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Effects of dietary heme iron and exercise training on abdominal fat accumulation and lipid metabolism in high-fat diet-fed mice

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Running title: Heme iron and exercise

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Animal by-products can be recycled and used as sources of essential nutrients. Water-soluble heme iron (WSHI), a functional food additive for supplementing iron, is produced by processing animal blood. In this study, we investigated the effects of dietary supplementation of 3% WSHI and exercise training for 4 weeks on the accumulation of abdominal fat and lipid metabolism in mice fed high-fat diet. Exercise-trained mice had significantly less perirenal adipose tissue, whereas WSHI-fed mice tended to have less epididymal adipose tissue. In addition, total weight of abdominal adipose tissues was significantly decreased in the Exercise + WSHI group. Dietary WSHI significantly increased the mRNA levels of lipoprotein lipase and hormone sensitive lipase. WSHI-fed mice also tended to show increased mRNA levels of adipose triglyceride lipase in their epididymal adipose tissue. Dietary WSHI also significantly decreased the mRNA levels of fatty acid oxidation-related enzymes in the liver, but did not influence levels in the gastrocnemius muscle. Exercise training did not influence the mRNA levels of lipid metabolism-related enzymes in the epididymal adipose tissue, liver, or the gastrocnemius muscle. These findings suggest that the accumulation of abdominal fat can be efficiently decreased by the combination of dietary WSHI and exercise training in mice fed high-fat diet.

Keywords: exercise, fat, heme iron, mice.

INTRODUCTION

The farming of pigs, cattle, and poultry for human consumption produces byproducts including blood, feathers, and manure, which have the potential to be recycled and converted into useful products of high value (Toldrá *et al*. 2012; Jayathilakan *et al*. 2012). For example, protein-rich biomass can be used a useful substrate in biogas production (Kovacs *et al.* 2015) or the gelling properties of porcine plasma could be improved to meet specific food requirements by reformulating its natural composition and by controlling its pH (Dàvila *et al*. 2007). Heme iron, which is found in blood and meat products, is an efficient source of dietary iron compared with non-heme iron (Seligman *et al*. 2000; Zimmermann *et al.* 2005, 2007; Winter *et al*. 2014). Heme iron is absorbed from the intestinal tract through the enterocytes as an intact metalloporphyrin. It travels across the intestinal brush border via a specific transporter, where it is then split by heme oxygenase (Raffin *et al*. 1974). Water-soluble heme iron (WSHI), a functional food ingredient for iron supply, is composed of heme iron and hemoglobin peptides and is sourced from animal blood (Fig. 1). Therefore, consuming WSHI would increase the use of animal blood, which is a byproduct of meat production.

In humans, serum iron concentrations are lower in obese individuals (Zhao *et al*. 2015), and serum iron concentration is also negatively correlated with body mass index (BMI) (Choma *et al.* 2015). Sharif *et al.* (2014) suggested that children with elevated BMIs should be screened for iron deficiency. Thus, WSHI could be a useful iron supplement for obese individuals. In addition, consuming globin digest, which is enzymatically hydrolyzed from red blood cells, suppresses post prandial hypertriglyceridemia in humans, mice, rats, and dogs, suggesting that hemoglobin-

derived peptides suppress lipid absorption (Kagawa *et al*. 1996, 1998). Since WSHI contains hemoglobin peptides (Fig. 1), we hypothesized that WSHI could be used as a functional food ingredient not only for the treatment of iron deficiency but also for the treatment of obesity.

There is evidence that the combination of treatment with anti-obesity food ingredients and exercise can efficiently treat obesity. For example, Shimotoyodome *et al*. (2005) found that the combination of treatment with dietary green tea extract and regular treadmill exercise stimulated whole body fat utilization. The authors suggested this might be attributed to the stimulation of fat catabolism, not only in the liver but also in the skeletal muscle, of mice fed high-fat diet. Seo *et al*. (2012) found that aged garlic extract enhanced the ability of exercise to reducing visceral fat in rats fed high-fat diet.

In this study, we investigated the effect of WSHI and exercise on abdominal fat accumulation in mice fed high-fat diet.

