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Experimental Research Article

Caffeine and exercise training alter expression of genes involved in inflammation and the browning of adipose tissue in High-Fat Diet-Fed Rats

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ABSTRACT

Introduction: Caffeine, as a popular drink, along with exercise training, may help restore altered gene expres-sion in high-fat diet (HFD)-induced obesity. This study examined the effects of exercise training, caffeine consumption, and their interaction on inflammation and genes involved in metabolism in rats fed an HFD.

Methods: Eighty male Wistar rats were separated into two groups: HFD and normal diet (ND). Each group was subsequently divided into four groups: sedentary, caffeine-only, exercise, and caffeine-plus-exercise. For eight weeks, the animals in the training groups engaged in aerobic exercise on a mo-torized treadmill for 60 minutes, five times per week. Animals in the caffeine group ingested a solution containing caffeine daily (6 mg/kg/bw). The expression of Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) and fibronectin type III domain-containing (FNDC5) genes in the calf muscle, uncoupling protein-1 (UCP1) in subcutaneous adipose tissue, NF-KB and TLR4 in vis-ceral adipose tissue, and fetuin-A (Fet-A) in the liver were investigated.

Results: The findings demonstrated that HFD significantly elevated the NF-Kβ gene and downregulated the skeletal muscle PGC-1α and FNDC5 genes, as well as serum fetuin-A. UCP-1 (366% vs. 56%), FNDC5 (26% vs. 54%), and PGC-1α (40% vs. 1700%) genes were all considerably elevated by exercise training and caffeine supplementation, respectively. Additionally, exercise training reduced TLR4 and NF-Kβ expression in visceral adipose tissue and liver Fet-A gene expression. Furthermore, following HFD, when compared to the sedentary group, exercise training with and without caffeine consumption decreased the NF-Kβ gene and liver Fet-A and increased PGC1-α, FNDC5, UCP1, and serum Fet-A.

Conclusion: These findings support the idea that exercise and caffeine may reduce inflammation by downregulating genes involved in inflammation and adipose tissue browning.

Keywords:
Exercise training
Caffeine
Supplementation
Brown adipose tissue uncoupling protein
In-flammation

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Introduction

Obesity, particularly when caused by a high-fat diet (HFD), is strongly linked to chronic low-grade inflammation due to excess adipose tissue infiltrated by immune cells that secrete inflammatory cytokines. This inflammation contributes to the development of various health issues, including metabolic disorders and cardiovascular diseases. Reducing body weight can mitigate obesity-induced inflammation by lowering pro-inflammatory cytokine production and enhancing adipose tissue function, thereby reducing the risk of chronic diseases like cardiovascular disease and type 2 diabetes (Villarroya et al., 2018). It is well-documented that both skeletal muscle and brown adipose tissue (BAT) have the same oxidative capacities (Porter et al., 2016). The similar oxidative capacities of muscle and BAT support the idea that browning white adipose tissue (WAT) can be a useful approach for weight loss due to the high metabolic activity and energy expenditure associated with BAT. Both muscle and BAT are known for their capacity to oxidize substrates and generate heat through a process called thermogenesis, which is facilitated by the presence of uncoupling protein 1 (*UCP1*) in BAT. This thermogenic process significantly increases energy expenditure and can lead to the reduction of fat stores (Zhang et al., 2022). Thus, it appears that increasing the browning of WAT can be considered a weight-loss strategy.

In addition to exercise, overexpression of the transcriptional co-activator Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (*PGC-1α*) has been found to increase the expression of fibronectin type III domain-containing (*FNDC5*) in skeletal muscle, which is associated with the expression of uncoupling protein-1 (*UCP1*), a marker of browning adipocytes, in adipose tissue (Boström et al., 2012). *PGC-1α*, as a muscle master metabolic regulator, is activated by muscle contraction (Boström et al., 2012; Egan et al., 2010). It has also been demonstrated that irisin, an exercise-responsive and PGC-1α-dependent myokine cleaved from the *FNDC5* gene (Boström et al., 2012), is secreted primarily by skeletal muscle (72%) and, to a lesser extent, by adipose tissue (28%) (Roca-Rivada et al., 2013). This secretion stimulates the browning of subcutaneous WAT by upregulating *UCP-1* (Boström et al., 2012).

Exercise training has been recognized as an effective weight loss strategy, promoting WAT browning and reducing meta-inflammation by increasing *FNDC5/irisin* levels. It was demonstrated that following 12 weeks of combined exercise training, circulating irisin decreased while the *PGC-1α* and *FNDC5* genes in skeletal muscle were elevated in human subjects (Norheim et al., 2014).

On the other hand, research has shown caffeine, a key component in many popular drinks, can combat diabetes (Natella and Scaccini 2012), obesity-related inflammation (Paiva et al., 2019), and boost metabolism. Through the central nervous system, it may also help boost thermogenic activity and energy expenditure (Clark et al., 2019). Additionally, caffeine has been discovered to upregulate *UCP1* in obese mice (Kogure et al., 2002).

A growing body of research indicates that exercise training and calorie restriction are not enough to reduce weight (Vettor et al., 2020). As a result, using pharmaceutical treatment in conjunction with physical exercise may be a successful plan to enhance energy expenditure and subsequently promote weight loss. Caffeine consumption, when combined with exercise training, appears to enhance thermogenic activity by influencing skeletal muscle and adipose tissue gene expression, promoting adipose tissue browning. However, to our knowledge, no study has investigated the combined effects of exercise training and caffeine consumption on the expression of adipose tissue browning-related genes in diet-induced obesity. Therefore, this study examined how exercise, caffeine intake, and their interaction influence skeletal muscle and adipose tissue gene expression related to adipose tissue browning, as well as their relationship with inflammatory markers.

