Na\textsuperscript{+}-K\textsuperscript{+}-ATPase \(\alpha_2\)-gene and skeletal muscle characteristics in response to long-term overfeeding

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METABOLIC PROPERTIES OF SKELETAL MUSCLE may play a role in the etiology of obesity and related comorbidities (6, 16). For instance, the skeletal muscle glycolytic-to-oxidative enzyme ratio has been shown to be strongly related to insulin resistance (17). Moreover, we have reported earlier that baseline skeletal muscle oxoglutarate dehydrogenase (OGDH) activity, a marker of the oxidative pathway, correlated negatively with both baseline percent body fat and the changes in fat mass in response to long-term caloric surplus (21). Therefore a low capacity for fat oxidation in skeletal muscle could be metabolically unfavorable.

Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is an essential plasma membrane enzyme catalyzing the transport of Na\textsuperscript{+} and K\textsuperscript{+} across the cell membrane in skeletal muscle (3). Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is constituted of a catalytic subunit (\(\alpha\)) and a \(\beta\)-subunit of unknown function. The \(\alpha\)-subunit has three isoforms (\(\alpha_1\), \(\alpha_2\), and \(\alpha_3\)), which are coded by separate genes (10). The \(\alpha_2\)-gene is expressed mainly in skeletal muscle. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity contributes \(\sim20\%\) of the whole body resting metabolic rate (RMR; Ref. 22), and the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase \(\alpha_2\)-gene may play a role in fuel oxidation (8). Recent studies have suggested a relationship between Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and metabolic properties, such as aerobic glycolysis, in skeletal muscle (7). The aim of this study was to explore the role of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase \(\alpha_2\)-gene BglII polymorphism in the changes of selected skeletal muscle metabolic properties observed in response to a long-term (100 days) overfeeding protocol conducted with 12 pairs of monozygotic twins.

SUBJECTS, MATERIALS, AND METHODS

Twelve pairs of young (aged 21 \(\pm\) 2 yr) male identical twins provided written consent to participate in an overfeeding study approved by the Laval University Medical Ethics Committee and the Office for the Protection from Research Risks of the National Institutes of Health (Bethesda, MD). The men were housed in a closed section of a dormitory on the campus of Laval University. During the period of overfeeding, the men ate a 4.2 MJ/day (1,000 kcal) energy surplus, 6 days/wk, during a period of 100 days. On the seventh day of each week, they consumed their habitual daily energy intake. The subjects were under 24-h supervision by members of the project staff who lived with them. The specific aims, study design, and methodology of this overfeeding protocol have been described elsewhere (2). In this study, the twins are considered for analysis purposes as 24 subjects and as 12 pairs. Each man stayed in the unit for 120 consecutive days: 14 days for the assessment of baseline daily energy intake, 3 days for testing before the period of overfeeding, 100 days for the period of overfeeding, and 3 days for testing after the period of overfeeding.

Body composition and regional fat distribution measurements. The data on body weight represent the mean measurement of 3 days, in each case the day on which the underwater weighing was done and the days before and after. Body density was determined by the hydrostatic weighing technique (1), and percentage body fat was calculated with a standard equation (19). Abdominal computerized tomography scanning was performed before and after the over-
feeding period with a Siemens Somatom DRH scanner (Erlangen, Germany) as reported earlier (20).

**RMR.** On the first day of the 3-day test period, the measurement of RMR was performed in a 12-h fasted state by use of an open-circuit indirect calorimeter, as previously described (23). The subject was placed in a comfortable reclining seat with his head inside a Beckman ventilated hood system (Schiller Park, IL). Then the subjects rested for 30 min to reduce previous disturbing influences, and RMR was assessed over the next 30 min. The concentrations of oxygen and carbon dioxide were measured by use of the paramagnetic and infrared analyzers (OM-11 and LB-2, Beckman), whereas pulmonary ventilation was determined with a Fleish respirometer. The analyzers were calibrated with standardized gases just before the measurements.