MATERIALS AND METHODS

Animals

Male ICR mice, 5-weeks old, were purchased from SLC Japan (Shizuoka, Japan), and were housed individually in plastic cages at 25℃ in a room with an automatically controlled 12-h light:dark cycle. Upon arrival, the mice fed a nonpurified diet (MF, 23.1% crude protein and 3,590 kcal/kg, Oriental Yeast. Co., Ltd., Tokyo, Japan) and acclimated to the facility for 7 d before feeding of the experimental diets. The mice had free access to water and assigned diets throughout the experimental period. Feed intake was measured twice a week. Body weight was measured every

week. The animal experiment in the present study was approved by the Institutional Animal Care and Use Committee (Permission number: 27-05-01) and carried out according to the Kobe University Animal Experimentation Regulation.

Experimental design

For doses of WSHI, we carried out a screening experiment to examine the effect of dietary WSHI (dose: 1-3 %) on the weight of abdominal adipose tissue in high-fat diet-fed mice. We found that the weight of epididymal adipose tissue tended to be lower in the mice fed 3% WSHI diet ($P = 0.079$). Twenty eight 6-week-old male ICR mice were assigned to four groups: a sedentary, high-fat diet group (Control); an exercise-trained, high fat diet group (Exercise); a sedentary, high-fat diet supplemented with 3% WSHI group (WSHI); or an exercise-trained, high-fat diet supplemented with 3% WSHI group (Exercise and WSHI) (Table 1). Average body weight was evenly distributed among each group. WSHI was obtained from ILS Inc. (Ibaraki, Japan). The WSHI comprised 77.1% crude protein, 12.0% carbohydrate, and 0.1% crude fat. The energy values for each diet were calculated from the macronutrient composition using the values of 4 kcal/g of carbohydrate, 4 kcal/g of protein, and 9 kcal/g of fat. Mice in the exercise-trained groups were run on a 2-lane motorized rodent treadmill (Melquest Ltd. Toyama, Japan). Mice were trained to run at 25 m/min following a 5 d training program of 5 m/min for 10 min, 10 m/min for 10 min, and 15 m/min for 10 min for the first day; at 10 m/min for 10 min, 15 m/min for 10 min, and 20 m/min for 10 min for the second day; 15 m/min for 10 min, 20 m/min for 10 min, and 25 m/min for 10 min for the third and fourth days; and at 15 m/min for 5 min, 20 m/min for 5 min, and 25 m/min for 20 min for the fifth day. After the training program, mice in the two exercised-

trained groups were run 3 d/wk on a treadmill at 25 m/min for 30 min during 3 weeks.

At the end of the experimental period, mice were anesthetized by the inhalation of isoflurane (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and their blood was collected from the abdominal vein. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant at a concentration of 1 mg/ ml blood. Plasma was immediately separated by centrifugation at 3,000 × g for 10 min at 4°C. It was then frozen using liquid nitrogen and stored at -80°C for plasma component analysis. The liver, epididymal adipose tissues, perirenal adipose tissues, and gastrocnemius muscles were excised, weighed, and then immediately frozen in liquid nitrogen for storage at -80°C before real-time PCR analysis.

Real-time PCR analysis.

Total RNA was extracted from the liver, epididymal adipose tissue and gastrocnemius muscle using Sepazol®-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from total RNA using a ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan). Complementary DNAs of lipid metabolism-related genes and an internal standard (ribosomal protein S17) were amplified with primers described in Table 2. THUNDERBIRD™ SYBR® qPCR Mix was purchased from TOYOBO CO. LTD. (Osaka, Japan), and mRNA expression was quantified in duplicate using the Applied Biosystems 7300 Real-Time PCR system according to the supplier's recommendations.

Plasma and hepatic components analysis.

