High Fat Diet Leads to Adiposity and Adipose Tissue Inflammation: The Effect of Whey Protein Supplementation and Aerobic Exercise Training

Farhad Ahmadi-Kani Golzar¹, Rozita Fathi¹ and Soleiman Mahjoub²

1. Faculty of Sport Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran.
2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Science, Babol, Iran.

Running title: Anti-inflammatory properties of whey protein and aerobic training

Corresponding author: Dr. Farhad Ahmadi-Kani Golzar, Ph.D. of Exercise Physiology, Exercise Biochemistry Division, Faculty of Sport Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran.

Telephone: +98 910 449 3599, e-mail: f.kani@stu.umz.ac.ir

E-mail addresses:

Farhad Ahmadi-Kani Golzar: f.kani@stu.umz.ac.ir

Rozita Fathi: r.fathi@umz.ac.ir

Soleiman Mahjoub: smahjoub20@gmail.com
ABSTRACT

There is little understanding about dietary proteins and their potential contribution to obesity-induced inflammation. This study investigates the effect of 10 weeks of aerobic training and whey protein (WP) supplementation on visceral adipose tissue inflammation in rats fed a high fat diet (HFD). In the first phase, 40 male Wistar rats were randomly divided into two groups: 1- normal diet (n=8), 2- high-fat diet (n=32). After this period—which lasted nine weeks—in the second phase, rats fed a HFD were randomly assigned into four groups: (n=8/group): 1- sedentary, 2- WP, 3- aerobic training, and 4- WP + aerobic training. The aerobic training was performed for 10 weeks, five days/week at 21 m/min, 15% incline, 60 minutes/day. HFD significantly increased body weight, adiposity index, fat pads weight, glucose levels, and insulin resistance index compared to the normal diet. Also, levels of TNF-α, MCP-1, HIF-1α, and VEGF-A in adipose tissue and serum levels of TNF-α were increased in the HFD group. Glucose levels, insulin resistance index, and triglyceride (TG) were reduced only by WP, independently of aerobic training. Both the aerobic training and WP reduced the fat pads weight, levels of TNF-α, HIF-1α, and VEGF-A in adipose tissue. Nevertheless, the levels of MCP-1 in adipose tissue and serum levels of TNF-α and MCP-1 were not reduced significantly by WP or aerobic training. These findings suggest that both aerobic training and WP supplementation lead to a reduction in adiposity and ameliorate obesity-induced inflammation in visceral adipose tissue.

Keywords: Whey protein; Inflammation; Hypoxia; Exercise training; High-fat diet
List of abbreviations

BMI, body mass index
GLUT-4, glucose transporter type 4
HDL, high density lipoprotein
HFD, high fat diet
HIF-1α, hypoxia inducible factor 1 α
HOMA, homeostatic model assessment
IL, interleukin
LDL, low-density lipoprotein
MAPK, mitogen-activated protein kinase
MCP-1, monocyte chemoattractant protein-1
NF-κB, nuclear factor-κB
TC, total cholesterol
TG, triglyceride
TNF-α, tumor necrosis factor α
VEGF, vascular endothelial growth factor
WAT, white adipose tissue
WP, whey protein
Introduction

Over the past several decades, overnutrition (high-fat intake) and the lack of daily physical activity have significantly increased worldwide obesity rates and the attendant increases in insulin resistance, type II diabetes, and cardiovascular disease, have created a global public health concern (Chawla et al., 2011; Huh et al., 2014; Makki et al., 2013). Recent studies show that white adipose tissue (WAT) is one of the primary contributors to the chronic low-grade inflammation characteristic of obesity (Baynard et al., 2012), which mainly results from increased adipocyte size and infiltrated macrophages in WAT (Yamashita et al., 2010). Increased adipocyte size leads to an elevation in expression and secretion of pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-6, and monocyte chemoattractant protein-1 (MCP-1) (Choe et al., 2016).

