The effects of exercise and adipose tissue lipolysis on plasma adiponectin concentration and adiponectin receptor expression in human skeletal muscle

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Abstract

Objective: It has been suggested that adiponectin regulates plasma free fatty acid (FFA) clearance by stimulating FFA uptake and/or oxidation in muscle. We aimed to determine changes in plasma adiponectin concentration and adiponectin receptor 1 and 2 mRNA expression in skeletal muscle during and after prolonged exercise under normal, fasting conditions (high FFA trial; HF A) and following pharmacological inhibition of adipose tissue lipolysis (low FFA trial; LF A). Furthermore, we aimed to detect and locate adiponectin in skeletal muscle tissue.

Methods: Ten subjects performed two exercise trials (120 min at 50% VO₂max). Indirect calorimetry was used to determine total fat oxidation rate. Plasma samples were collected at rest, during exercise and during post-exercise recovery to determine adiponectin, FFA and glycerol concentrations. Muscle biopsies were taken to determine adiponectin protein and adiponectin receptor 1 and 2 mRNA expression and to localise intramyocellular adiponectin.

Results: Basal plasma adiponectin concentrations averaged 6.57±0.7 and 6.63±0.8 mg/l in the HF A and LF A trials respectively, and did not change significantly during or after exercise. In the LF A trial, plasma FFA concentrations and total fat oxidation rates were substantially reduced. However, plasma adiponectin and muscle adiponectin receptor 1 and 2 mRNA expression did not differ between trials. Immunohistochemical staining of muscle cross-sections showed the presence of adiponectin in the sarcolemma of individual muscle fibres and within the interfibrillar arterioles.

Conclusion: Plasma adiponectin concentrations and adiponectin receptor 1 and 2 mRNA expression in muscle are not acutely regulated by changes in adipose tissue lipolysis and/or plasma FFA concentrations. Adiponectin is abundantly expressed in muscle, and, for the first time, it has been shown to be present in/on the sarcolemma of individual muscle fibres.

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Introduction

Adiponectin, also known as Acrp30 (1, 2), adipoQ (3), aPML-1 (4) and GBP28 (5) is a circulating hormone secreted by adipose tissue. Correlation studies have demonstrated that reduced adiponectin expression and/or low plasma adiponectin concentrations are associated with obesity (6), insulin resistance (7) and/or coronary artery disease (8). Moreover, exogenous adiponectin administration has been shown to improve insulin sensitivity in obese diabetic (9) and adiponectin-knockout mice (10). Although the exact physiological function of adiponectin remains to be established, it has been suggested that adiponectin plays a regulatory role in substrate metabolism in liver (11) and skeletal muscle (2).

Both weight loss and exercise have proven effective in the prevention and treatment of arteriosclerosis and skeletal muscle insulin resistance (12–14). Whereas weight loss and/or energy intake restriction have been associated with elevated plasma adiponectin concentrations (7, 8), studies investigating the effects of exercise on adiponectin release have provided inconsistent findings (15–22). Kraemer et al. (15) investigated the acute effects of short-term, running exercise on plasma adiponectin secretion in healthy subjects but observed no changes. Other studies, exploring the more long-term benefits of exercise training, also reported no changes in basal plasma adiponectin concentration (16–18, 22). In contrast, Kriketos et al. (19) recently reported substantially elevated fasting plasma adiponectin levels (260% above baseline content).
obtained. This study was approved by the local Medical
Ethical Committee and performed according to the
guidelines set by the Declaration of Helsinki.

Pre-testing
W_max and VO_2max were measured on an electronically
braked cycle ergometer (Lode Excalibur, Groningen,
The Netherlands) during an incremental exhaustive
exercise test one week before the first experimental
trial (28). Body composition was assessed using the
hydrostatic weighing method in the morning after an
overnight fast. Simultaneously, residual lung volume
was measured by the helium-dilution technique. Body
fat percentage was calculated using Siri’s equation
(29). Fat free mass (FFM) was calculated by subtracting
fat mass (FM) from total body weight.

