Determination of inflammatory and prominent proteomic changes in plasma and adipose tissue after high-intensity intermittent training in overweight and obese males

Melanie Leggate, Wayne G. Carter, Matthew J. C. Evans, Rebecca A. Vennard, Sarah Sribala-Sundaram, and Myra A. Nimmo

School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom

Submitted 26 August 2011; accepted in final form 17 January 2012

Leggate M, Carter WG, Evans MJC, Vennard RA, Sribala-Sundaram S, Nimmo MA. Determination of inflammatory and prominent proteomic changes in plasma and adipose tissue after high-intensity intermittent training in overweight and obese males. J Appl Physiol 112: 1353–1360, 2012. First published January 19, 2012; doi:10.1152/japplphysiol.01080.2011.—This study aimed to determine whether 2 wk of high-intensity intermittent training (HIIT) altered inflammatory status in plasma and adipose tissue in overweight and obese males. Twelve participants [mean (SD): age 23.7 (5.2) yr, body mass 91.0 (8.0) kg, body mass index 29.1 (3.1) kg/m²] undertook six HIIT sessions over 2 wk. Resting blood and subcutaneous abdominal adipose tissue samples were collected and insulin sensitivity determined, pre- and posttraining. Inflammatory proteins were quantified in plasma and adipose tissue. There was a significant decrease in soluble interleukin-6 receptor (sIL-6R; P = 0.050), monocyte chemotactic protein-1 (MCP-1, P = 0.047), and adiponectin (P = 0.041) in plasma posttraining. Plasma IL-6, intercellular adhesion molecule-1 (ICAM-1), tumor necrosis factor-α (TNF-α), IL-10, and insulin sensitivity did not change. In adipose tissue, IL-6 significantly decreased (P = 0.036) and IL-6R increased (P = 0.037), while adiponectin tended to decrease (P = 0.056), with no change in ICAM-1 posttraining. TNF-α, MCP-1, and IL-10 were not detectable in adipose tissue. Adipose tissue homogenates were then resolved using one-dimensional gel electrophoresis, and major changes in the adipose tissue proteome, as a consequence of HIIT, were evaluated. This proteomic approach identified significant reductions in annexin A2 (P = 0.046) and fatty acid synthase (P = 0.016) as a response to HIIT. The present investigation suggests 2 wk of HIIT is sufficient to induce beneficial alterations in the resting inflammatory profile and adipose tissue proteome of an overweight and obese male cohort.

Obesity is associated with chronic low-grade inflammation and underpins many long-term debilitating health conditions, including Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (20, 53, 57). The current World Health Organisation (WHO) estimate is that 1.5 billion adults are overweight worldwide, of which over 500 million individuals are obese.

Adipose tissue produces a number of inflammatory cytokines and cell adhesion molecules that contribute to chronic low-grade inflammation. Increased adiposity will drive localized inflammation deriving from cellular hypoxia (64) and macrophage infiltration into adipose tissue (15, 68), which increases the capacity for production of inflammatory proteins.

As a consequence, adipose tissue in obese individuals produces greater amounts of inflammatory proteins, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and intercellular adhesion molecule-1 (ICAM-1), than lean individuals (11, 29, 32, 33). These inflammatory proteins can be released into the circulation resulting in chronic low-grade inflammation, which is associated with insulin resistance (32).

Exercise has many health benefits, including maintaining a healthy weight, reducing the risk of developing chronic diseases such as T2DM and subsequent CVD (67), and reducing chronic low-grade inflammation in both healthy and disease states (1, 4, 6, 73). In addition, a lifestyle intervention involving exercise has been shown to improve insulin sensitivity in individuals with elevated fasting glucose levels, above that of the application of the antihyperglycemic drug metformin (36). Therefore, exercise plays an important role in the prevention of T2DM and associated comorbidities.

