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

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OBJECTIVE — To understand relationships between exercise training-mediated improvements in insulin sensitivity (S_I) 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_I.

RESULTS — PCA reduced the number of intermediates from 90 to 24 factors composed of biologically related components. With exercise training, improvements in S_I 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.

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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_I 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_I 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 described 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_I 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_I using linear regression (2). Analyses were controlled for age, sex, and baseline waist circumference, determinants of S_I readily obtained in a clinical setting before initiation of an exercise training program.

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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_I change model: $R^2_{SI} = 0.59$, $P < 0.0001$					
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_I change (sustained change) model: $R^2_{SI} = 0.34$, $P < 0.003$					
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 S_I

Because we observed a broad range of S_I changes across exercisers (supplementary Fig. 2, available in an online appendix), when evaluating relationships between changes in concentrations of metabolic intermediates and S_I, 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 S_I: 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 S_I 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 S_I (age, sex, and waist circumference).

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

Relations between sustained changes in metabolic intermediates and S_I 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 S_I 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 S_I 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 S_I change. A model containing glycine change explained a comparable 39% of variance in sustained S_I 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) S_I 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 S_I 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 S_I 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.

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