Diet and activity prior to testing
All subjects maintained normal dietary and physical
activity patterns throughout the experimental period.
They refrained from heavy physical labour and exercise
training for at least 3 days prior to each trial and filled
out a food intake diary for 2 days prior to the first trial
to keep dietary intake as identical as possible prior to
the other trial. The evening before each trial, subjects
received the same standardised meal (41.2 kJ/kg body
weight; consisting of 72 energy % (En%) carbohydrate,
11 En% fat and 17 En% protein).

Experimental trials
Each subject performed two similar trials, separated by
at least one week. Each trial consisted of 90 min of rest-
ing measurements, followed by 120 min of cycling exer-
cise (50% W_max) and a 120 min recovery period. In
both trials, blood samples were collected at regular
time intervals. In addition, percutaneous muscle biop-
sies were taken from the vastus lateralis muscle before,
immediately after and 2 h after exercise. In one trial
(low FFA; LFA), adipose tissue lipolysis was inhibited
by oral administration of a nicotinic acid analogue
(500 mg Acipimox). In the other trial (high FFA;
HFA), a placebo was administered. Both trials were
performed in randomised order and executed in a
double blind fashion.

Protocol
After an overnight fast, subjects arrived at the labora-
atory at 0800 h by car or public transportation. After
30 min of supine rest, a percutaneous muscle biopsy
was taken from the vastus lateralis muscle. A Teflon
catheter (Baxter, Utrecht, The Netherlands) was
inserted into an antecubital vein for blood sampling,
after which a resting blood sample was taken. At
t = 0 and t = 165 min, a capsule containing 250 mg
Acipimox (Nedios, Byk, Zwanenburg, The Netherlands)
or a placebo was orally administered. At t = 90 min subjects started to exercise on a cycle ergometer at a workload of 50% Wmax for a 2-h period. Whilst at rest, oxygen uptake (VO2) and carbon dioxide excretion (VCO2) were measured continuously (Oxycon-β, Mijnhart Bunnik, The Netherlands), during exercise, measurements were performed for 5 min every 15 min before blood collection. After cessation of exercise a second muscle biopsy was obtained, after which subjects rested supine for 2 h during which VO2 and VCO2 were again measured continuously. Blood samples for plasma FF A, glycerol, glucose, and adiponectin analyses were collected at t = 0 min and at 90 min (at rest), at t = 120, 150, 180, 210 min (exercise) and at t = 240, 300 and 330 min (post-exercise recovery). After 2 h of recovery (t = 330 min) a third muscle biopsy was collected.

**Blood sample analysis**

Blood samples (7 ml) were collected in EDTA-containing tubes and centrifuged at 1000 × g at 4°C for 10 min. Plasma aliquots were frozen immediately in liquid nitrogen and stored at −80°C until analyses. Plasma glucose (Uni Kit III, Roche, Basel, Switzerland), FFA (NEFA-C, Wako Chemicals, Neuss, Germany), and free glycerol (148270, Roche, Indianapolis, IN, USA) concentrations were analysed by radioimmunoassay (Linco Diagnostics Inc., St Charles, MO, USA).

**Immunohistochemistry**

Muscle samples were dissected and frozen in liquid nitrogen. About 15 mg muscle were frozen in nitrogen-cooled isopentane and embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands). Multiple serial sections (5 µm) were thaw mounted on uncoated, pre-cleaned glass slides, fixed in formaldehyde and incubated overnight with an Acrp30/ adiponectin monoclonal antibody (MAB 10651, R&D Systems Inc, Minneapolis, MN, USA), and a laminin polyclonal antibody (Sigma Diagnostics, Steinheim, Germany). After washes with PBS, sections were incubated with appropriate conjugated secondary antibodies: GAMlgG2B Alexa555, GARlgGAlexa488 (Molecular Probes, Leiden, The Netherlands). Thereafter, sections were embedded in Mowiol-Tris (Calbiochem, Omnilabo, Etten-Leur, The Netherlands) containing 0.5 µg/ml 4′,6′-diamino-2-phenylindole (DAPI, Molecular Probes). Slides were examined using a Nikon E800 fluorescence microscope (Uvikon, Bunnik, The Netherlands) coupled to a Basler A113 C progressive scan colour charge-coupled device (CCD) camera. Appropriate controls, applying only primary or secondary antibodies, showed no signal.