Plasma triglyceride (TG) and nonesterified fatty acid (NEFA) levels were

131 measured by colorimetric enzymatic methods using commercial kits (LabAssay™
132 Triglyceride and LabAssay™ NEFA, respectively, Wako Pure Chemical Industries, Ltd.
133 Osaka, Japan). Total lipids were extracted from the liver by the method of Folch *et al*.
134 (1957), and TG content was determined by the method of Fletcher (1968).
135
136 **Statistical analysis**
137 Data were analyzed by two-way analysis of variance. Significant difference in
138 the total weight of abdominal adipose tissues between groups was analyzed by
139 Bonferroni/Dunn method. All statistical analyses were performed using a commercial
140 software package (StatView version 5, SAS Institute, Cary, NC, USA, 1998). Difference
141 with $P < 0.05$ was considered significant.
142
143 **RESULTS**
144
145 Exercise training significantly ($P < 0.05$) decreased body weight and the weight of
146 perirenal adipose tissue. The weight of epididymal adipose tissue tended to be lower in
147 the mice fed WSHI ($P = 0.064$). In addition, the total weight of abdominal adipose
148 tissues was significantly decreased in the Exercise + WSHI group (Fig. 2). The weights
149 of liver and skeletal muscle were not influenced by either dietary WSHI or exercise
150 training. The content of TG in the liver and the levels of NEFA and TG in the blood
151 plasma were not affected by either dietary WSHI or exercise training.
152 There was significant changes in the mRNA levels of lipid metabolism-related
153 genes in the WSHI-fed mice. The WSHI-fed mice had significantly ($P < 0.05$) greater
154 mRNA levels of lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) (Table 4).

The mRNA levels of adipose triglyceride lipase (ATGL) also tended to be greater in the WSHI-fed mice ($P = 0.084$, Table 4). The WSHI-fed mice had significantly ($P < 0.05$) decreased mRNA levels of fatty acid oxidation-related genes such as peroxisome proliferator-activated receptor (PPAR) α, carnitine palmitoyltransferase 1a (CPT1a), and acyl-coenzyme A oxidase (ACO) in their livers (Table 5). In contrast, the mRNA levels of fatty acid synthesis-related genes in the liver were not significantly changed. The mRNA levels of fatty acid synthase (FAS) tended to be lower in the WSHI-fed mice ($P = 0.059$).

The exercise-trained mice showed no significant differences in the mRNA levels of fatty acid metabolism-related genes in their liver, adipose tissue, or gastrocnemius muscle (Tables 4-6). The mRNA levels of PPARδ in the gastrocnemius muscle tended to be greater in the mice that exercised ($P = 0.062$, Table 6). However, the mRNA levels of PPARδ target genes in the gastrocnemius muscle were not changed by exercise (Table 6).

DISCUSSION

Exercise significantly decreased the body weight of mice and the weight of their perirenal adipose tissue. The weight of the epididymal adipose tissue was also likely to be lower in the WSHI-fed mice. There was no significant interaction between exercise level and diet type on any of the parameters we measured. However, to evaluate whether WSHI and exercise showed an additive effect on abdominal fat accumulation, significant difference in the total weight of abdominal fat between groups was statistically analyzed by Bonferroni/Dunn multiple comparison analysis. The total

weight of abdominal adipose tissue was significantly decreased in the Exercise + WSHI group (Fig. 2). This finding suggests that the accumulation of abdominal fat can be efficiently decreased by the combination of dietary WSHI and exercise training in mice fed high-fat diet. The detailed mechanisms underlying this additive effect is still unclear. However, it seems likely that WSHI and exercise suppressed abdominal fat accumulation in high-fat diet-fed mice by different mechanisms.

ATGL and HSL are the rate-limiting enzymes of TG hydrolysis in white adipose tissue (Schweiger *et al.* 2006). ATGL has significantly higher TG lipase activity which catalyzes the first step in TG hydrolysis, whereas HSL has higher diacylglycerol lipase activity (Zimmermann *et al.* 2004). A genetic study by Schweiger *et al*. (2006) showed that ATGL and HSL are responsible for more than 95% of the TG hydrolase activity present in murine white adipose tissue. In the present study, WSHI-fed mice showed increased mRNA levels of HSL in their epididymal adipose tissue, while the mRNA levels of ATGL were also likely to be greater (Table 4). Therefore, the increase in the mRNA levels of these genes might be the cause of the reduction in epididymal adipose tissue weight in mice fed WSHI.

LPL, which plays a critical role in the uptake of fatty acids from serum TG to the white adipose tissue, showed significantly higher mRNA levels in the epididymal adipose tissue of the WSHI-fed mice (Table 4). However, plasma TG levels were not affected in the mice fed WSHI (Table 3). In addition, epididymal adipose tissue weight did not increase in the mice fed WSHI (Table3). These results suggest that the increase in the mRNA levels of LPL is not related to the decrease in epididymal adipose tissue in the WSHI-fed mice.