**Activities of enzyme markers in muscle biopsies.** Muscle biopsies were obtained from the middle region of the vastus lateralis muscle and ~2 cm away from the epimysium by the percutaneous needle biopsy technique as described earlier (21). The enzymatic markers considered here are OGDH (key enzyme in aerobic oxidation) and phosphofructokinase (PFK; key enzyme in glycolysis) and the ratio of PFK to OGDH (PFK/OGDH; an indication of glycolysis to aerobic oxidation). A piece of the frozen muscle sample (~10 mg) was mixed in a small Duall glass homogenizer with 39 vol (weight/volume) of extracting medium (0.1 M K, Na-phosphate, 2 mM EDTA, pH 7.2). The muscle sample was homogenized with several passes of the glass pestle and was used for the enzyme activity measurements. Maximal activity of OGDH and PFK was fluorometrically assayed the day of the biopsy at 25°C for OGDH and 30°C for PFK, according to the procedures described in previous studies (18, 23).

**Plasma glucose and insulin determinations.** The plasma glucose level was measured enzymatically (15) and the plasma insulin by radioimmunoassay. Glucose and insulin total areas under the curve during an oral glucose tolerance test (OGTT) test were determined with the trapezoidal method. Subjects ingested 75 g of glucose and were monitored for 3 h as described earlier (13).

**Plasma lipid measurements.** Blood samples were collected from an antecubital vein into Vacutainer tubes containing EDTA after a 12-h overnight fast. Plasma was separated immediately after blood collection by centrifugation at 3,000 rpm for 10 min at 4°C for the measurement of plasma lipid and lipoprotein levels. Triglyceride and cholesterol concentrations in plasma and lipoprotein fractions were determined enzymatically on a Technicon RA-500 automated analyzer (Bayer, Tarrytown, NY). Plasma very-low-density lipoproteins (density < 1.006 g/ml) were isolated by ultracentrifugation (50,000 rpm) by using a Beckman 50.3Ti rotor (Beckman, Palo Alto, CA) as reported earlier (11). High-density lipoprotein (HDL) particles were isolated from the bottom fraction (>1.006 g/ml) after precipitation of low-density lipoprotein (LDL) cholesterol with heparin and MnCl₂ (5). The triglyceride and cholesterol contents of the infranatant fraction were measured before and after the precipitation step for measurement of LDL and HDL compositions.

**Genotype measurements.** Genomic DNA was isolated from lymphoblastoid cell lines (12) and digested with BglII restriction enzyme, and the resulting DNA fragments were separated by agarose gel electrophoresis. Southern blot analysis was performed as described earlier (14).

**Statistical analysis.** Differences in phenotype changes (percent and absolute changes) between the Na⁺-K⁺-ATPase α2-gene BglII genotypes were assessed by t-tests. However, because both series of results were highly concordant, the emphasis is put on percent changes in this report. Percent changes were calculated from individual scores. Analyses were performed with the phenotype mean of each of the 12 pairs. Statistical analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

### RESULTS

Changes in body weight and measures of body composition with overfeeding in the Quebec Overfeeding Study have been reported previously for the 12 pairs of twins (2) and will not be detailed here. The 3.3-kb allele homozygous subjects (n = 1 pair) of the Na⁺-K⁺-ATPase α2-gene exon 1 BglII polymorphism were pooled together with the 8.0/3.3-kb heterozygotes (n = 10) of the Na⁺-K⁺-ATPase α2-gene BglII genotype to define the heterozygous group.

### Table 1. Effect of overfeeding on obesity-related phenotypes in 12 pairs of male twins in relation to the Na⁺-K⁺-ATPase α2 BglII genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>3.3-kb⁻ (n = 7 pairs)</th>
<th>3.3-kb⁺ (n = 5 pairs)</th>
<th>P Value for Differences Between Genotypes for Change in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Change, %</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>56.4±1.9</td>
<td>64.7±2.2</td>
<td>+14.8±1.1</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>5.0±0.7</td>
<td>10.3±1.1</td>
<td>+127.0±15.1</td>
</tr>
<tr>
<td>RMR, kJ/kg FFM</td>
<td>0.088±0.003</td>
<td>0.083±0.003</td>
<td>+5.2±2.3</td>
</tr>
<tr>
<td>RQ</td>
<td>0.76±0.02</td>
<td>0.73±0.01</td>
<td>-3.2±2.9</td>
</tr>
<tr>
<td>Abdominal fat in CT, cm²</td>
<td>31.9±3.0</td>
<td>59.5±4.5</td>
<td>+99.0±13.5</td>
</tr>
<tr>
<td>Visceral</td>
<td>68.6±10.8</td>
<td>132.4±12.2</td>
<td>+130.0±17.0</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>100.5±12.3</td>
<td>191.9±13.6</td>
<td>+118.1±14.9</td>
</tr>
<tr>
<td>Total</td>
<td>48.4±3.1</td>
<td>62.5±8.0</td>
<td>+62.1±37.7</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>55.0±5.5</td>
<td>71.8±9.2</td>
<td>+29.8±9.6</td>
</tr>
<tr>
<td>OGTT insulin area, 10³ PM × min</td>
<td>959.9±35.6</td>
<td>1,026.3±26.2</td>
<td>+8.2±3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. RMR, resting metabolic rate; FFM, fat-free mass; RQ, respiratory quotient; OGTT, oral glucose tolerance test; NS, not significant. Body weight was higher (P < 0.05) before and fat mass was higher both before (P = 0.01) and after (P = 0.05) overfeeding in 3.3-kb⁻ than in 3.3-kb⁺ subjects. OGTT glucose area tended to be higher (P = 0.08) in 3.3-kb⁻ than in 3.3-kb⁺ subjects after the caloric surplus only.