Adipose tissue expansion needs compensatory capillary density. Several studies suggest that during obesity, the development of the vascular network is not adequate to supply sufficient oxygen to all adipocytes, and eventually leads to local hypoxia (Hosogai et al., 2007; Pasarica et al., 2009; Ye et al., 2007; Ludzki et al., 2018). Hence, it is probable that hypertrophic adipocytes receive insufficient oxygen supply and hypoxia occurs. The activation of hypoxia-inducible factor 1α (HIF-1α), a key transcription factor mediating hypoxic responses, has been implicated in the adipose tissue inflammation, fibrosis, adipocyte dysfunction, and insulin resistance in obesity (Choe et al., 2014; Lee et al., 2014). In contrast, vascular endothelial growth factor A (VEGF-A) plays a significant role in stimulating angiogenesis in adipose tissue. An increase in angiogenesis may protect animals against adipose tissue hypoxia and inflammation (Elias et al., 2012).

Therefore, in order to design strategies to mitigate obesity-associated risk factors, the important factors that may prevent the development of obesity should be identified (Siddiqui et al., 2008). The recent evidence suggests that high intake of dairy products was inversely correlated with the risk of type II diabetes, obesity, and obesity-related diseases (Shi et al., 2012). Milk contains two major sources of protein—casein (80%) and whey (20%). Whey protein (WP) has been identified as an excellent preventer
of obesity because of the high biological value of its bioactive peptides (de Souza et al., 2014). Some studies have indicated the beneficial effects of WP supplementation on body weight, fat free mass, and insulin sensitivity and satiety in obese and diabetic individuals (McAllan et al., 2013; Siddiqui et al., 2008). WP may also have anti-inflammatory properties in different kinds of diseases, such as acute ischemic stroke (de Aguilar-Nascimento et al., 2011), hepatitis (Kume et al., 2012), and chronic obstructive pulmonary disease (Sugawara et al., 2012). These promising findings can be studied in other inflammation-related diseases.

On the other hand, evidence suggests that exercise training has anti-inflammatory effects with minimal side effects in adipose tissue (Linden et al., 2014; Speretta et al., 2012). However, the mechanisms responsible for its anti-inflammatory effects are not fully understood (Bradley et al., 2008; Yan et al., 2013). A limited number of animal studies reported that exercise training alters gene expression of angiogenic factors and hypoxia in adipose tissue (Disanzo & You, 2014; Hatano et al., 2011; You et al., 2013). However, the effects of regular exercise on adipose tissue VEGF-A and hypoxia in obesity are still unknown, and the results of previous studies are contradictory.

Notwithstanding, beyond investigations which have reported that short-term WP supplementation reduces the inflammatory response, few studies have directly addressed the effect of chronic supplementation in combination with exercise training on inflammatory markers in adipose tissue. There is little understanding about dietary proteins and their potential contribution to angiogenesis modulation (Medina & Quesada, 2014). Therefore, this study examines the effect of aerobic training and WP supplementation, either separately or combined, on inflammation (TNF-α, MCP-1), HIF1-α, and VEGF-A in visceral (epididymal) adipose tissue in rats fed a HFD.
Method

Animals and study design

Forty male Wistar rats, weighing 145±17.1 g, were purchased from Pasteur Institute, Iran. The rats were then maintained in individual cages with free access to food and tap water at the controlled temperature of 24–26°C and 12-hour light-dark cycle (light from 5:00 until 17:00). All experiments and procedures were performed in accordance with the Institutional Animal Care and Use Committee (IACUC). In addition, this study was approved by the University of Mazandaran ethical committee (#1247085). The duration of the experiment was 19 weeks. After one week for acclimatization to the new environment, in the first phase, the rats were randomly divided into two groups: 1- normal diet (N=8), 2- HFD (N=32), were fed with HFD (62.1% Kcal from fat) or normal diet (10.4% Kcal from fat). The normal diet was purchased from Behparvar Industries (Tehran, Iran). HFD was provided in accordance with the Research Diets instructions (D12492). The nutritional composition of ND and HFD is given in Table 1. After this period, which lasted nine weeks, the second phase (the last 10 weeks), the HFD-fed rats were randomly assigned into four groups (n=8/group): 1- sedentary control with vehicle fed a HFD (SC + HFD), 2- WP supplementation fed a HFD (WP-SC + HFD), 3- aerobic training with vehicle fed a HFD (ET+ HFD), and 4- WP + aerobic training fed a HFD (ET-WP + HFD). The sedentary group fed the normal diet (ND-SC) was also subject to a standard diet (ND) until the end protocol.