Obesity induces macrophage infiltration of the adipose tissue in mammals.

Adipose-resident macrophage (M1) secretes pro-inflammatory cytokines (Cao 2014). Monocyte chemoattractant protein-1 (MCP-1), an important chemokine for macrophage recruitment, and tumor necrosis factor-α (TNF- α), a pro-inflammatory cytokine, are involved in an inflammatory response in the adipose tissue (Kanda *et al*. 2006). Studies have shown that the mRNA levels of MCP-1 and TNFα were increased in obesity with adipocyte hypertrophy (Huh *et al.* 2014; Cani *et al*. 2007). There is also evidence that TNFα stimulates lipolysis in adipocytes (Wang *et al*. 2008). We analyzed the mRNA levels of these genes, and found that mRNA levels were not affected by treatment with dietary WSHI (Table S1). However, further studies will be required to clarify whether the inflammatory response in the adipose tissue is affected by dietary WSHI and exercise.

In the liver, the mRNA levels of PPARα and its target genes, including CPT-1a and ACO, significantly decreased in the WSHI-fed mice (Table 5). CPT-1a and ACO are known to be the rate-limiting enzymes in fatty acid oxidation. Silva *et al.* (2015) showed that intraperitoneal injection of iron dextran could decrease fatty acid oxidation in the liver of rats by the inhibition of PPARα and its target genes. In addition, Bonomo *et al.* (2012) showed a similar effect of intraperitoneal injection of iron dextran on the expression of PPARα in hypercholesterolemic hamsters. It is well known that heme iron is better absorbed than non-heme iron (Seligman *et al*. 2000; Zimmermann *et al.* 2005, 2007; Winter *et al*. 2014). Therefore, it is possible that WSHI, which contains 1.2% iron, efficiently supply iron to the body, which in turn results the decrease of mRNA levels of PPARα and its target genes.

There is evidence that mRNA levels of PPARα-related genes are influenced by the sympathetic nervous system (Nogueiras *et al*. 2010) and various hormones including

227 growth hormone, sex hormones (Jalouli *et al*. 2003), glucocorticoids (Lemberger *et al*.
228 1994; Sterchele *et al*. 1996), glucagon (Berglund *et al*. 2010), and leptin (Nogueiras *et*
229 *al*. 2010). Iron status can influence neurodegeneration in the brain (Schneider 2016) and
230 plasma hormone levels including glucocorticoid (Zinker *et al*. 1993) and leptin (Gao *et*
231 *al*. 2015). The exact mechanism underlying WSHI-induced downregulation of mRNA
232 expression of PPARα-related genes is not clear. However, these findings and our results
233 raise the possibility that WSHI affected iron metabolism in the body and downregulated
234 the expression of hepatic PPARα-related genes through a complex pathways. Further
235 studies are required to clarify this possibility.
236 It is unclear what molecular mechanism underlying the reduction in perirenal
237 adipose tissue in the exercise-trained mice. There was no significant effect of exercise
238 on lipid metabolism-related genes in the liver and epididymal adipose tissue (Tables 4
239 and 5). The mRNA levels of PPARδ were elevated in the exercise-trained mice ($P = 0.062$). However, the mRNA levels of PPARδ target genes in the gastrocnemius muscle
241 were unchanged in the exercise-trained mice (Table 6). Therefore, it seems likely that
242 exercise did not affect fatty acid oxidation in skeletal muscles in our experiments.
243 Exercise significantly decreased the weight of perirenal adipose tissue, which indicates
244 that TG metabolism in the perirenal adipose tissue could be altered by exercise (Table
245 3). Further study is needed to clarify the effect of exercise on TG metabolism in the
246 perirenal adipose tissue.
247 In summary, we investigated the effect of dietary WSHI and exercise training
248 on abdominal fat accumulation and lipid metabolism in mice fed high-fat diet. Our
249 findings suggest that the accumulation of abdominal fat can be efficiently decreased by
250 the combination of dietary WSHI and exercise training in mice fed high-fat diet.

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365
366 **FIGURE LEGENDS**
367

368 Figure 1　Preparation of water-soluble heme iron (WSHI)

369

370 Figure 2　Effects of exercise and WSHI on abdominal fat weight in high-fat diet-fed

371 mice. Abdominal fat weight is the sum of the weights of epididymal and perirenal

372 adipose tissues. Data represent means±SEM. Groups with different letters are

373 significantly different ($P < 0.05$).