**Immunoblotting**

About 30 mg muscle tissue were sonicated in ice-cold buffer (20 mmol/l HEPES (pH 7.4), 100 mmol/l KCl, 50 mmol/l beta-glycerophosphate, 50 mmol/l NaF, 1 mmol/l dithiothreitol, 0.5 mmol/l Na3VO4, 0.2 mmol/l EDTA, 2 mmol/l EGTA, 0.1 mmol/l PMSF and 1 mmol/l benzamidine). Homogenates were centrifuged for 5 min at 10 000 × g and at 4°C. Thereafter, the supernatant was centrifuged (at 10 000 × g and at 4°C for 10 min) and resolved in SDS-buffer. Human plasma was used as a positive control for adiponectin protein expression. First, albumin was filtered from the plasma sample, using the SwellGel Blue albumin removal kit (89845, Pierce Biotechnology, Rockford, IL, USA). After boiling the samples, denatured protein from a subset of 20 plasma samples (each containing ~6 µg protein) and 10 different muscle samples (each containing ~50 µg protein) were run on 15% SDS-polyacrylamide gels and transferred onto 0.45 µm nitrocellulose membranes. Membranes were exposed to a 1:1000 dilution of a monoclonal antihuman Acrp30/adiponectin antibody (MAB 10651) and incubated in a 1:10 000 dilution of horseradish peroxidase-conjugated antibody (Pierce). Light sensitive film (CL-Xposure; Pierce) was used to detect immunoreactive bands using chemiluminescent substrate (SuperSignal CL, Pierce).

**PCR methodology**

Total RNA was extracted from 10–15 mg wet muscle as previously described (30). cDNA was obtained using AMV Reverse Transcriptase and Oligo (dT)15 Primers (Promega, Madison, WI, USA). Samples were analysed in triplicate to detect adiponectin 1 and 2 receptor and cyclophilin mRNA transcripts using real time RT-PCR (Gene Amp 5700 sequence detection system, Applied Biosystems, Foster City, CA, USA) as previously described (30) using primer sequences obtained from GenBank (adiponectin receptor 1 – NM_015999: forward primer, cgccatggagaagatggaa; reverse primer, tcatatgggatgaccctc(aa); adiponectin receptor 2 – NM_024551: forward primer, catctgcactgccaagactga; reverse primer, gaacgcttttt; reverse primer, tgagacaccatggaagtgaacaa). A BLAST search confirmed homologous binding (31).

Input cDNA concentrations were normalised for the housekeeping gene (cyclophilin-X52851: forward primer, catctgacctgccaagactg; reverse primer, tcatgacctttcactttg).

**Calculations**

From respiratory measurements, total fat and carbohydrate oxidation rates were calculated using the
non-protein respiratory quotient (32).

Fat oxidation rate \(= 1.695 \text{VO}_2 - 1.701 \text{VCO}_2\)

Carbohydrate oxidation rate

\[= 4.585 \text{VCO}_2 - 3.226 \text{VO}_2\]

with \(\text{VO}_2\) and \(\text{VCO}_2\) in l/min and oxidation rates in g/min.

**Statistics**

All data are expressed as means±S.E.M. To compare substrate utilisation rates and plasma metabolite concentrations over time within a trial, a one-way repeated measures analysis of variance (ANOVA) was applied. To enable comparisons between trials, a two-way repeated measures ANOVA was applied. A Scheffé’s post-hoc test was applied in case of a significant F-ratio to locate specific differences. For non-time-dependent variables, a Student’s \(t\)-test for paired observations was used.

**Results**

**Plasma metabolites**

Plasma FFA concentrations increased substantially during exercise in the HFA trial \((P < 0.001)\). During recovery, plasma FFA levels declined but remained well above pre-exercise resting levels \((P < 0.001)\). In contrast, in the LFA trial, plasma FFA levels declined following Acipimox administration at rest, after which they remained below or at near baseline values throughout exercise and post-exercise recovery. Plasma FFA levels were significantly lower in the LFA versus the HFA trial \((P < 0.001; \text{Fig. 1A})\). Plasma glycerol concentrations increased substantially during exercise in the HFA trial \((P < 0.001)\). During recovery, plasma glycerol levels declined but remained well above pre-exercise resting values \((P < 0.001)\). In the LFA trial, small but significant increase in plasma glycerol was observed following the onset of exercise \((P < 0.01)\). Thereafter, glycerol levels declined to pre-exercise resting values. Plasma glycerol levels were significantly lower in the LFA compared with the HFA trial \((P < 0.001; \text{Fig. 1B})\). Plasma glucose concentrations declined during exercise in both trials \((P < 0.001)\). During post-exercise recovery, plasma glucose levels remained below pre-exercise resting values. No significant differences in plasma glucose concentrations were observed between trials \((\text{Fig. 1C})\).