WP supplementation

The supplemented groups were given WP powder dissolved in distilled water by oral feeding (gavage) within 30 minutes after the exercise (five days/week). The concentration of WP in water was 267 mg per 1 ml of water. Non-supplemented rats received a similar volume of vehicle (water) following the same protocol. WP was purchased from Optimum Nutrition (GOLD STANDARD WHEY; Optimum Nutrition, Inc., USA). The recommended value for using WP by humans is about 20 g per one intake with a normal diet and exercise program (Chen et al., 2014). The rat WP dose (2.05 g.kg) used in this study was
estimated from a human-equivalent dose based on the body surface area, using the following formula by the FDA of USA: assuming a 60-kg human (20 g in humans, corresponding to 0.333 g/kg for a 60 kg adult) = 0.333 * 6.17 = a rat dose of 2.05 g.kg⁻¹; the conversion coefficient of 6.17 was used to account for differences in the body surface area between rats and humans (Reagan-Shaw et al., 2008).

**Exercise training protocol**

Exercise training was performed on a motor-driven treadmill within the first hour of the dark cycle (its active phase in rats). The aerobic training protocol was administered with a starting speed of 15 m/min on a 15% incline for 5 min/day. Duration and speed were gradually increased by 2–3 min/day and 1–2 m/min per week respectively until reaching a speed of 21 m/min on a 15% incline for 60 min/day and five days/week. In Week 4, rats achieved this speed, and were then maintained at 21 m/min for the remaining six weeks (Linden et al., 2015).

**Blood and tissue collection**

WP supplementation and exercise were withheld prior to anesthesia for 48 hours. After eight hours of fasting, animals were euthanized. The blood was collected by cardiac puncture and centrifuged (1,500 g for 10 min at 4°C). The serum layer was collected and stored for biochemical analyses. The target organs, including liver and fat pads (mesenteric, retroperitoneal, perirenal, epididymal, and inguinal), were immediately excised, weighed, flash frozen in liquid nitrogen, and stored at -80°C for analysis.

**Anthropometric measurements**

Body weight and food intake were monitored once a week throughout the experiment. The body length was considered as the nasal-anal length. Thereafter, using the following formula, the body mass index (BMI) was calculated: BMI= bodyweight (g) / body length² (cm²) (Taylor & Phillips, 1996). The adiposity index was calculated by sum up the fat pad weights (epididymal, retroperitoneal, and inguinal) (Taylor & Phillips, 1996): adiposity index = 100 * sum of fat pad weights (g) / body weight (g).
Assessment of Insulin Resistance and lipid profile

The blood glucose was measured by the glucose oxidase method. Fasting insulin was measured using a commercial ELISA kit (Mercodia AB, Uppsala, Sweden, Cat#10-1250-01). The insulin resistance index was estimated by HOMA with the following formula: HOMA-IR= blood glucose (mmol/L) × fasting insulin (μIU/ml)/22.5 (Matthews et al., 1985). The levels of Triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured by an autoanalyzer (Hitachi, Japan).

Epididymal adipose tissue protein content

First, adipose tissue samples (100 mg) were ground in liquid nitrogen to a fine powder by using a mortar and pestle. Then, the powder and liquid nitrogen were transferred to an appropriately sized tube, and the liquid nitrogen was allowed to evaporate. After that, 1 ml of PBS (Containing 10 μg/ml of protease inhibitor cocktail; Goldbio, US, Cat#GB-326-1) was added to a 100 mg of powder. Next, homogenates were centrifuged in 12,000g for 10 min at 4°C and the lysates were collected. Finally, quantitative assessment of TNF-α (Diaclone, France, Cat#865.000.096), MCP-1 (Zellbio, Germany, Cat#ZB-10193-R9648), HIF1-α (Eastbiopharm, China, Cat#CK-E30271), and VEGF-A (Eastbiopharm, China, Cat#CK-E91384) was determined by ELISA in accordance with the manufacturer’s instructions. Adipose tissue TG content was measured by enzymatic colorimetric methods using commercially available kits (Pars Azmoon Laboratories, Iran).