374

高脂肪食給与マウスにおける腹部脂肪蓄積と脂質代謝関連遺伝子の発現に及ぼす食餌ヘム鉄と運動の影響

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食肉生産における副生物は必須栄養素の重要な供給源として再利用できる。動物の血液から生産される水溶性ヘム鉄は，鉄分供給用の機能性食品素材として利用されている。本研究では，4週間の飼料への3%のヘム鉄添加と運動負荷が，高脂肪食給与マウスにおける腹部脂肪蓄積と脂質代謝に及ぼす影響について調べた。運動は腎周囲脂肪組織重量を有意に減少させ，水溶性ヘム鉄は精巣上体周囲脂肪組織重量を減少させる傾向を示した。加えて，腹部脂肪組織の総重量はヘム鉄添加と運動負荷の併用により有意に減少した。水溶性ヘム鉄は精巣上体周囲脂肪組織におけるホルモン感受性リパーゼとリポタンパク質リパーゼのmRNA量を増加させ，肝臓の脂肪酸酸化関連酵素のmRNA量を減少させたが，腓腹筋の脂質代謝関連酵素の発現には影響しなかった。運動は精巣上体周囲脂肪組織，肝臓，及び腓腹筋の脂肪酸酸化関連酵素のmRNA量に影響を及ぼさなかった。これらの結果から，高脂肪食マウスにおける腹部脂肪蓄積は，水溶性ヘム鉄の給与と運動の組み合わせによって効率的に減少させ得ることが示唆された。

キーワード：運動，脂肪，ヘム鉄，マウス

Table 2. Primer sequences for real-time PCR analysis

Gene			Primer sequences	Accession number*
Liver				
	PPARα	sense	5’-CGG CAG TGC CCT GAA CA-3’	NM_001113418
		antisense	5’-TGG TAC CCT GAG GCC TTG TC-3’	
	CPT1a	sense	5’-GAA CCC CAA CAT CCC CAA AC-3'	NM_013495
		antisense	5’-TCC TGG CAT TCT CCT GGA AT-3’	
	ACO	sense	5’-CCC AAG ACC CAA GAG TTC ATT C-3’	NM_015729
		antisense	5’-CAG GCC ACC ACT TGA TGG A-3’	
	SREBP-1c	sense	5' -GCT ACC GGT CTT CTA TCA ATG ACA-3'	NM_011480
		antisense	5' -GCA GAT TTA TTC AGC TTT GCT TCA-3'	
	ACCα	sense	5' -CGC TCA GGT CAC CAA AAA GAA T-3'	NM_133360
		antisense	5' -GTC CCC GCC ACA TAA CTG AT-3'	
	FAS	sense	5’-TCC TGG AAC GAG AAC ACG ATC T-3’	NM_007988
		antisense	5’-GAG ACG TGT CAC TCC TGG ACT TG-3’	
Adipose tissue				
	ATGL	sense	5’-CCT CAG GAC AGC TCC ACC AA-3’	NM_001163689
		antisense	5’-TTG AAC TGG ATG CTG GTG TTG-3’	
	HSL	sense	5’-CTC CTA TGA CCT ACG GGA AGG A-3’	NM_001039507
		antisense	5’-TCA GAT TTT GCC AGG CTG TTG-3’	
	LPL	sense	5’-GAT GGA CGG TAA CGG GAA TG-3’	NM_008509
		antisense	5’-TAC AGG GCG GCC ACA AGT-3’	
Skeletal muscle				
	PPARδ	sense	5’-GCC ACA ACG CAC CCT TTG-3’	NM_011145
		antisense	5’-CCT TCT CTG CCT GCC ACA GT-3’	
	CPT1b	sense	5’-GTC CAA GCA GCC CGT CTA G-3’	NM_009948
		antisense	5’-TTG CGG CGA TAC ATG ATC AT-3’	
	UCP2	sense	5’-TGA TGT GGT CAA GAC GAG ATA CAT G-3’	NM_011671
		antisense	5’- CAG TGA CCT GCG CTG TGG TA-3’	
	UCP3	sense	5’-TTT TGC GGA CCT CCT CAC TT-3’	NM_009464
		antisense	5’-TGG ATC TGC AGA CGG ACC TT-3’	
Internal standard				
	RPS17	sense	5' -CCG GGT CAT CAT CGA GAA GT-3'	NM_009092
		antisense	5' -GCG CTT GTT GGT GTG GAA GT-3'	