Material and Methods

Animal Care

The University of Kurdistan's Ethics Committee approved this study (Approval ID: IR.UOK.REC.1397.024), which was based on the Guide for the Care and Use of Laboratory Animals (Council 2010). For this purpose, 80 male Wistar rats, aged 8 weeks and with an average weight of 180 g, were purchased from the Pasteur Institute of Iran and housed in cages with four rats per cage at a temperature of 21-23°C (12 hours of light and 12 hours of darkness). The rats were divided into two groups depending on their body weight after a one-week acclimation period: high-fat diet (*HFD*) or normal diet (*ND*). Then each of the ND and HFD groups was further divided into four subgroups: (1) normal sedentary (NS, n=10), (2) ND + caffeine (NC, n=10), (3)

ND + endurance training (NE, n=10), and (4) ND+ caffeine + endurance training (NCE, n=10). Similarly, the HFD group was divided into (1) sedentary HFD (HS, n=10), (2) HFD+ caffeine (HC, n=10), (3) HFD+ endurance training (HE, n=10), and (4) HFD + caffeine + endurance training (HCE, n=10). The rats had ad libitum access to an *HFD* or *ND* for the duration of the study (8 weeks). The *ND* consisted of carbohydrate (64% kcal), protein (24%), and fat (10% kcal), while the *HFD* consisted of soybean oil (60% kcal), protein (16% kcal), and carbohydrate (24% kcal). Rats in the exercise training groups were subjected to aerobic exercise for eight weeks (5 times/week). Additionally, rats in the caffeine-containing groups received daily gavage doses of caffeine (6 mg/kg/body weight) (Kobayashi-Hattori et al., 2005).

In this study, randomization was performed by numbering each sample (tagged on the back) and assigning them to control and treatment groups using an online randomization tool (https://randomizer.org). The sample size for each group was determined using an online sample size calculator (https://www.calculator.net/sample-size-calculator.html).

Exercise protocols

After one week of habituation to treadmill running (3 times per week, 15 minutes per session), animals in the training groups were subjected to progressive aerobic exercise training, starting with 20 minutes per day at an intensity of 15 meters per minute. Until the end of the third week, the speed and duration increased each week by 3 m/min and 5 min, respectively, so that the speed and duration reached 21 m/min and 30 min/session. Thereafter, the speed and duration gradually increased to 27 m/min and 60 min/session, respectively, throughout the remaining weeks (Høydal et al., 2007). The training protocol was carried out in the evening. To reduce the stress on the cage and the feeding tube, all animals were kept under the same conditions.

Tissue sampling, PCR quantification, and blood sampling

Following eight weeks of the intervention, the rats were anesthetized 48 hours after the last exercise training session while they were fasting. Then, blood samples were taken directly from the heart and centrifuged. Next, serum samples were kept at -20 °C for future analysis of irisin (MBS2601445) and Fet-A (MBS3809225). For tissue analysis, tissue samples of visceral and subcutaneous adipose tissue, the liver, and the gastrocnemius muscle were dissected and used for RNA extraction and real-time PCR quantification.

Total RNA was isolated from tissues using the RNX kit (Cinnagene). RNA sample concentration was determined using a biophotometer spectrophotometer (Eppendorf). Messenger RNA was reverse transcribed into cDNA using a Takara cDNA Synthesis Kit (Takara) according to the manufacturer's instructions. cDNA was quantified using the biophotometer and spectrophotometer to ensure equivalent concentrations for real-time analysis. A quantitative real-time polymerase chain reaction was used to validate a subset of differentially expressed genes. Gene-specific primers were designed using the Primer3 program (http://frodo.wi.mit.edu). Primer design criteria included a base-pair length of 100 to 200 and a guanine-cytosine (GC) content of 40% to 60%. Primers were designed to span exon/intron boundaries where possible and were tested using the same cDNA sample pool to ensure that there was no genomic contamination (Table 1).

SYBR Green (Ampliqon- Denmark) was used for real-time PCR analysis. Each 10 µL reaction contained 2X SYBR Green Master Mix I, 0.5 µmol/L gene-specific forward and reverse primers, and 100 ng cDNA. Polymerase chain reaction analysis was accomplished using the Rotor-Gene Q PCR System with the following cycle conditions: 95°C for 15 minutes, followed by 40 cycles of 95 °C for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. A melt curve analysis confirmed the amplification of a single cDNA product. Relative quantification of studied genes, toward the samples was calculated by using the comparative $2^{-\Delta\Delta Ct}$ method. Beta-Actin was used as a housekeeping gene for muscle tissue and the liver, and RPL13a was used as a housekeeping gene for adipose tissue.

Statistical analysis

Data values are expressed as a mean ± standard error of the means (*SEM*). Data normalization was done using Kolmogorov- Smirnov test. Data were analyzed using a univariate analysis of variance for diet, exercise, and caffeine. To set up a comparison within the groups (*HFD* or *ND*), two-way ANOVA and Bonferroni's post hoc test were applied. Pearson's correlation was also applied to

TABLE1: Primer's sequence

Genes	sequence	Primers (5'→3')	Length (bp)	Product size (bp)
PGC-1α	F	TTGAAAAAGCTTGACTGGCGT	21	94
	R	AGGGCAGCACACTCTATGTC	20	
UCP1	F	AGTGCCACTGTTGTCTTCAGGG	22	190
	R	CCTTGGATCTGAAGGCGGACT	21	
FNDC5	F	GTGGGCAGGTGTCATAGCTC	20	103
	R	GCTGGTCTCTGATGCACTCT	20	
NF-KB	F	AAC ATG GCA GAC GAT CC	20	129
	R	AAG GTA TGG GCC ATC TGT TGA C	22	
TLR4	F	TCA TGC TTT CTC ACG GCC TC	20	143
	R	TAG GAA GTA CCT CTA TGC AGG G	22	
Fetuin-A	F	GAC CCG GAA ACA GAG CAT GT	20	114
	R	CCG AGA CCA CAC CTT GAC TT	20	
Bactin	F	AGA GGG AAA TCG TGC GTG AC	20	150
	R	CAA TAG TGA TGA CCT GGC CGT	21	
RPL13a	F	TTG AGG ACC TCT GTG TAT TTG TCA A	23	200
	R	CCT GGA GGA GAA GAG GAA AGA GA	25	

determine the relationship between variables. The significant differences were considered to be P≤0.05. Data analysis was done using SPSS software (version 25).

Results

The effect of caffeine and exercise training on expression of genes PGC-1a and FNDC5 in gastrocnemius muscle

We found that diet (p<0.001), exercise (p<0.003), and caffeine (p<0.001) had a significant main influence on the expression of the *PGC-1α* gene in skeletal muscle. Additionally, the interaction effects of diet, caffeine, and exercise were all significant (P<0.005). When compared to *ND* animals, the *HFD* resulted in a decrease in *PGC-1a* gene expression after 8 weeks (P<0.001). However, caffeine consumption and exercise training significantly increased the *PGC-1α* gene (P< 0.001, P<0.001, respectively).

