carolina University and Duke University. We appreciate technical support from Lauren C. Naliboff and Erin Chu and helpful discussions from Dr. Deborah M. Muoio, all affiliated with the Sarah W. Stedman Nutrition and Metabolism Center at Duke University Medical Center.

References

1. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: moving from information to knowledge. *Diabetes* 2009;58:2429–2443
2. Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, Tanner CJ, Kuchibhatla M, Houmard JA, Newgard CB, Kraus WE. Relationships between circulating metabolic intermediates and insulin action in overweight to obese inactive men and women. *Diabetes Care* 2009;32:1678–1683
3. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq 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
4. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, Ilkayeva OR, Wenner BR, Bain JR, Lee JJ, Lim SC, Khoo CM, Shah SH, Newgard CB. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* 2010;53:757–767
5. Shah SH, Hauser ER, Bain JR, Muehlbauer MJ, Haynes C, Stevens RD, Wenner BR, Dowdy ZE, Granger CB, Ginsburg GS, Newgard CB, Kraus WE. High heritability of metabolomic profiles in families burdened with premature cardiovascular disease. *Mol Syst Biol* 2009;5:258
6. 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
7. Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, Dohm GL, Yan Z, Newgard CB, Muoio DM. Peroxisome proliferator-activated receptor- γ co-activator 1 α -mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J Biol Chem* 2005;280:33588–33598
8. Costa CG, Guérard WS, Struys EA, Holwerda U, ten Brink HJ, Tavares de Almeida I, Duran M, Jakobs C. Quantitative analysis of urinary acylglycines for the diagnosis of β -oxidation defects using GC-NCI-MS. *J Pharm Biomed Anal* 2000;21:1215–1224