Enhanced insulin sensitivity after acute exercise is not associated with changes in high-molecular weight adiponectin concentration in plasma

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Abstract

Background and objective: The effect of exercise on the plasma concentration of high-molecular weight (HMW) adiponectin (i.e. the biologically active form of circulating adiponectin) and the possible role of HMW adiponectin in mediating the exercise-induced enhancement of insulin action are not known. The aim of this study was to evaluate the relationship between the post-exercise increase in insulin sensitivity and plasma HMW adiponectin concentration.

Design and methods: We measured total and HMW adiponectin concentrations in plasma using an ELISA kit, and insulin sensitivity using the updated homeostasis model assessment of insulin sensitivity (HOMA2-IS) score in the basal, overnight fasted state, once 12 h after a single bout of moderate-intensity endurance exercise and once after an equivalent period of rest, in 27 healthy men and women (age: 29 ± 1 years and body mass index: 24.7 ± 0.8 kg/m²).

Results: The HOMA2-IS score was 18 ± 7% greater after exercise than after rest (229 ± 20 and 196 ± 17 respectively; \( P = 0.006 \)), whereas the concentrations of total adiponectin (7.8 ± 0.5 and 7.7 ± 0.5 mg/l respectively; \( P = 0.597 \)) and HMW adiponectin (3.0 ± 0.3 and 3.0 ± 0.3 mg/l respectively; \( P = 0.625 \)) were not different. The exercise-induced change in HOMA2-IS score was not related to changes in total and HMW adiponectin concentrations (\( P > 0.3 \)).

Conclusions: Changes in HMW adiponectin concentration are not involved in the acute exercise-induced enhancement of insulin action.

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Introduction

Adiponectin, in particular, the high-molecular weight (HMW) adiponectin isoform (1–4), has potent anti-atherogenic, anti-inflammatory, and anti-diabetic effects (5). Decreased plasma adiponectin concentrations are characteristic of insulin-resistant states, and in animals, adiponectin administration or over-expression improves insulin sensitivity (6, 7). In human subjects, adiponectin concentrations are reduced in obese, glucose-intolerant, and type 2 diabetic subjects, and weight loss and treatment with thiazolidinediones or rimonabant which improve insulin sensitivity also increase plasma adiponectin concentrations (7–9). Aerobic exercise is well known to enhance insulin action, both acutely, i.e. after a single bout of exercise, and chronically, i.e. after regular exercise training (10–12). However, it is not known whether the exercise-induced change in insulin sensitivity is accompanied by and possibly mediated by changes in adiponectin availability (13). Some studies report an increase, some report no change, and some even report a decrease in total plasma adiponectin concentration after a single session of aerobic exercise or endurance training (14). Part of this discrepancy could be due to the fact that only total plasma adiponectin but not HMW adiponectin concentration was measured in these studies. Furthermore, in studies that evaluated changes in HMW adiponectin concentration after a single bout of aerobic exercise, measurements were made within 30 min of exercise cessation (15, 16), whereas the acute exercise-induced enhancement of insulin action is typically not evident until later during recovery (12). Failure to observe changes in HMW adiponectin concentration (15, 16) might therefore have been due to improper timing of the measurements.

The purpose of this study was to determine the relationship between the exercise-induced changes in insulin sensitivity and HMW adiponectin concentration in plasma during the late phase of recovery from a single bout of moderate-intensity aerobic exercise. We made our measurements in healthy men and women in the basal state after an overnight fast, once 12 h after a single evening bout of endurance exercise and once...
after a time-matched resting trial. Insulin sensitivity was determined using the updated homeostasis model assessment of insulin sensitivity (HOMA2-IS) score, which is based on improved modeling algorithms (17).

Materials and methods

Subjects and preliminary testing

Twenty-seven men and women (age: 29 ± 1 years; body mass index: 24.7 ± 0.8 kg/m²; peak oxygen consumption (VO₂ peak): 39 ± 2 ml/kg per min; means ± s.e.m.) volunteered for the study. All the subjects were considered to be in good health after completing a medical evaluation, which included a history and physical examination and standard blood tests. All were normoglycemic and normolipidemic; none consumed tobacco products or took medications known to affect metabolism. VO₂ peak was determined on a bicycle ergometer as described previously (18–20). Written informed consent was obtained from all the subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Advisory Committee at the Washington University School of Medicine in St Louis, MO, USA.