Considering differences between groups in each diet regimen, compared to the sedentary group, the combination of exercise and caffeine in the *HFD* groups significantly upregulated the expression of the *PGC-1α* gene (P<0.007). Furthermore, there were no significant effects of either caffeine or exercise training on the *PGC-1*a gene in the *HFD* groups (P>0.05). When compared to the sedentary group in the *ND* groups, exercise training, caffeine, and the combination of those factors significantly elevated the *PGC-1α* gene (P<0.009, Fig. 1A).

In addition, the findings revealed that the major effects of diet (p<0.001), exercise (p<0.001), and caffeine (p<0.002) on *FNDC5* gene expression were significant. Additionally, the interactions between diet, exercise, and caffeine were all significant (P<0.001), although the interaction between diet, exercise, and caffeine was not significant (P>0.082). In comparison to the *ND* group, the expression of the *FNDC5* gene was downregulated by almost 60% after HFD feeding (P< 0.001). However, *FNDC5* gene expression was significantly elevated by 54% (P< 0.001) and 26% (P<0.001), respectively, in response to exercise training (vs. sedentary) and caffeine consumption (vs. placebo).

Regarding differences within groups, caffeine consumption alone in the *HFD* groups did not differ significantly from the sedentary group in terms of its effects on *FNDC5*(P > 0.05). However, compared to the sedentary group, exercise training dramatically upregulated the *FNDC5* gene by 194% (P<0.001), although this upreg-

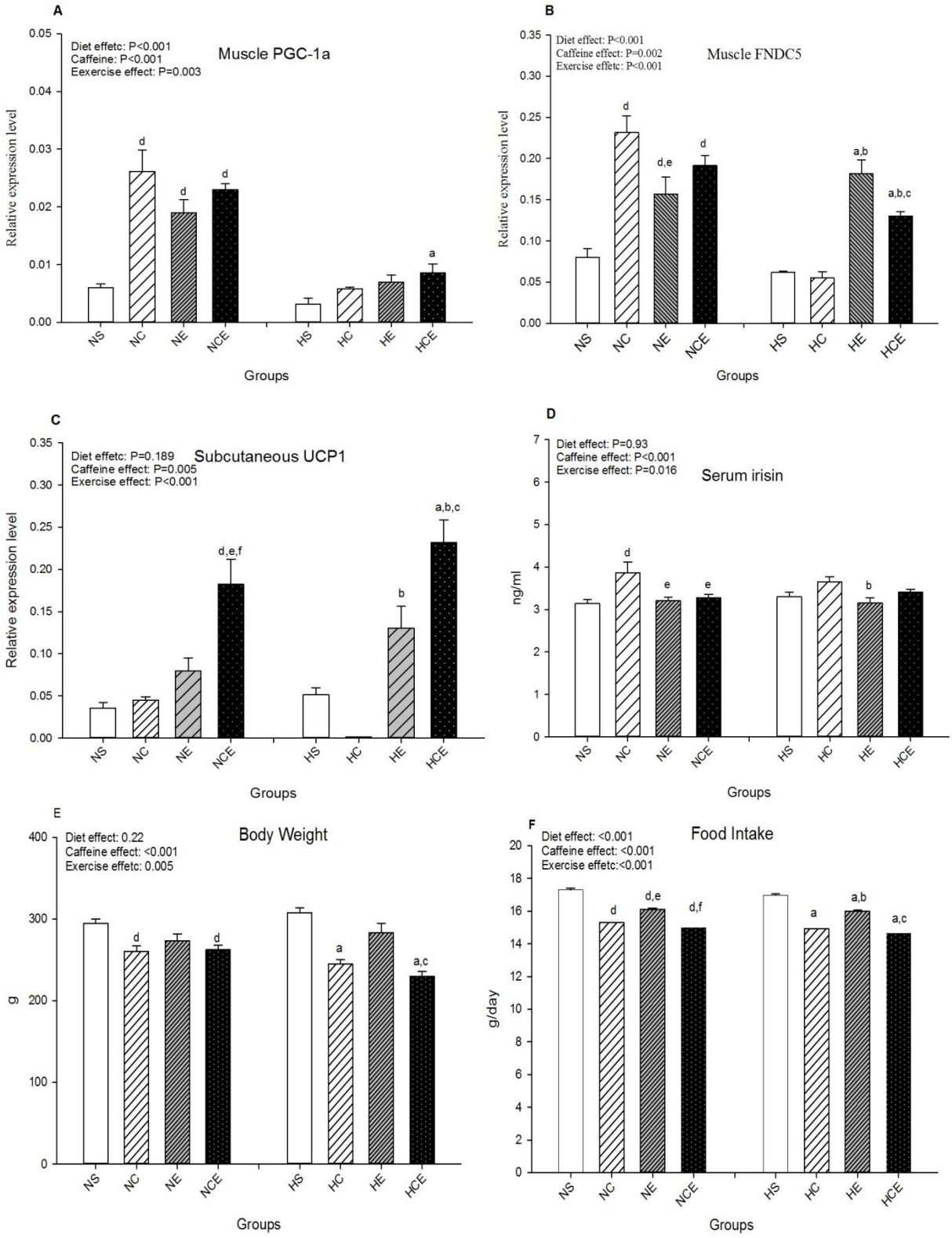

FIGURE 1. Muscle gene expressions, UCP1, Serum irisin, body weight, and food intake. Values are presented as mean ± SEM in each column, n=10/group. NS: Normal Sedentary, NC: Normal Caffeine, NE: Normal Endurance, NCE: Normal Caffeine Endurance, HS: High Fat diet Sedentary, HC: High-fat diet Caffeine, HE: High-fat diet Endurance, and HCE: High-fat diet Caffeine Endurance.

ulation was reduced in response to the combination of exercise and caffeine consumption (110%, P<0.001). As opposed to the sedentary group, caffeine consumption, exercise training, and the combination thereof in the *ND* group significantly increased the expression of the *FNDC5* gene (189%, P<0.001; 95%, P<0.016; 139%, P<0.001, respectively) (Fig. 1B).