**Substrate utilisation**

Total fat and carbohydrate oxidation rates as well as total energy expenditure at rest, during exercise and during post-exercise recovery are provided in Table 1.

![Figure 1](image-url)  
**Figure 1** Plasm FFA (A), glycerol (B) and glucose (C) concentrations at rest, during prolonged submaximal exercise and during post-exercise recovery following administration of Acipimox (low FFA trial (LFA), solid circles) or a placebo (high FFA trial (HFA), open circles). Times for rest, exercise and post-exercise recovery are \(t = 0–90\) min, \(t = 90–210\) min and \(t = 210–330\) min, respectively. Data provided are means±S.E.M.; \(*P < 0.05\), significant differences over time between trials in the resting, exercise and/or post-exercise recovery periods.

Total fat oxidation rates were substantially lower at rest, during exercise as well as during recovery in the LFA compared with the HFA trial \((P < 0.05)\). Total carbohydrate oxidation rates were greater in the LFA trial when compared with the HFA trial. Energy expenditure at rest, during exercise and during subsequent recovery were similar between trials \((\text{Table 1})\).
Plasma adiponectin concentrations

Fasting plasma adiponectin concentrations averaged $6.57 \pm 0.7$ and $6.63 \pm 0.8$ mg/l before the HF A and LF A trials respectively (not significantly different). No significant changes in plasma adiponectin concentrations were observed over time in the LF A or HF A trial. No differences in circulating adiponectin were observed between trials ($P = 0.989$) (Fig. 2).

Immunolocalisation

Immunohistochemistry performed on skeletal muscle cross-sections showed adiponectin present in the sarcolemma of skeletal muscle fibres (Fig. 3A). As we applied an antilaminin antibody as a marker for the sarcolemmal and vascular walls (Fig. 3B), adiponectin is shown to be present on the sarcolemma of the individual muscle fibres as well as in/on the vascular endothelial lining of the interfibrillar arterioles (Fig. 3A). Adiponectin does not seem to be present in the cytosol nor does it seem to be associated with the muscle fibres’ nuclei (Fig. 3C). These findings have been verified with two different anti-human adiponectin antibodies (MAB 10 651 and AF1065; R&D Systems, Inc.). No differences in adiponectin signal intensity or localisation were observed between biopsies collected before, immediately after or 2 h after exercise. No differences were observed between trials.

Adiponectin protein expression

Adiponectin protein expression in plasma and skeletal muscle tissue was visualised by Western blotting. Membranes probed with a primary monoclonal antibody (MAB 10 651) raised against an epitope in the globular domain (gAcrp30, amino acids 104–244) of full-length adiponectin protein showed three bands at 28 kDa, 55 kDa and 85 kDa according to the molecular weight marker. These three bands likely correspond with the adiponectin monomeric (1, 5, 33, 34), dimeric (2, 5, 34) and trimeric (1, 6, 34) forms. The band observed between 28 and 34 kDa likely represents one (or more) of the glycosylated isoforms (35). Whereas in plasma all three bands were shown, only the 28 kDa and ~85 kDa bands were observed in muscle (Fig. 4). The blot depicted is representative for the muscle samples analysed.