Experimental protocol

Each subject completed two time-matched studies within 4 weeks in a randomized order: one after resting and one after cycling on the preceding afternoon. Female subjects performed both trials in the same phase and one after cycling on the preceding afternoon. At 0700 h the following morning, an arterialized blood sample was obtained from a heated hand vein for the determination of fasting plasma glucose, insulin, and adiponectin concentrations. Blood was collected in chilled tubes containing heparin (for glucose) or sodium EDTA plus aprotinin (for insulin and adiponectin) and placed immediately on ice. Plasma was separated by centrifugation within 30 min of collection, and samples were stored at − 80 °C until analysis.

Sample analysis

Plasma glucose concentration was determined using the glucose oxidase method on an automated glucose analyzer (YSI 2300 STAT PLUS, Yellow Spring Instruments, Yellow Springs, OH, USA). Plasma insulin concentration was measured with a commercially available RIA kit which is specific for insulin (Linco Research, St Louis, MO, USA) (22). Total plasma adiponectin and HMW adiponectin concentrations were determined using a commercially available sandwich ELISA kit (American Laboratory Products Company, Windham, NH, USA), which uses monoclonal antibodies against human adiponectin and protease pretreatment to selectively digest low- and middle-molecular weight adiponectin isoforms (23, 24). Insulin sensitivity was assessed with the HOMA2-IS score using the HOMA Calculator v2.2.2 (Diabetes Trials Unit, The Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK) (17). Validation studies report good correlations (r = 0.7–0.9) between HOMA scores and estimates of insulin sensitivity derived from the hyperinsulinemic–euglycemic clamp technique and minimal model analysis (25), and the correlation between plasma adiponectin concentration and HOMA scores (r = 0.4) (26) is as good as the correlation between adiponectin and other measures of insulin sensitivity (r = 0.3–0.6; hyperinsulinemic–euglycemic clamp, minimal model analysis, and oral glucose tolerance test) (27–29).

Statistical analysis

Data were analyzed with SPSS v17.0 for Windows (SPSS Inc., Chicago, IL, USA). All data sets were normally distributed according to the Kolmogorov–Smirnov...
Exercise, insulin sensitivity, and adiponectin

Table 2 Glucose, insulin, and adiponectin concentrations and insulin sensitivity after rest and after exercise. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.8±0.1</td>
<td>4.6±0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>30±3</td>
<td>27±3</td>
<td>0.083</td>
</tr>
<tr>
<td>HOMA2-IS score</td>
<td>196±17</td>
<td>229±20</td>
<td>0.006</td>
</tr>
<tr>
<td>Total adiponectin (mg/l)</td>
<td>7.7±0.5</td>
<td>7.8±0.5</td>
<td>0.597</td>
</tr>
<tr>
<td>LMW/MMW adiponectin (mg/l)</td>
<td>4.7±0.3</td>
<td>4.8±0.3</td>
<td>0.189</td>
</tr>
<tr>
<td>HMW adiponectin (mg/l)</td>
<td>3.0±0.3</td>
<td>3.0±0.3</td>
<td>0.625</td>
</tr>
<tr>
<td>Ratio of HMW-to-total adiponectin</td>
<td>0.37±0.02</td>
<td>0.36±0.02</td>
<td>0.295</td>
</tr>
</tbody>
</table>

HOMA2-IS, updated homeostasis model assessment of insulin sensitivity; LMW+MMW, low- and middle-molecular weight; HMW, high-molecular weight. Data represent averages of duplicate measurements in the basal, overnight fasted state ~12 h after exercise or an equivalent period of rest.

Criteria. Data are presented as means ± S.E.M. Results after rest and after exercise were compared with Student’s paired, two-tailed t-test. Relationships between variables of interest were examined with Pearson’s correlation analyses. A P value of <0.05 was considered statistically significant.