The effect of caffeine and exercise training on the expression of gene UCP1 in subcutaneous adipose tissue

Here, we found a significant main effect of exercise (p<0.001) and caffeine (p<0.005) on *UCP1* gene expression in the subcutaneous adipose tissue. On the expression of the *UCP1* gene, we also indicated significant interaction effects between diet and exercise (P<0.031) and exercise and caffeine (P<0.001). Exercise training significantly increased *UCP1* gene expression by 366% compared to the sedentary group (P<0.001), regardless of the effects of caffeine or diet. In addition, caffeine consumption increased *UCP1* gene expression by 56% compared to the placebo group (P<0.001).

Taking into account variations between groups, compared to the sedentary group, the exercise and caffeine interventions in the *HFD* groups significantly raised the *UCP1* gene (351%, P<0.001). Additionally, it was also significantly higher in the exercise training plus caffeine consumption group than in the exercise training and caffeine consumption groups separately (P<0.001, P< 0.021, respectively). Likewise, compared to the sedentary group, *UCP1* was significantly elevated in response to the combination of exercise and caffeine consumption and the mono-interventions following *ND* feeding (P<0.001, Fig. 1C).

The effect of caffeine and exercise training on serum irisin, body weight, and food intake

According to the findings, caffeine and exercise had a significant (P<0.001, P<0.016, respectively) main effect on serum irisin levels. However, the main effect of diet was not statistically significant (P>0.84). In addition, the interaction between exercise and caffeine had a significant impact on serum irisin (P<0.031). Furthermore, compared to the sedentary group, exercise training reduced serum irisin levels by 3.6% (P<0.008). However, compared to the placebo group, caffeine consumption significantly boosted serum irisin by 10% (P<0.001).

Considering variations within groups, caffeine, exercise training, and their combination following the *HFD* regimen had no discernible impact on serum irisin in comparison to the sedentary group (P>0.05). However, compared to exercise training, the serum irisin levels were considerably higher after caffeine consumption (P<0.019). In addition, relative to the control and exercise training groups, caffeine intake during the *ND* regimen significantly raised serum irisin levels (P<0.006 and P<0.034, respectively). Moreover, raising serum irisin with caffeine alone outperformed increasing serum irisin with exercise training (P<0.05, Fig. 1D).

The main effects of caffeine and exercise on the final body weight were significant (P<0.001, P<0.005, respectively). In fact, caffeine consumption significantly reduced body weight compared to the placebo group (P<0.001). Likewise, animals in the exercise training group were significantly lighter than those in the sedentary group (P<0.032). Additionally, a substantial interaction effect between diet and caffeine on body weight is found (P<0.001).

Taking into account variations between groups, after *HFD* feeding, caffeine supplementation decreased body weight in comparison to the control group, both with and without exercise (P<0.001). When compared to the control group, the animals in the exercise training group were lighter (P<0.006). Likewise, compared to the control group, body weight significantly decreased in the *ND* groups when caffeine was consumed alone and in combination with exercise training (P<0.006 and P<0.01, respectively, Fig. 1E).

In addition, the results demonstrated that exercise, caffeine, and their combination had a statistically significant effect on food intake (P<0.001). In addition, the interaction effect between exercise and caffeine was found to be significant (P<0.001). Regardless of diet or caffeine intake, exercise training reduced food intake significantly compared to the sedentary group (P<0.001). Similarly, caffeine consumption decreased food intake by 10% relative to the placebo group (P<0.001).

Compared to the control group, caffeine consumption, exercise training, and the combination of the two reduced food intake following *HFD* feeding (P<0.001). Caffeine consumption with or without exercise training reduced food intake relative to the exercise training group (P<0.001). Similarly, all *ND* interventions decreased food intake relative to the control group (P<0.001) In addition, caffeine consumption, alone or in