Despite adipose tissue being a major source of inflammatory mediators, there is limited research investigating the effects of repeated exercise on inflammatory proteins in adipose tissue. No changes in IL-6, TNF-α, or adiponectin mRNA expression in subcutaneous adipose tissue were found after 12 wk of aerobic training (44) or strength training (34) in obese groups, despite aerobic training causing a significant reduction in body mass. Christiansen and colleagues (16) did, however, find an increase in adiponectin mRNA expression in adipose tissue, in an exercise-only group, but no change in any cytokine mRNA expression. In contrast, lifestyle interventions over 12 wk (16, 17) and 15 wk (12), incorporating exercise and a hypocaloric diet, found a decreased expression of IL-6, TNF-α, and monocyte chemotactic protein-1 (MCP-1) mRNA in adipose tissue and an increase in adiponectin mRNA alongside a weight loss of 5–14% in obese individuals. Studies investigating protein levels have shown IL-6 to be reduced in adipose tissue of obese women after weight loss induced by a hypocaloric diet (6). Hence the existing literature suggests that exercise alone may not be sufficient to reduce inflammation in adipose tissue.

However, in all of these studies the exercise protocol is of a relatively low to moderate intensity, and more recently studies incorporating elements of high-intensity exercise within an exercise program have been shown to improve cardioprotective outcomes including glucose control and aerobic capacity (for review see 58). This can be achieved with just 2 wk of training of six or seven exercise sessions, with positive training outcomes including improved insulin sensitivity (45). However, this protocol involved four to six 30-s maximal Wingate sprints per session, and participants reported feelings of nausea and light-headedness. An alternate intermittent protocol has shown...
a marked increase in whole body and skeletal muscle capacity for fatty acid oxidation (60) with 10 longer exercise intervals (~90% peak oxygen uptake [V\textsubscript{O\textsubscript{peak}}]), each lasting 4 min interspersed with 2 min rest in a population of untrained individuals, without any report of the negative outcomes associated with Wingate sprints. In addition to the health benefits associated with intermittent exercise protocols it has also been reported as “more enjoyable” than continuous moderate-intensity exercise in healthy young males (5) and in coronary heart disease patients (26).

To investigate whether increasing the intensity of exercise is sufficient to drive decreases in inflammatory proteins this study examined the effects of 2 wk high-intensity intermittent training (HIIT) on metabolic and inflammatory changes in the circulation and subcutaneous adipose tissue in a cohort of overweight and obese males. Furthermore, the most visually prominent changes in the adipose tissue proteome as a consequence of this exercise training regimen were investigated.

**MATERIALS AND METHODS**

Twelve overweight and obese males [mean (SD); age 23.7 (5.2) yr (range 18–34 yr); body mass index (BMI) 29.1 (3.1) kg/m\(^2\); waist circumference 96.3 (8.0) cm (range 89.0–112 cm)] participated in this study. All participants had a BMI greater than 25 kg/m\(^2\) but were otherwise healthy and reported taking part in no more than two bouts of light to moderate intensity exercise per week. Participants were excluded if they smoked or were on any medication. The volunteers gave informed written and verbal consent after being advised of all possible risks and discomforts associated with the procedures used in the study, and all procedures were submitted to and approved by Loughborough University Ethical Advisory Committee.

**Preliminary measurements.** Participants performed a V\textsubscript{O\textsubscript{peak}} test to volitional exhaustion using a continuous incremental protocol on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). Expired air was measured continuously for oxygen uptake (V\textsubscript{O\textsubscript{2}}) using an online breath-by-breath gas analysis system (Ultima CPX, Medical Graphics, MN, USA). V\textsubscript{O\textsubscript{peak}} was identified as the highest V\textsubscript{O\textsubscript{2}} over a 30-s period during the test. After 7 days, participants attended the laboratory to complete a familiarization of the HIIT. During this visit, participants completed six 4-min bouts of cycling with 2 min of rest between intervals. The workload was manipulated during the trial to ensure that the average corresponding V\textsubscript{O\textsubscript{2}} during exercise equated to ~85% V\textsubscript{O\textsubscript{peak}}. Waist-to-hip ratio measurements were also taken during this visit. Waist circumference was measured as half-way between the iliac crest and the lowest rib, and hip circumference was measured at the widest point of the hips according to the procedures outlined by the WHO. Blood pressure was measured using an automated blood pressure monitor (Omron M7, Omron Healthcare, Milton Keynes, UK).