* Refer to GenBank accession number. Abbreviations are as follows: PPARα, peroxisome proliferator-activated receptor α; CPT1a, carnitine palmitoyltransferase 1a; ACO, acyl-coenzyme A oxidase; FAS, fatty acid synthase; ACCα, acetyl-CoA carboxylase α; SREBP-1c, sterol regulatory element binding protein-1c; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase; LPL, lipoprotein lipase; PPARδ, peroxisome proliferator-activated receptor δ; CPT1b, carnitine palmitoyltransferase 1b; UCP2, uncoupling protein 2; UCP3, uncoupling protein 3; RPS17, ribosomal protein S17.

Table 3. Effects of water-soluble heme iron (WSHI) and exercise training on the weights of body and tissues, hepatic triglyceride (TG), and blood components in mice fed high-fat diet

	Control	Exercise	WSHI	Exercise + WSHI	Exercise	WSHI	Interaction
Body weight (g)	43.3 ± 1.1	39.7 ± 1.2	41.3 ± 1.5	39.7 ± 0.5	P < 0.05*	P = 0.404	P = 0.398
Food intake (g)	105.5 ± 4.8	94.2 ± 10.3	97.9 ± 2.5	96.1 ± 1.8	P = 0.630	P = 0.277	P = 0.429
Liver weight (g)	1.49 ± 0.05	1.51 ± 0.09	1.51 ± 0.07	1.50 ± 0.04	P = 0.930	P = 0.969	P = 0.802
Epididymal adipose tissue weight (g)	1.92 ± 0.13	1.64 ± 0.19	1.52 ± 0.15	1.36 ± 0.21	P = 0.224	P = 0.064	P = 0.732
Perirenal adipose tissue weight (g)	0.59 ± 0.04	0.42 ± 0.05	0.48 ± 0.06	0.41 ± 0.06	P < 0.05*	P = 0.283	P = 0.313
Gastrocnemius muscle weight (g)	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	P = 0.412	P = 0.591	P = 0.745
Hepatic TG (mg/g)	51.5 ± 5.4	48.3 ± 11.0	43.1 ± 13.7	42.5 ± 6.3	P = 0.847	P = 0.470	P = 0.893
Plasma NEFA (μEQ/100 ml)	57.4 ± 5.5	59.1 ± 3.4	63.7 ± 2.9	60.3 ± 3.6	P = 0.357	P = 0.831	P = 0.518
Plasma TG (mg/100 ml)	69.6 ± 7.5	75.8 ± 8.4	74.8 ± 5.0	70.6 ± 7.0	P = 1.000	P = 0.892	P = 0.552

Data are means ± S.E.M. of seven mice in each group. Data were analyzed by two-way analysis of variance.

Table 4. Effects of water-soluble heme iron (WSHI) and exercise training on the mRNA levels of TG metabolism-related genes in epididymal adipose tissue in mice fed high-fat diet

	Control	Exercise	WSHI	Exercise + WSHI	Exercise	WSHI	Interaction
ATGL	1.00 ± 0.35	0.90 ± 0.22	1.66 ± 0.40	1.50 ± 0.40	P = 0.706	P = 0.084	P = 0.933
HSL	1.00 ± 0.37	1.04 ± 0.17	1.79 ± 0.40	1.66 ± 0.32	P = 0.883	P < 0.05	P = 0.798
LPL	1.00 ± 0.41	1.11 ± 0.31	2.39 ± 0.66	1.94 ± 0.55	P = 0.742	P < 0.05	P = 0.584

mRNA levels were normalized to respective controls (set as 1 arbitrary unit). Data are means ± S.E.M. of seven mice in each group. Data were analyzed by two-way analysis of variance.