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Table 2. Effect of overfeeding on plasma lipids and lipoproteins in 12 pairs of male twins in relation to the Na\(^+\)-K\(^+\)-ATPase \(\alpha2\) BglII genotype

<table>
<thead>
<tr>
<th></th>
<th>3.3-kb(^-) (n = 7 pairs)</th>
<th>3.3-kb(^+) (n = 5 pairs)</th>
<th>(P) Value for Differences Between Genotypes for Change in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Change, %</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.42 ± 0.22</td>
<td>5.11 ± 0.31</td>
<td>+15.9 ± 4.7</td>
</tr>
<tr>
<td>LDL</td>
<td>2.76 ± 0.18</td>
<td>3.27 ± 0.22</td>
<td>+20.2 ± 4.7</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.40 ± 0.06</td>
<td>0.69 ± 0.17</td>
<td>+56.4 ± 22.3</td>
</tr>
<tr>
<td>HDL</td>
<td>1.26 ± 0.04</td>
<td>1.18 ± 0.04</td>
<td>−6.2 ± 3.0</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.16 ± 0.13</td>
<td>1.85 ± 0.35</td>
<td>+49.4 ± 17.1</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.71 ± 0.13</td>
<td>1.31 ± 0.32</td>
<td>+88.6 ± 30.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein. High-density lipoprotein (HDL) cholesterol levels were higher both before and after overfeeding (\(P < 0.05\)) in 3.3-kb\(^-\) than in 3.3-kb\(^+\) subjects.

4 pairs) (8.0/3.3-kb + 3.3/3.3-kb; 5 pairs) and compared with the 8.0-kb allele homozygotes (8.0/8.0-kb; n = 7 pairs) in all analyses. Thus the response to overfeeding between 3.3-kb positive (+) and 3.3-kb negative (−) is compared.

Table 1 shows the effect of overfeeding on obesity-related phenotypes for the two Na\(^+\)-K\(^+\)-ATPase \(\alpha2\) gene exon 1 BglII genotypes. Body weight was higher before overfeeding (\(P < 0.05\)), and fat mass was higher both before (\(P = 0.01\)) and after (\(P = 0.05\)) overfeeding in 3.3-kb\(^+\) than in 3.3-kb\(^-\) subjects. However, overfeeding induced a significantly larger increase in total fat mass in 3.3-kb\(^-\) than in 3.3-kb\(^+\) subjects (\(P < 0.05\)). In addition, the amount of abdominal visceral fat tended (\(P = 0.1\)) to increase more in 3.3-kb\(^-\) than in 3.3-kb\(^+\) subjects. OGTT glucose area tended to be higher (\(P = 0.08\)) in 3.3-kb\(^-\) than in 3.3-kb\(^+\) subjects after the caloric surplus. RMR tended to be higher and respiratory quotient lower before overfeeding among subjects with 3.3-kb\(^-\) than in those with 3.3-kb\(^+\).

HDL cholesterol levels were higher both before and after overfeeding (\(P < 0.05\)), and LDL cholesterol increased more (\(P = 0.05\)) in response to overfeeding in 3.3-kb\(^-\) than in 3.3-kb\(^+\) subjects (Table 2).