Subcutaneous abdominal adipose tissue biopsy and oral glucose tolerance test. One week after the familiarization, participants attended the laboratory after a 12-h overnight fast (water was allowed). Participants lay in a semisupine position, and 10 ml of 1% (wt/vol) lidocaine was administered under sterile conditions to the lower abdominal area before ~1.5 g of adipose tissue was extracted 10–15 cm laterally to the umbilicus using a percutaneous needle biopsy technique (16). The excised adipose tissue was immediately washed with 0.9% (wt/vol) NaCl solution to limit blood contamination before it was divided into Eppendorfs using sterile forceps. The tissue was snap-frozen in liquid nitrogen before transferring to a −80°C freezer until required for analysis. A cannula was then inserted into a participant’s antecubital vein, and a resting blood sample was collected into K\(_2\)EDTA vacutainers (BD Biosciences, San Diego, CA). Participants then consumed a 75-g glucose load (82.5 g dextrose monohydrate) in 300 ml liquid within a 5-min period. Further venous blood samples were collected every 30 min over a 2-h period.

**HIIT.** Participants completed three sessions of HIIT per week for 2 wk, with 1–2 days rest between each session. Each session consisted of ten 4-min intervals, with expired air collected during the first session to monitor exercise intensity. The mean V\textsubscript{O\textsubscript{2}} during the intervals of the first HIIT session was 85.0 (4.6)% V\textsubscript{O\textsubscript{peak}}, which equated to 89.5 (2.4)% of maximal heart rate. Subsequently, during the remaining HIIT sessions the workload was kept the same as the first session and heart rate was recorded throughout. The workload was adjusted if heart rate dropped below 80% of maximal levels in the subsequent sessions. Forty-six to forty-eight hours after the last training session a subcutaneous adipose tissue biopsy was taken, a fasted resting blood sample was collected, and an oral glucose tolerance test (OGTT) undertaken. The posttraining adipose tissue biopsy was taken from the contralateral side of the abdomen. This time delay from the last training session was employed to minimize any influence of acute exercise (28). Fluid and dietary intake were standardized 24 h before both visits. Blood pressure, waist and hip circumference, and V\textsubscript{O\textsubscript{2peak}} measurements were repeated 72 h after the last training session. All participants were asked to maintain their normal diet and physical activity routine throughout the training period.

**Blood preparation.** Hemoglobin concentration was measured in duplicate in whole blood using a commercially available kit (Randox Laboratories, Antrim, UK), and hematocrit content was measured in triplicate with a HaematoSpin1300 centrifuge (Hawksley, Sussex, UK). The intra-assay coefficients of variance were 2.7 and 0.5% for hemoglobin and hematocrit, respectively. Changes in plasma volume posttraining were calculated according to methods outlined previously (mean −1.7%) (19). The remaining whole blood was centrifuged at 4,000 g for 10 min at 4°C, and the plasma was removed, aliquoted, and stored at −80°C for subsequent analysis. Posttraining protein concentrations in plasma were adjusted to account for individual plasma volume changes.

**Adipose tissue homogenization.** Adipose tissue samples (typically 200–300 mg) were homogenized in 500 μl, 5 mM Tris-HCl (pH 7.5) buffer, containing 1 mM EDTA, 10% (vol/vol) sucrose, 1 mM DTT, and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) prior to using a handheld TissueRuptor (Qiagen, Crawley, UK). Homogenate was clarified by centrifugation at 10,000 g for 10 min at 4°C and the internatant transferred to a fresh eppendorf and then stored at −80°C prior to protein analysis. Intact protein concentration was determined using the DC Protein Assay Kit with a bovine serum albumin standard set used as protein standards (Bio-Rad Laboratories, Hercules, CA).

**ELISAs and biochemical analysis.** IL-6 and IL-6R were determined in plasma and adipose tissue immunotarin pre- and posttraining via noncommercial sandwich ELISAs as described in detail previously (25, 37). Commercially available ELISA kits were used to determine plasma insulin (Mercodia, Uppsala, Sweden) and adiponectin, high sensitivity (hs)-TNF-α, MCP-1, ICAM-1, and hs-IL-10 (R&D Systems, Minneapolis, MN) levels in plasma and adipose tissue. All inter- and intra-assay coefficients of variance were below 10%. Plasma glucose concentrations were determined by an enzymatic, colorimetric method using a bench top analyzer (Pentra 400, HORIBA ABX Diagnostics, Montpelier, France). Insulin sensitivity was assessed as the insulin sensitivity index (SI) calculated using the OGTT values by the formula proposed by Matsuda and DeFronzo (40).