Adiponectin receptor 1 and 2 mRNA expression

Skeletal muscle adiponectin receptor 1 and 2 mRNA content at rest, after exercise and after 2 h recovery are shown in Fig. 5A and B respectively. Adiponectin receptor 1 mRNA was more abundantly (25- to 70-fold) expressed compared with receptor 2. Muscle mRNA contents are expressed relative to corresponding

Table 1 Energy expenditure and substrate metabolism. Whole-body substrate oxidation following administration of a placebo (high FFA trial; HFA) or Acipimox (low FFA trial; LFA): total fat (FAT) oxidation rates, total carbohydrate (CHO) oxidation rates (expressed in g/min) and total energy expenditure (expressed in kJ/min) at rest. During exercise (at a 50% $W_{\text{max}}$ workload), and during 2 hours of post-exercise recovery. Data are expressed as means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Placebo trial (HFA)</td>
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<tr>
<td>FAT oxidation (g/min)</td>
<td>0.087 ± 0.007</td>
<td>0.607 ± 0.043</td>
<td>0.112 ± 0.008</td>
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<tr>
<td>CHO oxidation (g/min)</td>
<td>0.124 ± 0.017</td>
<td>1.984 ± 0.130</td>
<td>0.095 ± 0.014</td>
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<tr>
<td>Energy expenditure (kJ/min)</td>
<td>5.54 ± 0.23</td>
<td>56.8 ± 1.8</td>
<td>6.08 ± 0.30</td>
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<tr>
<td>Acipimox trial (LFA)</td>
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<tr>
<td>FAT oxidation (g/min)</td>
<td>0.069 ± 0.007*</td>
<td>0.392 ± 0.003*</td>
<td>0.070 ± 0.008*</td>
</tr>
<tr>
<td>CHO oxidation (g/min)</td>
<td>0.150 ± 0.012*</td>
<td>2.536 ± 0.102*</td>
<td>0.177 ± 0.016*</td>
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<tr>
<td>Energy expenditure (kJ/min)</td>
<td>5.26 ± 0.24</td>
<td>57.0 ± 2.0</td>
<td>5.69 ± 0.24</td>
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<tr>
<td>Ergometry</td>
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<tr>
<td>50% $W_{\text{max}}$ workload (W)</td>
<td>194 ± 7</td>
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<tr>
<td>Relative workload (%$VO_{\text{2\max}}$)</td>
<td>58.1 ± 1.05</td>
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* $P < 0.05$ significantly different from HFA trial.
baseline values, set to equal 1. Adiponectin receptor 1 mRNA expression did not change with time in the HFA or LFA trial and did not significantly differ between groups \( (P > 0.05) \). Adiponectin receptor 2 mRNA content decreased significantly in the HFA trial only \( (P = 0.02) \). Differences in adiponectin receptor 2 mRNA content between trials did not reach statistical significance \( (P = 0.08) \).

**Discussion**

Since the discovery of the various adipocytokines, it has become apparent that adipose tissue is actively involved in the regulation of skeletal muscle metabolism. Recently, adiponectin has received much interest due to the observation that plasma adiponectin levels are reduced in the obese and/or type 2 diabetic state (3, 6–8, 36–43). In line with these observations, dietary interventions (8, 20, 44) as well as pharmacological treatment with thiazolidinediones (34, 45–50) to improve insulin sensitivity have been shown to elevate adiponectin concentrations. Although an acute bout of exercise and exercise training substantially improve insulin sensitivity (13, 14), their effect on plasma adiponectin remains to be elucidated (15–22). In rodent studies, adiponectin has been shown to regulate plasma FFA clearance by stimulating skeletal muscle FFA uptake (10) and/or oxidation (2, 9). With adipose tissue lipolysis and plasma FFA appearance rates reaching maximal levels during prolonged, low to moderate intensity exercise (51, 52), we speculated that potential exercise-induced changes in plasma adiponectin concentration would be most prominent during or immediately after such exercise tasks. However, in accordance with earlier observations in athletes during short, high-intensity running exercise (15), we did not observe any
changes in plasma adiponectin concentration under exercise conditions. Even though adipose tissue lipolysis and fat oxidation rates were substantially elevated in the placebo trial (HF A; Table 1 and Fig. 1), plasma adiponectin concentrations remained unaffected during or after prolonged exercise (Fig. 2).