Statistical analysis

Diagrams and data statistical analysis were performed by SigmaPlot 12.5 (SPSS Inc., Chicago, IL, USA). Student’s t-test was used to compare the ND sedentary group and the HFD sedentary group. To compare the HFD-fed groups, two-way ANOVA analysis was used to compare trained and untrained groups (ET + ET-WP × SC + WP-SC), supplemented and non-supplemented groups (WP-SC + ET-WP * SC + ET). The significance was considered as α=0.05.
Results

Body Weight, Food Intake, BMI, Obesity Index, and Fat Pads Weight

Sedentary ND and sedentary HFD groups were compared to investigate the effect of HFD on the variables at the end of the experiment. In the sedentary HFD group, the body weight at Week 9 (Fig. 1A), final body weight, BMI, adiposity index, liver weight, and fat pads weight (subcutaneous, epididymal, mesenteric, retroperitoneal, and perirenal) were significantly higher than the ND group (Table 2).

According to the results of the two-way ANOVA analysis, it was found that the trained groups (ET-WP and ET) had lower body weight, adiposity index, food intake, and fat pads weight compared to the sedentary groups (SC and WP-SC) (Table 2 and Fig. 1 B). Nevertheless, no significant difference was observed in the liver weight and BMI of the trained and sedentary groups (Table 2). Furthermore, lower fat pads weight and adiposity index were observed in the supplemented groups (WP-SC and ET-WP) compared to the non-supplement groups (SC and ET). However, no significant difference was observed in the body weight, BMI, food intake, and liver weight of the supplemented and non-supplemented groups. No significant interaction was observed for any of the variables (Table 2).

Lipid profile and insulin resistance

The sedentary HFD group had higher levels of TG in serum and adipose tissue compared to the sedentary ND group. No significant difference was observed in TC, HDL, and LDL levels between the two groups (Table 2). The results also indicated that the sedentary HFD group had higher glucose levels and insulin resistance index compared to the sedentary ND group. However, insulin levels showed no significant difference between the two groups (Table 3).

No significant difference was observed in the levels of TG in serum and adipose tissue, TC, LDL, and HDL between the trained and sedentary groups. Furthermore, lower levels of TG in serum and adipose tissue were observed in the supplemented groups compared to the non-supplemented groups. Additionally, no significant difference was observed in TC, HDL, and LDL between the supplemented...
and non-supplemented groups. The interaction effect was not significant for any of the variables (Table 2). Levels of glucose, insulin, and insulin resistance index were reduced only by WP, independently of aerobic training (Table 3). No significant interaction was observed.

**Levels of adipokines in serum and adipose tissue**

The sedentary HFD group had higher serum TNF-α levels compared to the sedentary ND group (Table 3). However, no difference existed for serum MCP-1 levels between the two groups (Table 3). There were no significant changes in serum TNF-α and MCP-1 levels in response to either aerobic training or WP supplementation (Fig. 2 A and C).

The results revealed that the sedentary HFD group had higher levels of TNF-α, MCP-1, HIF-1α, and VEGF-A in epididymal adipose tissue compared to the sedentary ND group (Table 3). Based on the two-way ANOVA, TNF-α, HIF-1α, and VEGF-A levels in epididymal adipose tissue were reduced by both aerobic training and the WP supplementation, but no significant interaction was observed. No difference was observed in the levels of MCP-1 of epididymal adipose tissue in response to either aerobic training or WP supplementation (Fig. 2).

**Discussion**

The present study evaluated the effects of WP supplementation and aerobic training on adiposity, adipose tissue inflammatory factors, and insulin resistance in obese rats. The main findings were that WP supplementation alone, and in combination with aerobic training inhibited the high fat diet-induced inflammation, including the reduction in levels of TNF-α, HIF-1α, and VEGF-A in epididymal adipose tissue. To our knowledge, this is the first study that showed the anti-inflammatory effect of WP in adipose tissue. This novel finding provides a new awareness of the potential of WP supplementation to decrease adipose tissue inflammation in populations at-risk of developing obesity.