Results

Plasma glucose concentration was ~5% lower (P=0.001), plasma insulin concentration was ~10% lower (P=0.083), and the HOMA2-IS score was ~20% greater (P=0.006) after exercise than after rest (Table 2). Total plasma adiponectin and HMW adiponectin concentrations and the proportional contribution of HMW adiponectin to total adiponectin concentration (i.e. the ratio of HMW-to-total adiponectin) were not different after rest and after exercise (Table 2). There was no relationship between exercise duration or exercise energy expenditure and the changes in plasma total and HMW adiponectin concentrations (all P values >0.175).

After the resting trial, the HOMA2-IS score was positively associated with both total (r=0.641, P<0.001) and HMW (r=0.621, P=0.001) adiponectin concentrations and the ratio of HMW-to-total adiponectin (r=0.423, P=0.028); however, these relationships were not readily apparent after the exercise trial (all P values >0.05).

The exercise-induced change in HOMA2-IS was not related to changes in circulating total and HMW adiponectin concentrations or the ratio of HMW-to-total adiponectin (Table 3).

Discussion

We evaluated the relationship between exercise-induced changes in insulin sensitivity, assessed by the HOMA2-IS score, and total and HMW adiponectin concentrations in the basal, overnight fasted state after a single bout of endurance exercise in healthy men and women. We found that the correlation between total adiponectin concentration and insulin sensitivity observed at rest in our present study and by others (26–29) disappears after exercise. Furthermore, changes in HMW adiponectin concentration (the biologically active isoform of adiponectin) are not involved in increasing insulin sensitivity during the late phase of recovery from a single bout of exercise. Therefore, circulating adiponectin concentration may be an important determinant of insulin action during resting conditions, but it does not mediate the exercise-induced changes in insulin sensitivity, indicating that other factors are likely to be responsible. The failure of a single bout of exercise to raise total and HMW adiponectin concentrations in plasma is consistent with the results obtained from studies that measured total and HMW adiponectin concentrations immediately (15) or shortly after (16) a single bout of aerobic exercise; however, they are at odds with the results of the studies of endurance training which reportedly raises plasma HMW adiponectin concentration (30, 31). Increased HMW adiponectin availability therefore appears to be an adaptation to chronic exercise, and is not necessary to mediate the increase in insulin action acutely after exercise.

Our findings complement and expand our current knowledge regarding the effect of exercise on adiponectin homeostasis. Although adiponectin secretion from subcutaneous adipose tissue increases significantly during exercise (32), only some but not all the studies

Table 3 Relationship between exercise-induced changes in insulin sensitivity and circulating adiponectin.

<table>
<thead>
<tr>
<th>Change in HOMA2-IS score</th>
<th>Percent change in HOMA2-IS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in total adiponectin concentration</td>
<td>-0.207 (0.301)</td>
</tr>
<tr>
<td>Percent change in total adiponectin concentration</td>
<td>-</td>
</tr>
<tr>
<td>Change in HMW adiponectin concentration</td>
<td>-0.201 (0.314)</td>
</tr>
<tr>
<td>Percent change in HMW adiponectin concentration</td>
<td>-</td>
</tr>
<tr>
<td>Change in the ratio of HMW-to-total adiponectin</td>
<td>-0.107 (0.596)</td>
</tr>
<tr>
<td>Percent change in the ratio of HMW-to-total adiponectin</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are Pearson’s linear correlation coefficients (r) with P values given in the parentheses. HOMA2-IS, updated homeostasis model assessment of insulin sensitivity; HMW, high-molecular weight.
report an increase in total adiponectin concentration after a single bout of exercise (14). More importantly, however, only two studies to date have measured the concentration of HMW adiponectin, i.e. the biologically active isof orm of adiponectin (1–4), during and/or after exercise and found no changes, even though measurements were made during the early phase of recovery from exercise (≤ 30 min post-exercise) (15, 16), whereas the acute exercise-induced enhancement of insulin action is typically not evident until > 3 h after exercise cessation (12). To our knowledge, acute exercise-induced changes in plasma HMW adiponectin concentration during the prolonged recovery period have never been examined. Actually, only one study has measured total adiponectin concentration up to 48 h after a single bout of exercise and did not observe any changes, but unfortunately the amount of exercise performed in that study was insufficient to increase insulin sensitivity (33). In our study, insulin sensitivity improved after exercise by 18 ± 7%, but we found no correlation between the exercise-induced changes in insulin sensitivity and plasma adiponectin concentration. We therefore conclude that the insulin-sensitizing effect of a single bout of exercise is not mediated by changes in plasma HMW adiponectin availability.