In accordance, studies exploring the benefits of participation in a long-term exercise training regimen generally report no changes in basal plasma adiponectin concentrations (16–18, 22). In contrast, Kriketos et al. (19) reported a massive increase in fasting plasma adiponectin levels after 2–3 bouts of low to moderate intensity exercise performed within a one-week period. Although it could be speculated that the inclusion of overweight, sedentary males in their study could be responsible for the apparent discrepant findings (19), others have failed to report such changes in a similar population after 6 months of exercise training (18). Therefore, further research is warranted to confirm the proposed effects of a short period of exercise training on plasma adiponectin levels. We conclude that adiponectin release is unrelated to the acute, temporary increase in adipose tissue lipolytic rate, plasma FFA concentration and/or whole-body fat oxidation rate during and immediately after prolonged, moderate intensity exercise in healthy, lean males.

Several groups have speculated on the proposed negative association between basal circulating adiponectin and plasma FFA and/or triglyceride concentrations (7, 53) and suggested that adiponectin release might be under acute negative feedback control by plasma FFA concentrations (23, 24). To investigate this, Staiger et al. (23) reduced plasma FFA availability in vivo in healthy males by administration of a nicotinic acid analogue (Acipimox) which specifically inhibits adipose tissue lipolysis (54, 55). The decline in plasma FFA levels observed in that study did not reduce plasma adiponectin concentrations (23). In contrast, Bernstein et al. (24) recently performed a similar study in which they reported a ~40% decline in plasma adiponectin concentrations following Acipimox administration. In the present study, plasma FFA availability was reduced at rest, during exercise and during post-exercise recovery by Acipimox administration in the LF A trial. We observed a pronounced reduction in plasma FFA and glycerol concentrations (Fig. 1) and whole-body fat oxidation rates (Table 1). However, the latter did not affect plasma adiponectin concentrations (Fig. 2). Furthermore, no differences were observed between the HF A and LF A trials. In accordance with Staiger et al. (23), we conclude that adiponectin release is unrelated to an acute, temporary decline in adipose tissue lipolytic rate, plasma FFA concentration and/or whole-body fat oxidation rate in healthy, lean males. As such, our data imply that there are other, more important, factors that mediate adiponectin production and/or release by adipose tissue. The latter likely include adipocyte size and/or lipid content of the adipocyte, circulating catecholamines, glucocorticoids, tumour necrosis factor α, and/or interleukin-6 (56). As animal work and human correlation studies still point towards an important regulatory role of circulating plasma adiponectin concentration on skeletal muscle metabolism (2), we speculated that adiponectin may be present in human skeletal muscle tissue. In accordance, Western blotting analyses showed adiponectin protein to be abundantly expressed in both plasma and skeletal muscle tissue (Fig. 4). Subsequently, we performed immunohistochemical staining on skeletal muscle cross-sections to show the presence of adiponectin on the sarcolemma of individual muscle fibres and within the endothelial lining of the interfibrillar arterioles (Fig. 3). The finding of adiponectin within the sarcolemma is surprising and has not been reported previously. The presence of adiponectin in the interfibrillar arterioles in human skeletal muscle tissue is consistent with the known actions of...
adiponectin as a potent anti-inflammatory and athero-
protective agent in vascular tissue (8, 57–59). More-
over, adiponectin has been reported to stimulate
vasodilatation and/or angiogenesis (57). As such,
these findings seem to suggest that adiponectin could
represent an important link between skeletal muscle
tissue perfusion and substrate use.