Similar to previous studies (Linden et al., 2014; Speretta et al., 2012), in this study, HFD increased adipose tissue mass and body weight. In addition, HFD resulted in the increases of glucose, insulin
resistance index, inflammatory indicators in epididymal adipose tissue, and circulating levels of TNF-\(\alpha\) and TG levels in serum and adipose tissue.

The results of this study demonstrated a significant decrease in adiposity index, and adipose tissue weight by WP supplementation. It has been shown that diets with higher protein content promote satiety (van Milgen et al., 2001). Our results showed that WP had no effect on food intake and body weight. It is possible that the rats fed WP may have received more kcal via gavage. A previous study reported that WP supplementation benefits body composition and reduces insulin resistance and fatty liver in HFD-fed rats via enhancement in basal metabolic rate and an elevation in oxygen consumed by mitochondria (Shertzer et al., 2011). In another study, whey supplementation in rats for nine days caused an increase in translocation of glucose transporter type 4 (GLUT-4) to the plasma membrane, which improves insulin sensitivity (Morato et al., 2013). With regard to the WP utilized in this study that reduced insulin resistance and glucose levels, it is possible that dietary modification and control may be an important therapeutic target in improving metabolic complications.

We have found that exercise training significantly prevented weight gain, decreased fat pads weight, and adiposity index, which is consistent with previous studies (Linden et al., 2014; Speretta et al., 2012; Yamashita et al., 2010). Our data showed that training groups had a lower caloric intake compared with the sedentary groups. Hence, the increase in energy expenditure and appetite suppression through exercise training and the lower caloric intake could have contributed to the lower body weight and fat gain found in the trained HFD groups.

Also, the findings of this study showed the increases of HIF-1\(\alpha\) and VEGF-A in epididymal adipose tissue following HFD consumption. Recently, Karki et al. (2017) showed that angiogenic disruption is associated with up-regulation of an anti-angiogenic isoform of VEGF-A; VEGF-A165b. Hence, it is likely that the increase of VEGF-A following HFD in our study would be caused by the expression of anti-angiogenesis isoform, which does not result in the increase in the adipose tissue capillary density.
We found that WP supplementation and aerobic training similarly reduced the level of TNF-α, HIF-1α, and VEGF-A in visceral adipose tissue. Hence, we can infer that WP can be considered a substitution of exercise training to lessen adipose tissue hypoxia during obesity. Furthermore, aerobic training may stimulate angiogenesis, and increase in adipose tissue blood flow, reducing obesity-induced inflammation and hypoxia (You et al., 2013).

The beneficial effects of WP are likely attributed to its rapid digestion and absorption and, so, the greater increase in blood amino acid concentration immediately after a single meal. Also, WP contains bioactive peptides to which anti-inflammatory properties are attributed (e.g. lactoferrin, β-lactoglobulin, and α-lactalbumin) (Cam & de Mejia, 2012; Rusu et al., 2010). Moreover, studies have shown that WP has antioxidant properties (Sheikholeslami & Ahmadi, 2012; Teixeira et al., 2016). Another possible mechanism by which WP may have modulated the inflammatory response is via improved antioxidant status (Pihlanto, 2006). Redox status and oxidative stress contribute to inflammatory response (Rahman et al., 2005).

Studies support the benefits of aerobic training in alleviating inflammatory state in adipose tissue (Bradley et al., 2008; Speretta et al., 2012; Yamashita et al., 2010). Recently, reductions in macrophage infiltration and macrophages polarization toward inflammatory properties in adipose tissue have been suggested. These mechanisms are of great importance since obesity promotes macrophage infiltration into adipose tissue, which triggers macrophage phenotype switch from an anti-inflammatory M2 phenotype to a pro-inflammatory M1 phenotype and its consequences for insulin resistance (Goh et al., 2016).

In the present study, there was a positive correlation between HIF-1α and VEGF-A levels (r=0.728, P=0.000414), and a decrease in HIF-1α was associated with a decrease in VEGF-A. Also, there was a positive correlation between epididymal fat pad and HIF-1α (r=0.538, P=0.00179) and VEGF-A (r=0.716, P=0.0000387) levels. Song et al. (2016) also showed a positive correlation between HIF-1α and VEGF-A
levels with epididymal fat pad and body weight. Thus, decreased levels of HIF-1α and VEGF-A in the current study may be partly caused by the smaller size of adipose tissue, which is the result of exercise training and WP supplementation. Although the evaluation of adipose tissue oxygenation is beyond the scope of this study, we assumed that this reduction in the fat pad size could result in lower micro-hypoxia and reduce the need for increasing capillarization, which may be associated with the improvement of adipose tissue inflammation.