Table 5. Effects of water-soluble heme iron (WSHI) and exercise training on the mRNA levels of fatty acid metabolism-related genes in the liver in mice fed high-fat diet

	Control	Exercise	WSHI	Exercise + WSHI	Exercise	WSHI	Interaction
PPARα	1.00 ± 0.15	0.81 ± 0.18	0.38 ± 0.17	0.72 ± 0.06	P = 0.622	P < 0.05	P = 0.090
CPT-1a	1.0 ± 0.13	1.0 ± 0.24	0.4 ± 0.19	0.8 ± 0.07	P = 0.386	P < 0.05	P = 0.256
ACO	1.0 ± 0.12	0.9 ± 0.18	0.4 ± 0.16	0.7 ± 0.03	P = 0.367	P < 0.05	P = 0.184
SREBP-1c	1.00 ± 0.15	1.02 ± 0.25	0.62 ± 0.15	1.06 ± 0.12	P = 0.196	P = 0.325	P = 0.242
ACCα	1.00 ± 0.14	1.08 ± 0.16	0.85 ± 0.13	1.09 ± 0.06	P = 0.235	P = 0.601	P = 0.553
FAS	1.00 ± 0.11	0.96 ± 0.25	0.39 ± 0.24	0.84 ± 0.06	P = 0.271	P = 0.059	P = 0.189

mRNA levels were normalized to respective controls (set as 1 arbitrary unit). Data are means ± S.E.M. of seven mice in each group. Data were analyzed by two-way analysis of variance.

Table 6. Effects of water-soluble heme iron (WSHI) and exercise training on the mRNA levels of fatty acid metabolism-related genes in the gastrocnemius muscle in mice fed hgh-fat diet

	Control	Exercise	WSHI	Exercise + WSHI	Exercise	WSHI	Interaction
PPARδ	1.00 ± 0.18	1.53 ± 0.11	1.18 ± 0.14	1.43 ± 0.34	P = 0.062	P = 0.850	P = 0.499
CPT1b	1.00 ± 0.16	1.26 ± 0.11	1.03 ± 0.17	1.28 ± 0.32	P = 0.211	P = 0.891	P = 0.981
UCP2	1.00 ± 0.19	1.30 ± 0.30	1.27 ± 0.17	1.23 ± 0.16	P = 0.551	P = 0.660	P = 0.445
UCP3	1.00 ± 0.15	1.30 ± 0.14	1.10 ± 0.20	1.07 ± 0.23	P = 0.455	P = 0.729	P = 0.387

mRNA levels were normalized to respective controls (set as 1 arbitrary unit). Data are means ± S.E.M. of seven mice in each group. Data were analyzed by two-way analysis of variance.