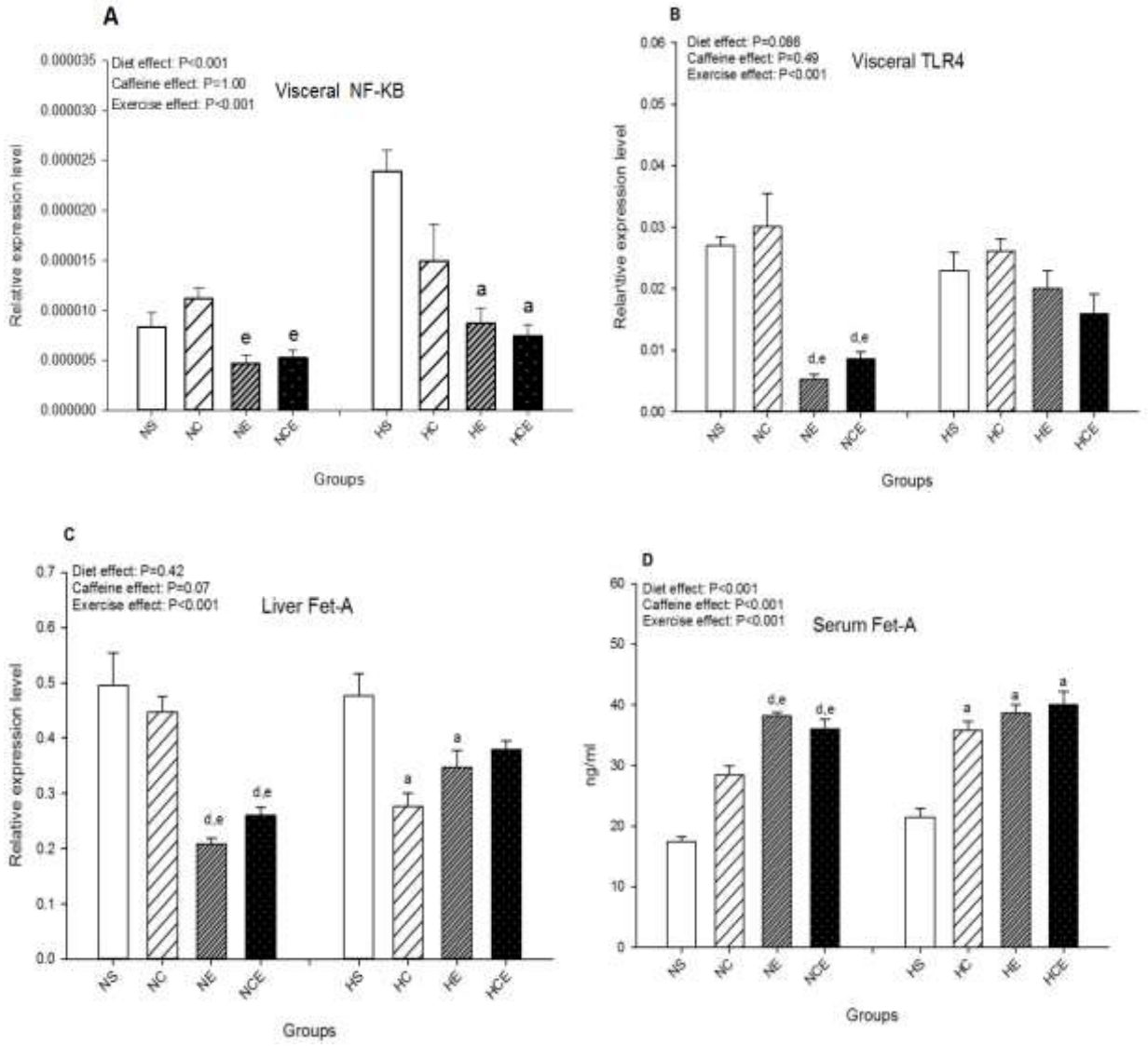

FIGURE 2. Visceral adipose tissue gene expressions, Liver Fet-A gene expression, and serum Fet-A, Values are presented as mean ± SEM in each column, n=10/group. NS: Normal Sedentary, NC: Normal Caffeine, NE: Normal Endurance, NCE: Normal Caffeine Endurance, HS: High Fat diet Sedentary, HC: High-fat diet Caffeine, HE: High-fat diet Endurance, and HCE: High-fat diet Caffeine Endurance.

conjunction with exercise, significantly decreased food intake compared to the control group (P<0.001, Fig. 1F).

The effect of caffeine and exercise training on expression of genes NF-KB and TLR4 in visceral adipose tissue

The outcomes have also shown that diet and exercise significantly affected the expression of the *NF-KB* gene in visceral adipose tissue (both P<0.001). Additionally, it was found that the effects of diet and exercise and diet and caffeine interactions were both statistically significant (P<0.034 and P<0.026, respectively). In addition, exercise training downregulated (vs. sedentary) and *HFD* increased *NF-KB* gene expression (vs. *ND*) regardless of caffeine supplementation (P<0.001).

Regarding differences within groups, in response to exercise training with and without caffeine intake after consuming *HFD*, *NF-KB* gene expression was downregulated by 63% (P<0.001) and 68% (P<0.001), respectively, in comparison to the sedentary control group. However, caffeine consumption, exercise training, and the combination thereof had no significant effects on the *NF-KB* gene expression in the *ND* regime, in contrast to the sedentary control group (P>0.064). In addition, compared to caffeine consumption alone, the expression levels of the *NF-KB* gene were lower in the exercise training groups with and without caffeine consumption (P<0.004 and P < 0.009, respectively, Fig. 2A).

Moreover, the results demonstrated that exercise's major influence on *TLR4* gene expression was considerable (P<0.001). Additionally, we also found a highly significant interaction (P<0.001) between diet and exercise on *TLR4* gene expression in visceral adipose tissue. Exercise training significantly increased *TLR4* gene expression by 53% compared to sedentary rats, regardless of *HFD* or caffeine consumption (P<0.001).

Taking into account variations between groups, there were no discernible differences between groups following *HFD* feeding (P>0.05). In the *ND* regimen, however, exercise training with and without caffeine consumption significantly reduced the expression of the *TLR4* gene by 80% and 68%, respectively, in comparison to the sedentary control group (P<0.001). In comparison to the mono-caffeine consumption, it was likewise lower in the exercise training groups with and without caffeine consumption (P<0.001, Fig. 2B).

The effect of caffeine and exercise training on the expression of gene Fet-A in Liver tissue and serum Fet-A

According to Fig. 2C, exercise training had a significant main effect on the expression of the *Fet-A* gene in the liver (P<0.001). Exercise and diet, as well as exercise and caffeine, had significant interaction effects (P<0.001). However, neither the effects of diet or caffeine nor the interaction between diet and caffeine were found to be statistically significant (P>0.05). Regardless of caffeine consumption or diet habits, exercise training dramatically reduced *Fet-A* gene expression by 92% when compared to the sedentary group (P<0.001). Although caffeine consumption resulted in a 10% reduction in *Fet-A* gene expression, this reduction was not statistically significant (P>0.08).

Taking into account variations between groups, compared to the sedentary control group, exercise training, and caffeine consumption in *HFD* groups dramatically reduced the expression of the *Fet-A* gene (P<0.043, P<0.001, respectively). However, when compared to the sedentary control group, there was no discernible difference in the combination of exercise training and caffeine consumption effects on the expression of the *Fet-A* gene (P>0.08). Additionally, compared to the sedentary control and the mono-caffeine consumption groups in the *ND* regimen, exercise training with and without caffeine consumption significantly downregulated the *Fet-A* gene (P<0.005, Fig. 2C).

Data revealed that diet, exercise, and caffeine all had a substantial main influence on serum *Fet-A* (P<0.001). Additionally, it was found that the interaction impact between diet and caffeine was significant (P<0.001). The *HFD* significantly increased serum *Fet-A* compared to the *ND* (P<0.001), regardless of exercise training or caffeine consumption, and this rise was 36% greater in response to exercise training compared to the sedentary group (P<0.001). Likewise, caffeine intake significantly raised serum *Fet-A* by 21% compared to the placebo group (P<0.001).

Regarding differences within groups, caffeine use, exercise training, and their combination all elevated serum *Fet-A* levels in the *HFD* regimen compared to the sedentary group (P<0.001). Similarly, in the ND regimen, exercise training, caffeine, and the combination of the two significantly increased serum *Fet-A* compared to the sedentary group (P<0.001). Additionally, exercise training with and without caffeine consumption significantly enhanced *Fet-A* compared to caffeine consumption alone (P<0.001, Fig. 2D).