One study showed that IL-10 leads to suppression of VEGF derived from M1 macrophages, but does not have any effect on VEGF release from M2 macrophages (Wu et al., 2010). Since, given that aerobic training leads to increase in anti-inflammatory markers, especially IL-10 (Speretta et al., 2012), it is likely that the decreased levels of VEGF in exercise training and WP groups are caused by increase in anti-inflammatory profile and reduction in in M1 macrophages. Although some studies have focused on the effect of exercise training on blood flow in adipose tissue, the results obtained from a study indicate that when adipose tissue size decreases following exercise training, blood flow increases in the adipose tissue (Sakurai et al., 2013). Therefore, an increase in blood flow to the adipose tissue produced by exercise training may mitigate obesity-induced hypoxia, possibly causing attenuation of inflammatory changes in the adipose tissue.

In addition, a review study showed that three proteins present in milk are able to modulate angiogenesis (Medina & Quesada, 2014). The negative effects of WP on levels of HIF-1α and VEGF-A in visceral adipose tissue could be caused by a decrease in inflammatory markers induced by anti-inflammatory effects of WP. Nevertheless, there is no study that addresses the effect of WP on hypoxia and angiogenesis in adipose tissue. Therefore, justification of these findings seems to be difficult and more studies need to be conducted.

As regards the effect of exercise training on insulin resistance, Linden et al. (2014) showed that increases in fasting insulin and HOMA in sedentary mice fed an HFD is prevented by moderate intensity treadmill training, which are inconsistent with our findings. Recent clinical trials have shown that exercise training
may be associated with a lack of response or even a reverse response to glucose homeostasis (Böhm et al., 2016). The primary mechanism is not completely understood, but it appears that HFD prevents exercise training modulation effect on glucose and lipid metabolism (Batacan et al., 2016).

No significant exercise training effects or interactions were observed for lipid profile throughout the intervention period. Nevertheless, WP supplementation resulted in a decrease in TG levels in serum and adipose tissue but had no significant effect on TC, LDL-C, and HDL-C levels. In this line, a meta-analysis study showed the beneficial effects of WP on serum TG levels decreases. Their findings suggested that WP had no significant effect on TC, LDL-C, and HDL-C (Zhang et al., 2016). The positive effect of WP on TG reduction might be caused by increases in hepatic lipid metabolism, inhibition of intestinal lipids absorption, and an increase in excretion of fecal sterols (Graf et al., 2011). Also, body weight and body fat reduction could possibly improve the lipid profile.

Conclusion

In summary, our findings suggest that WP supplementation leads to a reduction in body fat, TG levels, and insulin resistance, and mitigates high fat diet-induced inflammation. Also, aerobic training reduced adipose tissue inflammation, fat pads, and body weight, though it had no effect on insulin resistance. In addition, both aerobic training and WP reduced levels of HIF-1α and VEGF-A in epididymal adipose tissue. Further studies are required to focus on the effect of exercise training and dietary proteins on vascularization, blood flow, oxygenation, and cellular metabolism in the adipose tissue of obese animal and human models.

Authors Contributions

F.A.-K. designed the study, collected and analyzed data, wrote the first draft of the manuscript, contributed to discussion, and reviewed the manuscript. R.F. designed the study, contributed to discussion, and reviewed the manuscript. S. M. collected and analyzed data, reviewed the manuscript.
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Conflict of interest

The authors have nothing to disclose

References


Table 1
Nutritional composition of the diets

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<th>High fat diet</th>
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<td>Carbohydrates</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Total fat</td>
<td>4.3%</td>
<td>10.4%</td>
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<th>Kcal</th>
<th>g</th>
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<td>-</td>
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<td>60%</td>
<td>64.4%</td>
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<tr>
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<td>24.8%</td>
<td>20%</td>
<td>16%</td>
</tr>
<tr>
<td>Total fat</td>
<td>4.3%</td>
<td>10.4%</td>
<td>31.4%</td>
<td>62.1%</td>
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</table>
Table 2