The mechanisms responsible for the increase in insulin sensitivity after acute exercise are not entirely clear. Depletion of muscle glycogen leads to enhanced insulin-mediated glucose uptake in the previously exercised muscles to facilitate glycogen replenishment (34, 35). Intramuscular triglyceride is also closely associated with insulin sensitivity (36), and the depletion of skeletal muscle lipid stores during exercise in conjunction with enhanced lipid oxidation after exercise could also facilitate muscle insulin action (37). On the other hand, although both chronic (12 months) (38) and acute (48 h) (39) diet-induced energy deficits improve insulin sensitivity, the negative energy balance induced by exercise in our study is unlikely to be the cause for the exercise-induced enhancement of insulin action because a single bout of prolonged endurance exercise brings about an increase in insulin sensitivity the next morning regardless of whether the calories expended during exercise are replaced by overfeeding (zero energy balance) or not (negative energy balance) (40). Likewise, total and HMW adiponectin concentrations increase after prolonged hypocaloric diets (41, 42); however, they are not affected by short-term (4 days of − 800 kcal/day) (43) or acute (48 h of food deprivation) (44) energy deficits.

The acute effect of exercise on plasma adiponectin concentration contrasts that of regular exercise training where increased HMW adiponectin concentrations and improved insulin sensitivity were observed 48–72 h after the last bout of exercise (30, 31). The reason for the different response of HMW adiponectin concentration to acute and chronic exercise is not entirely clear, but it could be due to the training-induced decrease in body weight and body fat in these studies (30, 31) rather than to an adaptive response to repeated bouts of exercise. Adipose tissue adiponectin gene expression and plasma concentration increase after weight loss (41, 45), and training-induced changes in total plasma adiponectin concentration correlate inversely with the corresponding changes in body weight and body fat (46, 47). In fact, two recent studies demonstrated that short-term (7 days) endurance (48) and long-term (12 weeks) resistance (49) exercise training do not increase total and HMW adiponectin concentrations in the absence of weight loss.

We used the HOMA score as an index of whole-body insulin sensitivity. Therefore, we cannot determine whether the exercise-induced changes in insulin sensitivity occurred in the muscle or the liver. However, this does not affect the conclusion derived from our study, i.e. that changes in total and HMW adiponectin concentrations are not responsible for the exercise-induced increase in insulin sensitivity. Furthermore, we only measured the plasma concentration of total and HMW adiponectin, so we do not know whether exercise might have caused an increase in the sensitivity to adiponectin. There is evidence that exercise (acute and chronic) increases adiponectin receptor expression in skeletal muscle (30, 50) and adipose tissue (51). Therefore, changes in adiponectin receptor tissue density after exercise, rather than changes in circulating total and HMW adiponectin, may be of physiological importance for the increase in insulin sensitivity after exercise. Unfortunately, assessing sensitivity to adiponectin in vivo is currently not feasible as many factors, both technical and physiological, make administration of the protein rather challenging (52).

In summary, despite increased insulin sensitivity we found no evidence of an exercise-induced change in the concentration of adiponectin (total and HMW) in plasma in the basal, post-absorptive state. These observations argue against the involvement of adiponectin in the acute exercise-induced enhancement of insulin action.

Declaration of interest

The authors have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
F Magkos and B Mittendorfer designed the research; F Magkos, B Selma Mohammed, and B Mittendorfer performed the studies; and F Magkos and B Mittendorfer wrote the manuscript.

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References
4 von Eynatten M, Lepper PM & Humpert PM. Total and high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care* 2006 29 1357–1362.