One-dimensional polyacrylamide gel electrophoresis (1D-PAGE). Protein homogenate from the adipose tissue samples was reduced by the addition of a quarter of a volume of 5× concentrated reducing solution [10% sodium dodecyl sulfate (SDS) and 500 mM diithreitol (DTT)] and then heat-denatured by incubation for 10 min at 50°C. After cooling, one quarter of a volume of 5× Laemmli sample buffer was added [250 mM Tris-HCl (pH 6.8), 40% (vol/vol) glycerol, 5% (wt/vol) SDS, 0.005% (wt/vol) bromphenol] and proteins (35 μg/gel

**J Appl Physiol • doi:10.1152/japplphysiol.01080.2011 • www.jappl.org**

1354 Inflammatory Response to HIIT in Plasma and Adipose Tissue • Leggate M et al.
Table 1. Pre- and posttraining values

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>91.0 (8.0)</td>
<td>90.7 (7.8)</td>
<td>0.518</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.1 (3.4)</td>
<td>29.0 (3.2)</td>
<td>0.501</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96.3 (8.0)</td>
<td>94.9 (8.4)</td>
<td>0.029*</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>109.8 (5.2)</td>
<td>109 (6)</td>
<td>0.052</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.88 (0.05)</td>
<td>0.87 (0.05)</td>
<td>0.269</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.6 (0.6)</td>
<td>5.0 (1.0)</td>
<td>0.151</td>
</tr>
<tr>
<td>Fasting insulin, mU/l</td>
<td>7.8 (3.2)</td>
<td>6.8 (3.5)</td>
<td>0.268</td>
</tr>
<tr>
<td>Insulin sensitivity index</td>
<td>6.7 (4.6)</td>
<td>7.7 (3.8)</td>
<td>0.374</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>126 (8)</td>
<td>129 (9)</td>
<td>0.870</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>77 (12)</td>
<td>79 (11)</td>
<td>0.651</td>
</tr>
<tr>
<td>V̇O₂peak, l/min</td>
<td>3.4 (0.6)</td>
<td>3.7 (0.5)</td>
<td>0.022*</td>
</tr>
<tr>
<td>V̇O₂peak, ml/kg·1·min⁻¹</td>
<td>38.4 (6.4)</td>
<td>41.6 (5.2)</td>
<td>0.037*</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 12. BMI, body mass index; V̇O₂peak, peak oxygen consumption. *Significantly different compared with pretraining (P ≤ 0.05).

Table 2. Concentration of IL-6, sIL-6R, ICAM-1, adiponectin, TNF-α, MCP-1, and IL-10 in plasma at pre- and post-2 wk high-intensity intermittent training

<table>
<thead>
<tr>
<th></th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/ml</td>
<td>3.1 (3.0)</td>
<td>2.6 (2.2)</td>
<td>0.373</td>
</tr>
<tr>
<td>sIL-6R, ng/ml</td>
<td>42.0 (12.3)</td>
<td>37.6 (9.6)</td>
<td>0.050*</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>145 (50)</td>
<td>128 (38)</td>
<td>0.047*</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>7.5 (3.5)</td>
<td>6.7 (3.4)</td>
<td>0.041*</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>1.3 (0.4)</td>
<td>1.3 (0.5)</td>
<td>0.918</td>
</tr>
<tr>
<td>ICAM-1, pg/ml</td>
<td>161 (25)</td>
<td>154 (19)</td>
<td>0.373</td>
</tr>
<tr>
<td>IL-10, pg/ml</td>
<td>2.1 (0.6)</td>
<td>1.9 (0.6)</td>
<td>0.455</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 12. sIL-6R, soluble IL-6 receptor; MCP-1, monocyte chemotactant protein 1. *Significantly different compared with pretraining (P ≤ 0.05).
taken on the adipose tissue homogenates from all participants. An example of the Western blotting from five participants is included (Fig. 2A, bottom). Reductions in protein staining were supported by Western blotting, which for all participants collectively resulted in a significant 24% reduction ($P = 0.046$) in annexin A2 levels (Fig. 2B) and a significant 23% reduction ($P = 0.016$) in FAS levels (Fig. 2C), as a consequence of the exercise training. Annexin A2 levels were reduced in 9 of the

Fig. 1. Concentration of IL-6, IL-6R, ICAM-1, and adiponectin in subcutaneous adipose tissue at pre- and post-2 wk high-intensity intermittent training (HIIT). Values are means (SD); $n = 12$. †Significantly different from pretraining ($P \leq 0.05$).