Figure 1

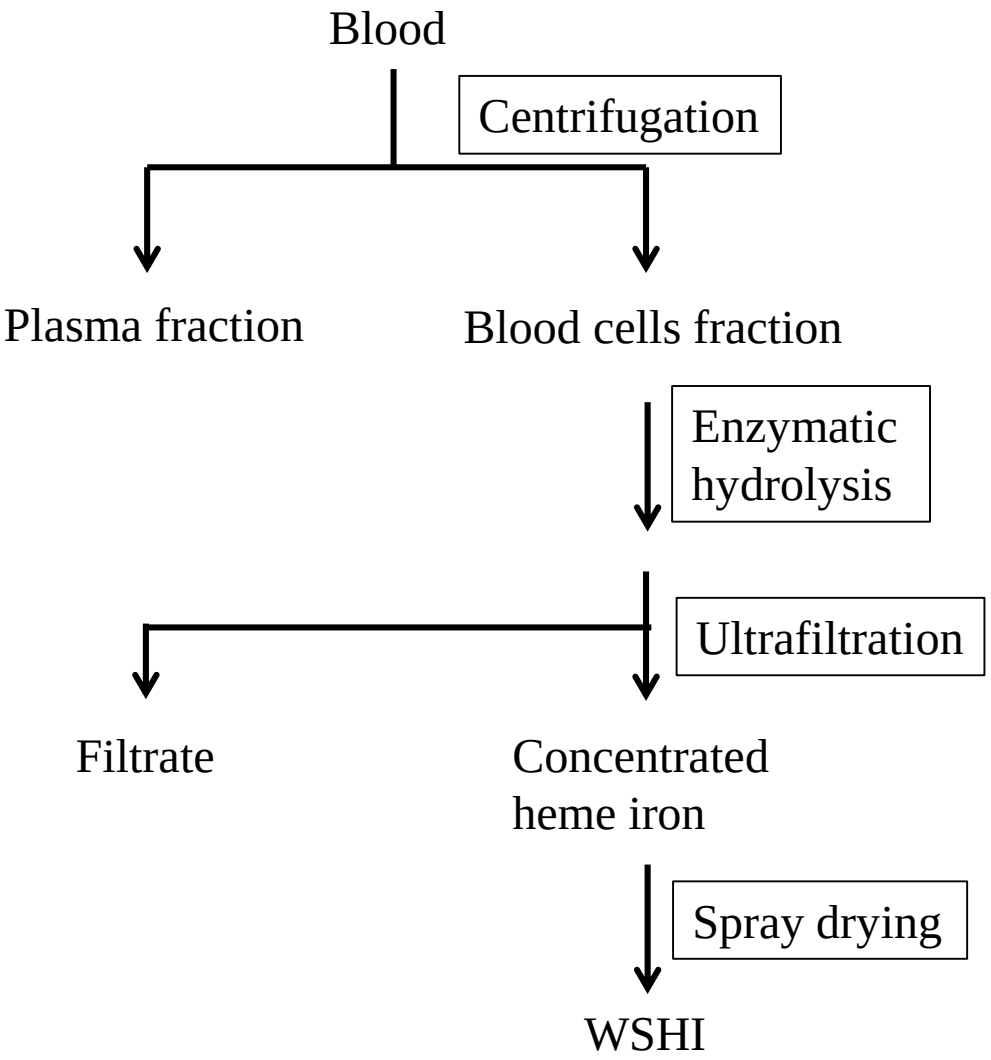
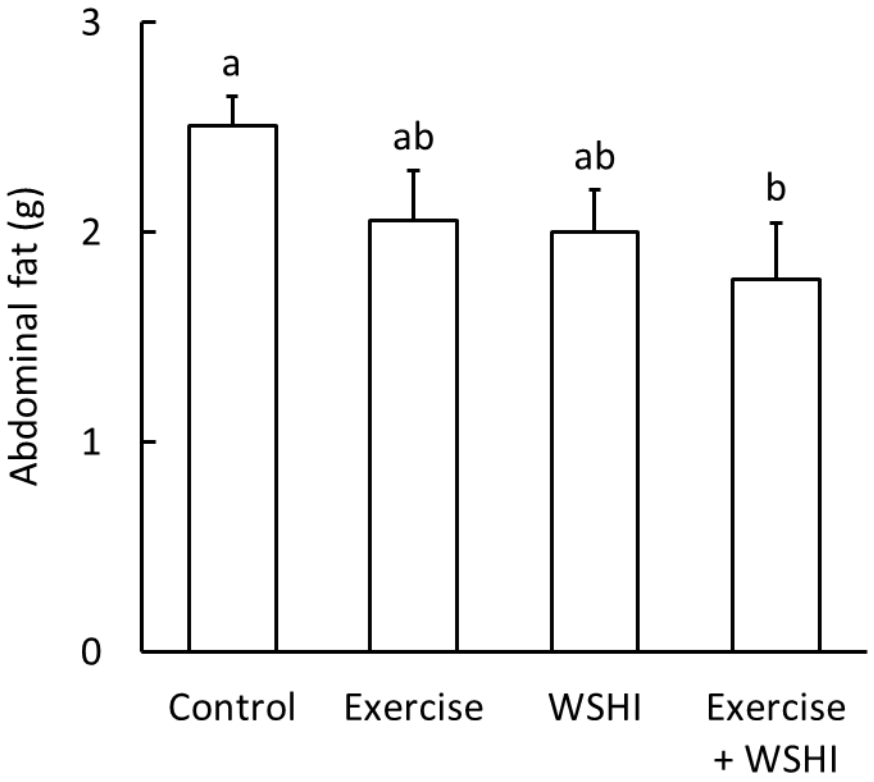

Blood
Centrifugation
Plasma fraction
Blood cells fraction
Enzymatic hydrolysis
Ultrafiltration
Filtrate
Concentrated heme iron
Spray drying
WSHI

Figure 2


Abdominal fat (g)
3
2
1
0
a
ab
ab
b
Control
Exercise
WSHI
Exercise + WSHI