In Table 3, the effects of overfeeding on skeletal muscle enzymes by genotype are shown. OGDH activity decreased in 3.3-kb\(^-\) subjects but increased in 3.3-kb\(^+\) subjects. In contrast, PFK as well as PFK/OGDH increased in 3.3-kb\(^-\) subjects but decreased in 3.3-kb\(^+\) subjects (\(P < 0.05\) and \(P < 0.01\) for the difference between genotype groups for the change in PFK and PFK/OGDH, respectively).

### DISCUSSION

The present study suggests that, in response to overfeeding, the Na\(^+\)-K\(^+\)-ATPase \(\alpha2\) gene exon 1 BglII polymorphism was associated with the change in PFK/OGDH, which is an indicator of the glycolytic relative to the oxidative pathways. OGDH activity decreased in 3.3-kb\(^-\) subjects, and PFK as well as PFK/OGDH increased with overfeeding. In contrast, among 3.3-kb\(^+\) subjects, OGDH increased together with a slight decrease in PFK resulting in a decrease in PFK/OGDH. In earlier studies, the skeletal muscle glycolytic-to-oxidative enzyme ratio has been shown to be strongly related to insulin resistance (17). In the present study, the changes in insulin areas under the OGTT curve were not different between the genotypes in response to overfeeding. However, the glucose area during OGTT tended to be higher in the 3.3-kb\(^-\) than in the 3.3-kb\(^+\) subjects after the overfeeding protocol. One could speculate that stimulated insulin secretion after the overfeeding protocol could only partially compensate for a diminished insulin action in 3.3-kb\(^-\) subjects. This is intriguing because the 3.3-kb\(^-\) subjects had significantly less total fat both before and after overfeeding. In addition, the plasma level of LDL cholesterol, total fat mass, and possibly visceral adiposity (trend only) increased more in the 3.3-kb\(^-\) subjects,
who also showed a greater increase in PFK/OGDH with overfeeding. This is in accordance with an earlier reported association between visceral obesity and reduced skeletal muscle utilization of free fatty acids and an increased ratio of glycolytic to oxidative enzyme capacity in skeletal muscle (4).

Earlier studies have found in cross-sectional designs that the Na\(^+\)-K\(^+\)-ATPase \(\alpha\)2-gene may play a role in metabolic fuel-oxidation rates (8). The mechanism for this effect is unknown. Na\(^+\)-K\(^+\)-ATPase is an essential plasma membrane enzyme (3) that regulates plasma membrane potential and may potentially influence the intracellular uptake of fatty acids. Na\(^+\)-K\(^+\)-ATPase \(\alpha\)2-gene exon 1 \(B_{gII}\) polymorphism, or another variant in linkage disequilibrium, may change the Na\(^+\)-K\(^+\)-ATPase activity and skeletal muscle uptake of free fatty acids. This could in turn reduce the capacity for fat oxidation and increase the reliance on carbohydrates in skeletal muscle.

Obesity is associated with decreased activities of skeletal muscle aerobic metabolism enzymes (9, 16). Thus, in the present study, 3.3-kb \(\alpha\) subjects, who were on average almost 10 kg heavier and had almost 5 kg more fat mass at baseline, tended also to have lower OGDH and higher PFK activities, i.e., a more glycolytic profile in skeletal muscle at baseline. The underlying cause could be an altered ATPase activity perhaps as a result of a point mutation in the Na\(^+\)-K\(^+\)-ATPase \(\alpha\)2-gene. Earlier studies have suggested a relationship between aerobic glycolysis and Na\(^+\)-K\(^+\)-ATPase \(\alpha\)2 activity (7). Moreover, we have observed here that the 3.3-kb \(\alpha\) subjects gained significantly less fat and slightly less abdominal fat. However, their PFK/OGDH activities or respiratory quotient did not change much in response to overfeeding. We speculate that 3.3-kb \(\alpha\) subjects chronically exposed to positive energy balance could have experienced an increase in ATPase activity that prevented them from gaining more body fat.

In conclusion, the Na\(^+\)-K\(^+\)-ATPase \(\alpha\)2-gene exon 1 \(B_{gII}\) polymorphism was associated with a change of the skeletal muscle glycolytic-to-oxidative enzyme activities ratio. The plasma level of LDL cholesterol and total fat mass increased more in the 3.3-kb \(\alpha\) subjects, who also showed a greater increase in the skeletal muscle glycolytic-to-oxidative enzyme ratio with overfeeding. The skeletal muscle properties observed among 3.3-kb \(\alpha\) subjects in response to long-term overfeeding seems to be metabolically unfavorable.

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