Variables correlation

Serum irisin levels were not linked with the expression of *UCP1, FNDC5, TLR4, PGC-1α,* and *Fet-A* genes, according to Pearson correlation (Table 2). Additionally, the expression of the *FNDC5* gene in skeletal muscle showed a high positive correlation with *PGC-1α* (r = 0.634, P< 0.001), a moderate correlation with serum *Fet-A* (r = 0.421, P = 0.037), and a negative correlation with *NF-kb* (r = -0.384, P< 0.001). In addition, the subcutaneous *UCP1* gene showed a positive connection with serum *Fet-A* (r = 0.529, P<0.001) and a negative correlation with *TLR4* (r = -0.411, P<0.001) and *NF-KB* (r = -408, P<0.014). The skeletal muscle *PGC-1α* gene had a substantial negative connection with the adipose tissue inflammatory gene, *NF-kb* (r = -0.322, P< 0.006) (Table 2).

Discussion

Caffeine, a key component in coffee, is commonly used to enhance physical performance, increase resting energy expenditure, and improve fat and carbohydrate metabolism (Van Schaik et al., 2021). The study's significant finding is that caffeine increased *UCP1* gene expression in white adipose tissue by 56%. Notably, exercise training alone boosted *UCP1* gene expression by

TABLE 2. Simple correlation (Pearson r) between data variables.

	UCP1	FNDC5	PGC-1α	NF-kb	TLR4	Serum irisin	Serum Fet-A
Fet-A	–0.19	–0.136	–0.226	0.325*	0.478*	0.054	–0.519**
UCP1		0.267	0.06	–0.348*	–0.403**	–0.026	0.509**
FNDC5			0.616**	–0.438**	–0.238	0.254	0.316*
PGC-1α				–0.376*	–0.185	0.150	0.163
NF-kb					0.301*	0.017	–0.370*
TLR4						0.004	0.419**

r values significant set at **P < 0.01, *P < 0.05.
Serum irisin levels did not correlate with the expression of the *UCP1*, *FNDC5*, *TLR4*, *PGC-1α*, and *Fet-A* genes. Pearson correlation analysis revealed that the *FNDC5* gene in skeletal muscle showed a strong positive correlation with *PGC-1α*, a moderate correlation with serum *Fet-A*, and a negative correlation with *NF-kB*. Additionally, subcutaneous *UCP1* gene expression positively correlated with serum *Fet-A* and negatively with *TLR4* and *NF-KB*. The *PGC-1α* gene in skeletal muscle was significantly negatively correlated with the adipose tissue inflammatory gene NF-kB. Values are presented as mean ± SEM in each column, n=10/group. **NS**: Normal Sedentary, **NC**: Normal Caffeine, **NE**: Normal Endurance, **NCE**: Normal Caffeine Endurance, **HS**: High Fat diet Sedentary, **HC**: High-fat diet Caffeine, **HE**: High-fat diet Endurance and **HCE**: High-fat diet Caffeine Endurance.

366%, irrespective of dietary differences. Interestingly, the combination of caffeine and exercise training resulted in an even stronger increase in *UCP1* gene expression.

Caffeine has been shown to upregulate *UCP1* in obese mice, aligning with our findings (Kogure et al., 2002). It was found to increase the *UCP1* gene expression by 20%. Contrary to our results, some studies reported that exercise training did not alter *UCP1* mRNA levels in subcutaneous adipose tissue, possibly due to the lack of *PGC-1α* induction. Exercise-induced IL-6 is considered crucial for enhancing subcutaneous *UCP1* expression, as demonstrated in IL-6-deficient mice, where exercise training had no significant effect on *UCP1*. Caffeine inhibits phosphodiesterase, raising intracellular cAMP levels, which improves *UCP1* function. It may also influence mitochondrial biogenesis by increasing *PGC-1α* through elevated Ca2+ levels (Yamada et al., 2022). Additionally, caffeine, as an A_{1A} receptor antagonist, promotes brown adipose tissue thermogenesis and oxygen consumption in obese individuals, as activating A1A receptors inhibits thermogenesis and reduces energy expenditure (Tupone et al., 2013).

Irisin, as an exercise-induced myokine, significantly contributes to energy expenditure through the browning of adipocytes (Boström et al., 2012). In this study, we found that drinking caffeine with or without exercise training did not have a big effect on serum irisin levels during the *HFD* regimen. However, serum irisin levels went up during the *ND* regimen compared to the exercise training and control groups. It has been reported that mice with *HFD* have lower irisin levels (Lu et al., 2016). There is debate regarding how exercise training affects serum irisin. In contrast to this work, Lu et al. (2016) showed that eight weeks of swimming exercise with *HFD* or *ND* significantly increased serum irisin levels in male Wistar rats (Lu et al., 2016).

Exercise training is reported to increase serum irisin levels more during *ND* than *HFD* feeding (Lu et al., 2016). It has also been suggested that the reduced levels of irisin in the blood of individuals with prediabetes who exercise may be associated with an increased rate of glucose metabolism (Norheim et al., 2014). In a different study, exercise training was found to significantly raise serum irisin levels, which were inversely linked to visceral adiposity (Miyamoto-Mikami et al., 2015). Through the upregulation of *PGC-1α*, exercise training may raise irisin concentration (Boström et al., 2012), most likely. It has been noted that high-intensity interval training (> 80% VO2max) elicits a higher *PGC-1α* response than low-intensity exercise (Egan et al., 2010). The increase in *PGC-1α* mRNA expression after high-intensity exercise (Egan et al., 2010; Stepto et al., 2012) may be the reason why the serum concentration of irisin was considerably higher after high-intensity exercise compared to low-intensity exercise (Tsuchiya et al., 2014). Additionally, it has been claimed that caffeine consumption has no appreciable effect on irisin levels (Peter et al., 2014). However, in this study, we did not find any significant effects of exercise training on serum irisin which may relate to the lower exercise intensity. Another explanation for these differences in irisin responses to chronic exercise training, according to a previous study, maybe the use of different peptide se-

quences (Raschke et al., 2013), as previously explained (Norheim et al., 2014).