The effect of WP and ET in HFD rats upon body composition and lipid profile at the end of the experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SC-ND</th>
<th>HFD</th>
<th>P value</th>
<th>Main effect</th>
<th>Main effect</th>
<th>Interaction</th>
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</thead>
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<tr>
<td></td>
<td>SC</td>
<td>SC-WP</td>
<td>ET</td>
<td>ET-WP</td>
<td></td>
<td>(WP - ET)</td>
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<tr>
<td>Initial body weight (g)</td>
<td>167 ± 21</td>
<td>165 ± 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Week 9 body weight (g)</td>
<td>329 ± 41</td>
<td>387 ± 28 *</td>
<td>377 ± 42</td>
<td>370 ± 38</td>
<td>389 ± 40</td>
<td>0.597</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>389 ± 50</td>
<td>479 ± 65 *</td>
<td>454 ± 60</td>
<td>428 ± 47</td>
<td>408 ± 36</td>
<td>0.241</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.732 ± 0.07</td>
<td>0.82 ± 0.09 *</td>
<td>0.80 ± 0.09</td>
<td>0.78 ± 0.08</td>
<td>0.75 ± 0.06</td>
<td>0.438</td>
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<tr>
<td>Adiposity index (%)</td>
<td>4.8 ± 1.8</td>
<td>11.3 ± 2.5 *</td>
<td>7.0 ± 2</td>
<td>6.5 ± 2.2</td>
<td>4.8 ± 1</td>
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<tr>
<td>Food intake (g/day)</td>
<td>18.2 ± 1.1</td>
<td>18.64 ± 1.28</td>
<td>17.5 ± 0.98</td>
<td>15.2 ± 1</td>
<td>15.88 ± 1.3</td>
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<tr>
<td>Liver weight (g)</td>
<td>9.9 ± 1.6</td>
<td>13.8 ± 2 *</td>
<td>12 ± 2.1</td>
<td>13 ± 1.8</td>
<td>11.9 ± 2.2</td>
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<tr>
<td>Inguinal fat pad (g)</td>
<td>7.6 ± 2.6</td>
<td>22.5 ± 8.6 *</td>
<td>11.5 ± 4</td>
<td>10.7 ± 4.3</td>
<td>6.4 ± 1.6</td>
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</tr>
<tr>
<td>Epididymal fat pad (g)</td>
<td>6.6 ± 2.8</td>
<td>17.9 ± 5.7 *</td>
<td>11.3 ± 4.5</td>
<td>9.5 ± 3.2</td>
<td>7.3 ± 2</td>
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<td>Mesenteric fat pad</td>
<td>5.2 ± 3.4</td>
<td>11.9 ± 2.5 *</td>
<td>8 ± 4.2</td>
<td>6.3 ± 2.2</td>
<td>4.1 ± 1.6</td>
<td>0.007</td>
</tr>
<tr>
<td>Retroperitoneal fat pad (g)</td>
<td>4.3 ± 1.8</td>
<td>16.4 ± 4.9 *</td>
<td>9.7 ± 4</td>
<td>8.2 ± 3.8</td>
<td>6 ± 2.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Perirenal fat pad (g)</td>
<td>1.7 ± 0.8</td>
<td>5.2 ± 0.9 *</td>
<td>3.5 ± 1.8</td>
<td>2.5 ± 1.2</td>
<td>2 ± 0.6</td>
<td>0.024</td>
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<tr>
<td>TG (mg/dl)</td>
<td>73.6 ± 13.8</td>
<td>68 ± 12</td>
<td>75 ± 13</td>
<td>68.6 ± 6.8</td>
<td>75.2 ± 11</td>
<td>0.092</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>53.6 ± 1.8</td>
<td>118.8 ± 55.5 *</td>
<td>70.9 ± 25.1</td>
<td>96 ± 20.2</td>
<td>62 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>26.9 ± 4.1</td>
<td>25.9 ± 3.8</td>
<td>27.6 ± 4.8</td>
<td>26.8 ± 4.9</td>
<td>24.9 ± 3.8</td>
<td>0.968</td>
</tr>
<tr>
<td>Adipose tissue TG (mg/dl)</td>
<td>5.4 ± 2.4</td>
<td>6.3 ± 1.8</td>
<td>5 ± 1.2</td>
<td>6.3 ± 2</td>
<td>7.4 ± 2.2</td>
<td>0.934</td>
</tr>
</tbody>
</table>