Fig. 2. A, top: one-dimensional polyacrylamide gel electrophoresis protein profiles in subcutaneous adipose tissue homogenate of 5 representative participants at pre- and post-2 wk HIIT. A, bottom: Western blotting protein bands for annexin A2, fatty acid synthase, and actin in 5 participants. Change in annexin A2 (B) and fatty acid synthase (C) in subcutaneous adipose tissue at pre- and post-2 wk HIIT. Values are means (SD); $n = 12$. *Significantly different from pretraining ($P \leq 0.05$).
DISCUSSION

Exercise regimens of varying intensities may improve well-being and combat some of the basal increase in inflammation associated with excessive fat deposition and other adverse health conditions including T2DM and CVD (1, 4, 61, 73). The results of this study indicate that in overweight and obese males, a 2 wk HIIT regimen can reduce inflammation in the circulation as well as subcutaneous adipose tissue and can induce a reduction in waist circumference and increase $V_{O_2peak}$ with 100% of HIIT sessions completed.

IL-6 is a pleiotropic cytokine and exerts both pro- and anti-inflammatory actions (52). This is the first study to report a significant reduction in IL-6 in subcutaneous adipose tissue with exercise training. However, it is unclear whether this is due directly to the exercise or to a loss in fat, as reduced IL-6 in subcutaneous adipose tissue has been shown after weight loss in obese women through a hypocaloric diet but no exercise intervention (6). The same research group has also shown that IL-6 in subcutaneous adipose tissue is negatively correlated with insulin sensitivity (7). Previous studies have found no change in IL-6 mRNA expression in adipose tissue after 12 wk of aerobic training in obese females (44) or strength training in obese males (34), although these studies did not examine IL-6 protein changes in adipose tissue, and the exercise intensity was substantially lower. The finding of a decrease in IL-6 protein in adipose tissue in the present study could therefore be due to the higher exercise intensity elicited in this study. In the present study there was no significant change in circulating IL-6 posttraining, which could explain why insulin sensitivity was unaltered, as systemic IL-6 is strongly correlated with insulin resistance (6, 7, 22, 32). It is important to note that the present study focused on total changes in the protein concentration of inflammatory proteins within the adipose tissue as opposed to changes in protein secretion. Therefore, although there was a significant reduction of IL-6 in adipose tissue, the rate of IL-6 secretion from adipose tissue into the circulation may have been unaltered and could explain why there was no change in circulating IL-6. Other tissues and cells also contribute to circulating IL-6, with only ~15–35% of circulating IL-6 at rest deriving from subcutaneous adipose tissue (41). The lack of correlation between the changes in adipose tissue and circulating levels is therefore understandable.

Within adipose tissue, only around 4–10% of IL-6 comes from adipocytes (21, 23); therefore it is likely that other immune cells such as macrophages are the main source of IL-6 production. Macrophage recruitment into adipose tissue is greater in obese compared with lean individuals (15, 68), although it seems that the size of the adipocytes triggers macrophage infiltration rather than overall obesity (18). MCP-1 is a chemotactrant known specifically to stimulate macrophage and monocyte recruitment into adipose tissue. MCP-1 levels are increased in obesity, resulting in an influx of macrophages and monocytes into the adipose tissue (13). In the present study, MCP-1 was not detectable in subcutaneous adipose tissue and furthermore has been shown to be higher in visceral adipose tissue (13); therefore it is likely that the decrease of this chemokine in the circulation is due to a reduced MCP-1 production in other tissues such as visceral adipose tissue.

IL-6R in subcutaneous adipose tissue was significantly increased posttraining, which is consistent with findings in skeletal muscle after 10 wk of knee extensor exercise training (2, 30). IL-6R has previously been shown to be expressed on the plasma membrane of ~60% of adipocytes (7). The counter finding of a reduction in sIL-6R in plasma is also in support of previous studies showing similar changes in obese women after 6 mo of training (54, 72) and in chronic heart failure patients after a 12-wk exercise intervention (1). This finding is consistent with the current understanding that sIL-6R is independent of cell production and is derived from proteolytic cleavage of the membrane-bound IL-6R (37).