An increase in the irisin precursor protein *FNDC5* leads to a rise in *UCP-1* mRNA gene expression. Our findings showed that an *HFD* significantly reduced *FNDC5* gene expression, whereas caffeine and exercise training significantly increased it. However, these changes did not correlate with serum irisin or *UCP1* gene expression in adipose tissue. Previous studies have shown that aerobic exercise for 10 weeks raises skeletal muscle *FNDC5* mRNA levels and plasma irisin levels, though exercise training had no effect on plasma irisin and *FNDC5* in the skeletal muscle of obese rats (Boström et al., 2012). High-intensity interval training (HIIT) performed for 20 days also increased *FNDC5* mRNA in skeletal muscle (Timmons et al., 2012). The differences in results could be due to variations in exercise intensity, mode, and duration. In this study, despite the increase in *FNDC5* due to caffeine consumption and exercise training, circulating irisin levels did not significantly change. This suggests that increased *FNDC5* levels were not linked with higher circulating irisin levels. It has been suggested that white adipose tissue also secretes *FNDC5*, implying that during *HFD* regimens, exercise training or caffeine consumption may prompt adipose tissue to contribute to circulating irisin levels.

Skeletal muscles play a crucial role in fat and carbohydrate metabolism, with the increase in *PGC-1α* being essential for muscle adaptation to exercise (Egan and Zierath 2013). *PGC-1α* initiates cell signaling that impacts inflammation, browning of white adipose tissue, and energy metabolism. This study found that an *HFD* significantly reduced *PGC-1α* gene expression in skeletal muscle compared to a *ND*. However, exercise, caffeine, or their combination significantly increased *PGC-1α* expression under both diet regimens. Supporting this, Granata et al. (2020) showed that high-intensity interval training raised *PGC-1α* and p53 protein levels in resting skeletal muscle (Granata et al., 2020). Exercise training likely activates *PGC-1α* through increased intracellular Ca2+ and AMPK activation (Ojuka et al., 2003). Both exercise and caffeine enhance *PGC-1α* and *FNDC5* in skeletal muscle, suggesting a compensatory mechanism to counteract HFD-induced metabolic stress. Future research should investigate the long-term effects of *HFD* and caffeine consumption on these mechanisms.

Previous research indicates that caffeine can have both anti-inflammatory (Muqaku et al., 2016) and pro-inflammatory effects (Tunc et al., 2013). In our study, exercise training significantly reduced the expression of the *Fet-A* gene in the liver. Both caffeine consumption and exercise training also lowered *Fet-A* gene levels in the liver compared to sedentary controls, despite *HFD* feeding. Diet-induced obesity has been noted to increase *Fet-A* mRNA expression in the liver, and similarly, *HFD* increased serum *Fet-A* levels in obese rats, a response enhanced by exercise and caffeine (Jung et al., 2013).

This study found a positive correlation between *UCP1* and *FNDC5* gene expression and both blood and liver *Fet-A* levels. Adipose tissue is identified as the primary source of serum *Fet-A* (Chatterjee et al., 2013), with visceral adipose tissue secreting more *Fet-A* than subcutaneous adipose tissue, although this secretion decreases with fasting or exercise (Pérez-Sotelo et al., 2017). Long-term exercise training and weight loss have been shown to increase *Fet-A* levels, correlating with increased maximal oxygen consumption (Blumenthal et al., 2017). However, short-term exercise training has been linked to decreased circulating *Fet-A* levels, and aerobic exercise without weight loss does not affect *Fet-A* levels (Malin et al., 2013). Additionally, 4-week high-intensity or moderate-intensity exercise training did not alter serum *Fet-A* levels. The lack of caffeine's impact on circulating *Fet-A* suggests an unclear underlying mechanism. In some studies, increased circulating *Fet-A* was independent of improved glucose metabolism (Blumenthal et al., 2017). Responses to exercise and caffeine may differ between healthy and unhealthy subjects, necessitating further research (Malin et al., 2013).

A high-fat diet increases the production of *Fet-A* by enhancing *NF-kB's* binding to its gene promoter (Dasgupta et al., 2010; Lin et al., 1998). Fet-A serves as an endogenous ligand for *TLR4* (Pal et al., 2012). Exercise significantly lowered NF-kB gene expression in visceral adipose tissue, whereas *HFD* significantly increased it. Caffeine alone did not significantly impact *NF-kB* gene expression after either *HFD* or *ND* feeding. However, exercise, both with and without caffeine, reduced *NF-kB* gene expression even under *HFD* conditions. Similarly, exercise reduced *TLR4* gene expression regardless of diet or caffeine intake. Caffeine and exercise did not affect *TLR4* during *HFD* but reduced its expression following *ND* feeding.

Caffeine consumption reduces *TLR4* activation,

which in turn lowers inflammation and cytokine production (Kawasaki and Kawai 2014; Vargas-Pozada et al., 2022). Higher caffeine intake has been shown to significantly reduce inflammation, as seen in studies where eight cups of coffee daily for twelve weeks had a greater effect than four cups (Kempf et al., 2010). While research on the combined effects of exercise and caffeine on inflammatory genes in adipose tissue is limited, caffeine is known to prevent increases in liver *TLR4* and *NF-κB* protein levels, suggesting its anti-inflammatory properties.

Exercise training mitigates the interaction between free fatty acids (FFAs) and *Fet-A*, a crucial factor in reducing inflammation (Lee et al., 2017). *Fet-A* facilitates inflammation by binding to fatty acids and presenting them to *TLR4*, which then enhances pro-inflammatory cytokine release via the *TLR4-NF-κB* pathway (Pal et al., 2012). Additionally, *Fet-A* attracts macrophages to adipose tissue, shifting them from an anti-inflammatory to a pro-inflammatory state. Exercise improves adipose tissue oxygenation and encourages the anti-inflammatory polarization of macrophages, reducing inflammation even with a high-fat or normal diet.

This study has a few limitations. First, we lack information on two genes, *FNDC5* and *PGC-1*, in adipose tissue that could have an impact on how white adipose tissue turns brown. Second, we only examine these parameters at the transcriptional level. Nevertheless, examining these factors at the protein level is required to corroborate the browning effects of coffee and exercise, since gene expression may not translate to protein expression. Third, as we only examined the short-term effects of caffeine consumption and exercise training, more research is needed to examine the long-term effects of this combination on inter-organ crosstalk.

