Exercise-induced changes in metabolic intermediates, hormones, and inflammatory markers associated with improvements in insulin sensitivity

Short Title: Exercise Training-Induced Changes in Metabolites

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Objective: To understand relationships between exercise training (ET)-mediated improvements in insulin sensitivity ($S_I$) and changes in circulating concentrations of metabolic intermediates, hormones, and inflammatory mediators.

Methods: Targeted mass spectrometry and ELISAs were used to quantify metabolic intermediates, hormones and inflammatory markers at baseline, after six months of ET, and two weeks after ET 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 comprised of biologically-related components. With ET, 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 ET ($P<0.05$, $R^2=0.34$).

Conclusions: These observations support prior observations in animal models that ET promotes more efficient mitochondrial beta oxidation and challenges current hypotheses regarding ET and glycine metabolism.

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 ET 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. Specifically, we sought to elucidate relationships between changes in whole body $S_I$ and plasma metabolic and inflammatory biomarkers following six months of aerobic ET.

RESEARCH DESIGN AND METHODS

Study design and inclusion/exclusion criteria have been described previously(2). Participants were randomized to six months of inactivity or one of three groups of supervised aerobic ET(6). Assessments including FSIVGTTs and fasting blood sampling were performed at baseline, after six months of ET, and two weeks after cessation of ET.

Metabolic, Hormone and Inflammatory Markers: Methods for measuring circulating metabolic intermediates and $S_I$ via an intravenous glucose tolerance test (IVGTT) have been described previously(2). Adiponectin and leptin were measured at all three time points by ELISA (adiponectin-ALPCO Diagnostics: Salem, NH, samples diluted 1:1000; 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 six months of ET. Coefficients of variation for all assays were below 4.5%.

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

Data Analysis: Changes scores for metabolite concentrations were computed as post-training minus baseline concentrations. Intervention group differences in metabolite changes were compared with analyses of
Exercise Training-Induced Changes in Metabolites

RESULTS
Comparing Exercise Groups to Inactivity. Supplementary Table 1 in the online appendix (available at http://care.diabetesjournals.org) demonstrates mean baseline to post-training changes in clinical, metabolic, and inflammatory analytes. Significant changes were noted for arachidoyl carnitine (C20), leptin and MCP-1 (Supplementary Figure 1 in the online appendix).

Relationships Between Changes in Metabolic Intermediates and S_I. Since we observed a broad range of S_I changes across exercisers (Supplementary Figure 2), when evaluating relationships between changes in concentrations of metabolic intermediates and S_I, we chose to focus only on those individuals (n=53) randomized to ET.

PCA identified 24 factors, consisting of groups of metabolites and other analytes that changed similarly from baseline to post-training (Supplementary Table 2). We found four change factors were independently associated with change in S_I: Factors 1 (free fatty acids and by-products of fatty acid oxidation), 11 (glycine and proline), 22 (C20 acylcarnitine), and 23 (C18:1-OH acylcarnitine) (Table 1, P<0.05 for all, Supplementary Figure 3). This model indicated that improvements in S_I following six months of ET were associated with reductions in fatty acids and their by-products, and with increases in glycine, proline, and the C20- and C18:1-OH-acylcarnitine. These associations were independent of other known contributors to S_I (age, gender, waist circumference).

In order 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 variance in S_I change while a model containing proline change explained a smaller amount (44%) of variance in S_I change (Supplementary Table 3).

Relations Between Sustained Changes in Metabolic Intermediates and S_I Following Cessation of Training. PCA also identified factors of naturally grouped metabolic and inflammatory markers that changed similarly from baseline to the end of the two week training cessation period (Supplementary Table 4). Modeling indicated that improvements in S_I retained 15 days after cessation of ET were associated with gender (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, to better understand how glycine, alanine and proline concentrations were related to sustained S_I change, we repeated relationships for these three variables alone and in combinations of two. A model containing glycine change explained a comparable 39% of variance in sustained S_I change from baseline while model containing alanine, proline, or a combination of the two change explained smaller percentages (27%, 28%, 30%, respectively) of variance in sustained (Pre-15d) S_I change (Supplementary Table 3).

CONCLUSIONS
After six months of ET 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 ET 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. Importantly, these relationships were sustained despite two weeks of training cessation. These findings suggest that by providing an increased energy demand, ET 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, where improvements in insulin sensitivity were associated with a 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 efficiency and clearing of incompletely oxidized substrates from the mitochondria.

**Author Contributions.** KMH performed data analysis, participated in conceptual design and key discussions and wrote the manuscript. CAS and LAB participated in primary data collection, participated in key discussions, and edited the manuscript. DT, MJM, JRB, RDS, and BRW performed laboratory analyses, participated in key discussions and edited the manuscript. VBK, CBN, and WEK participated in conceptual design and key discussions, and edited the manuscript.

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**REFERENCES**


Table 1: Linear regression models for change in insulin sensitivity using backward stepwise variable selection controlling for age, gender, and waist circumference. Listed metabolite factors are ones that remained significant in multivariable regression models.

<table>
<thead>
<tr>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>Partial R^2</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline to Post-Training S\textsubscript{I} Change Model: R^2\textsubscript{SI} = 0.59, P&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.010</td>
<td>0.030</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Gender (men=0; women=1)</td>
<td>-0.818</td>
<td>0.455</td>
<td>0.04</td>
<td>3.24</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.003</td>
<td>0.023</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Factor 1: Free fatty acids and by-products of fatty acid oxidation</td>
<td>-0.847</td>
<td>0.188</td>
<td>0.21</td>
<td>20.38</td>
</tr>
<tr>
<td>Factor 11: Glycine and Proline</td>
<td>0.871</td>
<td>0.178</td>
<td>0.24</td>
<td>24.05</td>
</tr>
<tr>
<td>Factor 22: C20 Acylcarnitine</td>
<td>0.430</td>
<td>0.185</td>
<td>0.06</td>
<td>5.40</td>
</tr>
<tr>
<td>Factor 23: C18:1-OH Acylcarnitine</td>
<td>0.365</td>
<td>0.180</td>
<td>0.04</td>
<td>4.10</td>
</tr>
</tbody>
</table>

| Baseline to 15 days Post-Training S\textsubscript{I} Change (Sustained Change) Model: R^2\textsubscript{SI} = 0.34, P<0.003 |
| Age                | 0.030          | 0.035       | 0.01    | 0.74    | 0.39 |
| Gender (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 |

* Models were forced to include age, gender, waist circumference