ICAM-1 is a vascular cell adhesion molecule that is used as a biomarker of endothelial dysfunction and can independently predict CVD (46). In the present study, no alterations were found in ICAM-1 in plasma or adipose tissue. Some research has shown improvements in circulating ICAM-1 with exercise training in individuals with metabolic disorders and T2DM (47, 48, 73); however, there were no changes in a group with normal glucose tolerance after a 4-wk exercise intervention (63). In all of these studies preintervention ICAM-1 concentrations were two to three times greater than in the present study. As with MCP-1, it has been demonstrated that ICAM-1 is greater in visceral fat in obese compared with lean individuals, but there is no difference in subcutaneous adipose tissue (11). Therefore, the reason for the absence of a reduction in circulating ICAM-1 in the present study could be that decreases in circulating ICAM-1 found in other studies are caused by a reduction in ICAM-1 production in visceral as opposed to subcutaneous fat, as well as lower pretraining circulating ICAM-1 in the present study than in the aforementioned studies.

The literature shows clear evidence that adiponectin levels are inversely correlated with BMI (3, 10, 14, 31, 51, 66, 69) and is increased after 1 yr of high-intensity exercise in T2DM patients (4). The present finding of a reduction in adiponectin in plasma and a tendency for this decrease to be replicated in adipose tissue ($P = 0.056$) is inconsistent with some existing literature. However, in a recent review (55), only three of eight randomized control trials involving exercise training resulted in an increase in plasma adiponectin, and there is some evidence, supported by the present study, that to increase adiponectin levels, at least in plasma, dietary restriction with a 10% weight loss is required (39). Christiansen and colleagues (17) support this concept presenting a small nonsignificant decrease in plasma adiponectin after a 3-mo exercise regimen, yet adiponectin was increased in a diet-only group and a combined diet plus exercise group. This research group demonstrated adiponectin mRNA in subcutaneous adipose tissue was increased in all groups, whereas other exercise-only interventions have found no changes in adiponectin mRNA in adipose tissue (34, 44), although none of these studies measured protein changes. An alternative explanation may relate to the fact that there are different isoforms of adiponectin expressing both anti- and proinflammatory actions (27, 42, 43). Haugen and Drevon (27) demonstrated that globular adiponectin induced TNF-α secretion, demonstrating pro-inflammatory properties, whereas in studies measuring total adiponectin, it is thought to exhibit overall anti-inflammatory properties, includ-
ing enhancing insulin sensitivity (8, 71). Further work is required to determine whether functionality is related to changes in the various isoforms of adiponectin and to test the differences between dietary restriction and exercise interventions on the adiponectin response.

IL-10, MCP-1, and TNF-α were not detectable within subcutaneous adipose tissue, suggesting the dominant source of inflammation is visceral adipose tissue, which is consistent with other studies (11, 13, 23), although one ex vivo study found IL-6 to be greater in subcutaneous than visceral fat (24) and similarly adiponectin is more abundant in subcutaneous adipose tissue (21, 38). Despite the majority of evidence suggesting inflammatory proteins are present at greater concentrations in visceral than subcutaneous adipose tissue, visceral adipose tissue accounts for only 13% of total adipose tissue in obese men and 6% in obese women (49); therefore the contribution of subcutaneous adipose tissue to systemic low-grade inflammation could be substantial. Clarification of the protein concentration of inflammatory cytokines in adipose tissue is therefore required to substantiate the existing literature.

Additional proteomic analysis was carried out to determine if there were any gross changes in the wider subcutaneous adipose tissue proteome. Significant reductions of both annexin A2 and FAS were identified in adipose tissue after 2 wk HIIT. This is the first study to have shown a significant reduction of annexin A2 and FAS were identified in adipose tissue after 2 wk HIIT. Although one ex vivo study found IL-6 to be greater in subcutaneous than visceral fat (24) and similarly adiponectin is more abundant in subcutaneous adipose tissue (21, 38). Despite the majority of evidence suggesting inflammatory proteins are present at greater concentrations in visceral than subcutaneous adipose tissue, visceral adipose tissue accounts for only 13% of total adipose tissue in obese men and 6% in obese women (49); therefore the contribution of subcutaneous adipose tissue to systemic low-grade inflammation could be substantial. Clarification of the protein concentration of inflammatory cytokines in adipose tissue is therefore required to substantiate the existing literature.