The presence of adiponectin on the sarcolemmal
membrane of the individual muscle fibre implies that
adiponectin is bound to adiponectin receptors in
muscle tissue for signalling purposes. In that context,
the recently cloned adiponectin receptor 1 (expressed
abundantly in muscle) and receptor 2 (expressed pre-
eminently in the liver) are likely to play a key role (27).
These receptors mediate the elevated glucose uptake
and FFA oxidation induced by adiponectin through
activation of AMP kinase and peroxisome proliferator-
activated receptor-α ligand activities (27). The latter
could potentially stimulate the translocation and/or
expression of the various skeletal muscle fatty acid
transporters (9, 60). As plasma adiponectin concen-
trations were not affected by either exercise or pharma-
cological inhibition of adipose tissue lipolysis, we
speculated that the effects of adiponectin on muscle
metabolism could be mediated at the receptor level.
As such, we measured adiponectin 1 and 2 receptor
mRNA expression in muscle tissue in both conditions.
As expected, adiponectin receptor 1 was more abun-
dantly expressed in muscle tissue than receptor 2.
Prolonged exercise and/or lipolytic inhibition did not
change adiponectin receptor 1 mRNA expression
(Fig. 5A). Interestingly, adiponectin receptor 2 mRNA
content decreased significantly following exercise in
the control trial (HFA; Fig. 5B). However, differences
in adiponectin receptor 2 mRNA expression between
trials did not reach statistical significance (P = 0.08).
Although these data tend to suggest that there is
some negative feedback inhibition of circulating FFA
concentrations at the level of the adiponectin receptor,
it should be noted that the adiponectin receptor 2 is
predominantly expressed in liver and not in muscle
tissue. As such, we can only speculate on the physio-
logical relevance to muscle metabolism. It is tempting
to hypothesise that more long-term regulation of
substrate metabolism is co-regulated by adiponectin
production and/or release in adipose tissue, whereas
more short-term regulation is also realized at the level
of the adiponectin receptor in skeletal muscle tissue
and/or the intercellular arterioles. More research is
warranted to elucidate the physiological role of the
adiponectin receptors in various tissues.

In conclusion, prolonged moderate intensity exercise
does not affect plasma adiponectin concentrations or
skeletal muscle adiponectin receptor 1 and 2 mRNA
expression in healthy, lean males. Furthermore, adipo-
nectin concentrations and adiponectin receptor 1
mRNA expression in muscle are not modulated by
acute changes in adipose tissue lipolysis, circulating
plasma FFA levels and/or whole-body fat oxidation
rate. Adiponectin is abundantly expressed in human
skeletal muscle tissue, and is located in the sarcolemma
of individual muscle fibres and within the endothelial
lining of the arterioles. The latter findings imply that
there is a regulatory role for adiponectin and/or adipo-
nectin receptors in skeletal muscle metabolism.

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References

1 Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF. A
novel serum protein similar to C1q, produced exclusively in adipopo-
2 Fruebis J, Tsao TS, Jaworschi S, Ebbets-Reed D, Erickson MR,
Yen FT, Bihain BE & Lodish HF. Proteolytic cleavage product of
30-kDa adipocyte complement-related protein increases fatty
acid oxidation in muscle and causes weight loss in mice. PNAS
3 Hu E, Liang P & Spiegelman BM. Adipoq is a novel adipose-
pecific gene dysregulated in obesity. Journal of Biological Chem-
4 Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y &
Matsubara K. cDNA cloning and expression of a novel adipose
specific collagen-like factor. apM1. Biochemical and Biophysical
Research Communications 1996 221 286–289.
5 Nakano Y, Tobe T, Chot-Mitura NH, Mouda T & Tomita M. Isolation
and characterization of GBP28, a novel gelatin-binding protein
purified from human plasma. Journal of Biochemistry 1996 120
803–812.
6 Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J,
Hotta K, Shimomura I, Nakamura T, Miyaoaka K, Kuriyama H,
Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M,
Ohmoto Y, Funahashi T & Matsuzawa Y. Paradoxical decrease
of an adipose-specific protein, adiponectin, in obesity. Biochemical
and Biophysical Research Communications 1999 257 79–83.
7 Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M,
Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K,
Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K,
Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S,
Hanafusa T & Matsuzawa Y. Plasma concentrations of a novel,
adipose-specific protein, adiponectin, in type 2 diabetic patients.
Arteriosclerosis, Thrombosis, and Vascular Biology 2000 20
1595–1599.
8 Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL,
Chen CL, Tai TY & Chuang LM. Weight reduction increases
plasma levels of an adipose-derived anti-inflammatory protein,
adiponectin. Journal of Clinical Endocrinology and Metabolism
2001 86 3815–3819.
9 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K,
Mori Y, Ide T, Murakami K, Tsujobayama-Kasakoa N, Ezaki O,
Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H,
Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S,
Tomita M, Frouguel P & Kadowaki T. The fat-derived hormone
adiponectin reverses insulin resistance associated with both
Adiponectin and muscle metabolism


45 Smith SA. Central role of the adipocyte in the insulin-sensitising and cardiovascular risk modifying actions of the thiazolidinediones. Biochimica 2003 85 1219–1230.

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