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 ($P < 0.05, R^2 = 0.39$); 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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Factors 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, hydroxy-acylcarnitine) (Table 1). **Table 1—Linear regression models for change in insulin sensitivity using backward stepwise variable selection controlling for age, sex, and waist circumference**

| Parameter                          | Partial $R^2$ | $P$ value | $F$ value | SE | $R^2$ | estimate |
|------------------------------------|---------------|-----------|-----------|----|-------|----------|
| Age                                | 0.030         | 0.035     | 0.01      | 0.74 | 0.00  | 0.010    |
| Sex (men = 0; women = 1)            | -0.003        | 0.023     | 0.00      | 0.91 | 0.04  | -0.081   |
| Waist circumference                 | -0.003        | 0.023     | 0.00      | 0.91 | 0.04  | -0.081   |
| Factor 1: free fatty acids and by-products of fatty acid oxidation | -0.003 | 0.023 | 0.00 | 0.91 | 0.04 | -0.081 |
| Factor 11: glycine and proline     | 0.030         | 0.035     | 0.01      | 0.74 | 0.00  | 0.010    |
| Factor 22: C20, acylcarnitine      | -0.003        | 0.023     | 0.00      | 0.91 | 0.04  | -0.081   |
| Factor 23: C18:1, hydroxy-acylcarnitine | 0.030 | 0.035 | 0.01 | 0.74 | 0.00  | 0.010 |

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), lepntin, 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, hydroxy-acylcarnitine) (Table 1). **Table 1—Linear regression models for change in insulin sensitivity using backward stepwise variable selection controlling for age, sex, and waist circumference**

| Parameter                          | Partial $R^2$ | $P$ value | $F$ value | SE | $R^2$ | estimate |
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| Waist circumference                 | -0.003        | 0.023     | 0.00      | 0.91 | 0.04  | -0.081   |
| Factor 1: free fatty acids and by-products of fatty acid oxidation | -0.003 | 0.023 | 0.00 | 0.91 | 0.04 | -0.081 |
| Factor 11: glycine and proline     | 0.030         | 0.035     | 0.01      | 0.74 | 0.00  | 0.010    |
| Factor 22: C20, acylcarnitine      | -0.003        | 0.023     | 0.00      | 0.91 | 0.04  | -0.081   |
| Factor 23: C18:1, hydroxy-acylcarnitine | 0.030 | 0.035 | 0.01 | 0.74 | 0.00  | 0.010 |

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

**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 exercise cessation. These findings suggest that by providing an increased energy demand, exercise training either promotes more efficient mitochondrial function and $\beta$-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 overload, 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-
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ciency and clearing of incompletely oxidized substrates from the mitochondria.

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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.

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