Proteomic analysis also demonstrated a reduction of similar magnitude in the enzyme FAS in subcutaneous adipose tissue. Expression of FAS is elevated in both subcutaneous and visceral adipose depots in obese individuals, with increased subcutaneous mRNA FAS expression shown to be associated with high serum IL-6 and insulin resistance (9). The findings of the present study are in agreement with a report of reduced FAS in adipose tissue after 16 wk exercise training (62). The authors of this study also reported FAS was reduced to a greater extent after aerobic interval training compared with continuous moderate exercise. In contrast to these studies, an increase in mRNA expression of FAS has also been shown after 4 wk exercise training in subcutaneous fat (50), although this study did not look at translated FAS protein and therefore emphasizes the need to demonstrate any functional changes with protein analysis. The proteomic analysis was limited to an evaluation of the most visibly prominent protein level changes, but it is likely that other protein levels will be changed within the adipose tissue proteome as a consequence of this exercise regimen; however, a more extensive proteomic study was beyond the scope of this manuscript.

A decrease in waist circumference similar to the present study was found after 2 wk sprint interval training (70), although it seems unlikely that the decrease in waist circumference is simply due to the increased energy expenditure introduced due to the training protocol. Total energy expenditure in the present study was estimated to be ~14,500 kJ for the six HIIT sessions, with an estimated additional ~5,000 kJ due to excess postexercise oxygen consumption (35). This would theoretically cause a total body fat loss of ~600 g of adipose tissue, which is unlikely to induce a mean reduction in waist circumference of ~1.4 cm. Further studies are therefore required to determine the cause of the reduced waist circumference since abdominal adiposity was not measured in the present study.

Despite significant improvements in inflammatory proteins, waist circumference, and \( \text{V}_\text{O}_2\text{peak} \), the present investigation found no improvement of insulin sensitivity after HIIT. A possible explanation for the discrepancy in findings between this study and others that did detail an increase of insulin sensitivity after 2 wk sprint interval training (45, 70) could be due to the timing of the posttraining OGTT. In the present study, the OGTT took place 46–48 h after training to eradicate any acute effects on insulin sensitivity from the last training session (28). A previous investigation in overweight and obese males has shown that insulin sensitivity although augmented 24 h after the last bout of exercise was lost at 72 h posttraining, suggesting the augmentation may be due to the effect of the last acute exercise bout (70). In contrast to these findings, utilizing the gold standard methodology of the hyperinsulinemic euglycemic clamp, before and after a 2 wk sprint training protocol, and sampling 72 h posttraining, found insulin sensitivity to be increased (45). In this study, however, the preexercise glucose infusion rate of the training group appeared low, and the posttraining sample, although significantly different from pretraining, was comparable to the sedentary control group and much lower than an acute exercise group, suggesting the pretreatment value was unusually low. It is clear that the timing of the posttraining samples after the last exercise bout is critical when interpreting insulin sensitivity results.

In conclusion the present study provides novel evidence to support that HIIT, a high-intensity intermittent training protocol, is an appropriate form of exercise to induce both metabolic and inflammatory changes after only 2 wk. The protocol was suitable for an overweight and obese cohort, with all HIIT sessions completed by the participants. Although the training period is short and it is unlikely to have changed parameters independent of the training, future studies should consider introducing a control group when determining whether HIIT is suitable for different patient groups and if greater health benefits can be achieved over a longer training period.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the contribution of the study participants. We would like to thank Dr. David Tooth of the University of Nottingham for mass spectrometry analysis.

**GRANTS**

We are also thankful to the Wellcome Trust for the funding of a summer scholarship to Miss Sarah Sribala-Sundaram.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: M.L. and M.A.N. conception and design of research; M.L., W.G.C., M.J.E., R.A.V., S.S.-S., and M.A.N. performed experiments;
REFERENCES


Inflammatory Response to HIIT in Plasma and Adipose Tissue • Leggate M et al.  