Values are means ± SD. ND-SC, sedentary control - normal diet; SC, sedentary control; SC-WP, whey protein; ET, aerobic training; ET-WP, whey protein + aerobic training. * indicate significant differences between SC-HFD and SC-ND.
Table 3

The effect of HFD upon blood glucose, Insulin, HOMA-IR, and inflammatory factors

<table>
<thead>
<tr>
<th></th>
<th>ND-SC</th>
<th>HFD-SC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>7.02 ± 2.1</td>
<td>10.5 ± 3.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>1.24 ± 1.13</td>
<td>2.4 ± 0.95</td>
<td>0.084</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.358 ± 0.36</td>
<td>0.987 ± 0.18</td>
<td>0.023</td>
</tr>
<tr>
<td>Serum TNF-α (pg/ml)</td>
<td>11.6 ± 3.2</td>
<td>20.8 ± 11.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Epididymal TNF-α (pg/mgtissue)</td>
<td>3.6 ± 1.98</td>
<td>6.1 ± 1.5</td>
<td>0.025</td>
</tr>
<tr>
<td>Serum MCP-1 (ng/L)</td>
<td>203 ± 38</td>
<td>239 ± 30</td>
<td>0.133</td>
</tr>
<tr>
<td>Epididymal MCP-1 (µg/mgtissue)</td>
<td>0.49 ± 0.37</td>
<td>1.09 ± 0.12</td>
<td>0.004</td>
</tr>
<tr>
<td>Epididymal HIF-1α (ng/mgtissue)</td>
<td>9.5 ± 2.7</td>
<td>15.4 ± 0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Epididymal VEGF-A (pg/mgtissue)</td>
<td>0.54 ± 0.017</td>
<td>0.76 ± 0.071</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Values are means ± SD. ND-SC, sedentary normal diet; HFD-SC, sedentary high fat diet.
Figure captions

Figure 1. Changes of body weight after 9 weeks of high-fat diet (A), The effect of WP and ET in HFD rats upon body weight (B), blood glucose (C), Insulin (D), and HOMA-IR (E) at the end of the experiment. Values are mean ±SEM. Data were analyzed by two-way ANOVA. WP corresponds to diet effect (WP-SC + ET-WP × SC + ET); ET corresponds to exercise effect (ET + ET-WP × SC + WP-SC); and Interaction is the interaction between the corresponding parameters (SC × WP-SC × ET × ET-WP). ND-SC, sedentary control - normal diet; SC, sedentary control; SC-WP, whey protein; ET, aerobic training; ET-WP, whey protein + aerobic training.

Figure 2. The effect of WP and ET in HFD rats on serum TNF-α (A), epididymal TNF-α (B), serum MCP-1 (C), epididymal MCP-1 (D), epididymal HIF-1α (E), and epididymal VEGF-A (F) at the end of the experiment. Values are mean ±SEM. Data were analyzed by two-way ANOVA. WP corresponds to diet effect (WP-SC + ET-WP × SC + ET); ET corresponds to exercise effect (ET + ET-WP × SC + WP-SC); and Interaction is the interaction between the corresponding parameters (SC × WP-SC × ET × ET-WP). ND-SC, sedentary control - normal diet; SC, sedentary control; SC-WP, whey protein; ET, aerobic training; ET-WP, whey protein + aerobic training. * indicate significant differences with normal diet.
Figure 2.

A) Serum TNF-α (pg/mg)

B) Epididymal TNF-α (pg/mg tissue)

C) Serum MCP-1 (ng/mL)

D) Epididymal MCP-1 (pg/mg tissue)

E) Epididymal HIF-1 (ng/mg tissue)

F) Epididymal VEGF-A (pg/mg tissue)