Conclusion

The results showed that endurance exercise training caused a significant increase in the expression of *FNDC 5* and *Fet-1* genes and a significant decrease in *TLR 4* compared to caffeine. Also, the interaction of endurance exercise training with caffeine caused a further increase in *UCP-1* gene expression. Compared to the endurance exercise and caffeine groups, there was no significant difference between the effects of endurance exercise and caffeine on *PGC-1a* and *NF-kb* gene expression. In summary, our study highlights the significant effects of exercise training, caffeine supplementation, and their combination on the expression of genes in critical metabolic tissues such as skeletal muscle, liver, and adipose tissue. We found that an *HFD* reduced the expression of *PGC-1α* and *FNDC5* genes in skeletal muscle, but this was reversed by exercise and caffeine. Additionally, exercise and caffeine promoted the browning of white adipose tissue by increasing *UCP1* gene expression. These adaptive responses also helped reduce inflammation in visceral adipose tissue and the liver. Future research should explore the long-term effects of caffeine, exercise, and their combination on inter-organ communication.

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Conflict of interest

Nothing declared.

Ethics approval

The studies involving animals were reviewed and approved by Research Ethics Committee at the University of Kurdistan (Approval ID: IR.UOK.REC.1397.024).

References

Blumenthal J B, Gitterman A, Ryan A S, Prior S J. Effects of exercise training and weight loss on plasma Fetuin-a levels and insulin sensitivity in overweight older men. J Diabetes Res 2017; 2017. https://doi.org/10.1155/2017/1492581

Boström P, Wu J, Jedrychowski M P, Korde A, Ye L, Lo J C, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481: 463-468. https://doi.org/10.1038/nature10777

Chatterjee P, Seal S, Mukherjee S, Kundu R, Mukherjee S, Ray S, et al. Adipocyte fetuin-A contributes to macrophage migration into adipose tissue and polarization of macrophages. Journal of Biological Chemistry 2013; 288: 28324-28330. https://doi.org/10.1074/jbc.C113.495473

Clark K S, Coleman C, Shelton R, Heemstra L A, Novak C M. Caffeine enhances activity thermogenesis and energy expenditure in rats. Clin Exp Pharmacol Physiol 2019; 46: 475-482. https://doi.org/10.1111/1440-1681.13065

Council N R. Guide for the care and use of laboratory animals: National Academies Press, 2010.

Dasgupta S, Bhattacharya S, Biswas A, Majumdar S S, Mukhopadhyay S, Ray S, et al. NF-κB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. Biochemical journal 2010; 429: 451-462. https://doi.org/10.1042/BJ20100330

Egan B, Carson B P, Garcia-Roves P M, Chibalin A V, Sarsfield F M, Barron N, et al. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor γ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. The Journal of physiology 2010; 588: 1779-1790. https://doi.org/10.1113/jphysiol.2010.188011

Egan B, Zierath J R. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell metabolism 2013; 17: 162-184. https://doi.org/10.1016/j.cmet.2012.12.012

Granata C, Oliveira R S, Little J P, Bishop D J. Forty high-intensity interval training sessions blunt exercise-induced changes in the nuclear protein content of PGC-1α and p53 in human skeletal muscle. Am J Physiol Endocrinol Metab 2020; 318: 224-236. https://doi.org/10.1152/ajpendo.00233.2019

Høydal M A, Wisløff U, Kemi O J, Ellingsen Ø. Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. Eur J Prev Cardiol 2007; 14: 753-760. https://doi.org/10.1097/HJR.0b013e3281eacef1

Jung T W, Youn B-S, Choi H Y, Lee S Y, Hong H C, Yang S J, et al. Salsalate and adiponectin ameliorate hepatic steatosis by inhibition of the hepatokine fetuin-A. Biochemical pharmacology 2013; 86: 960-969. https://doi.org/10.1016/j.bcp.2013.07.034

Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Frontiers in immunology 2014: 461. https://doi.org/10.3389/fimmu.2014.00461

Kempf K, Herder C, Erlund I, Kolb H, Martin S, Carstensen M, et al. Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial. Am J Clin Nutr 2010; 91: 950-957. https://doi.org/10.3945/ajcn.2009.28548

Kobayashi-Hattori K, Mogi A, Matsumoto Y, Takita T. Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. Biosci Biotechnol Biochem 2005; 69: 2219-2223. https://doi.org/10.1271/bbb.69.2219

Kogure A, Sakane N, Takakura Y, Umekawa T, Yoshioka K, Nishino H, et al. Effects of caffeine on the uncoupling protein family in obese yellow KK mice. Clin Exp Pharmacol Physiol 2002; 29: 391-394. https://doi.org/10.1046/j.1440-1681.2002.03675.x

Lee S, Norheim F, Gulseth H L, Langleite T M, Kolnes K J, Tangen D S, et al. Interaction between plasma fetuin-A and free fatty acids predicts changes in insulin sensitivity in response to long-term exercise. Physiological reports 2017; 5: e13183. https://doi.org/10.14814/phy2.13183

Lin X, Braymer H, Bray G, York D. Differential expression of insulin receptor tyrosine kinase inhibitor (fetuin) gene in a model of diet-induced obesity. Life sciences 1998; 63: 145-153. https://doi.org/10.1016/S0024-3205(98)00250-1

Lu Y, Li H, Shen S-W, Shen Z-H, Xu M, Yang C-J, et al. Swimming exercise increases serum irisin level and reduces body fat mass in high-fat-diet fed Wistar rats. Lipids Health Dis 2016; 15: 1-8. https://doi.org/10.1186/s12944-016-0263-y

Malin S K, Mulya A, Fealy C E, Haus J M, Pagadala M R, Scelsi A R, et al. Fetuin-A is linked to improved glucose tolerance after short-term exercise training in nonalcoholic fatty liver disease. J Appl Physiol 2013; 115: 988-994. https://doi.org/10.1152/japplphysiol.00237.2013

Miyamoto-Mikami E, Sato K, Kurihara T, Hasegawa N, Fujie S, Fujita S, et al. Endurance training-induced increase in circulating irisin levels is associated with reduction of abdominal visceral fat in middle-aged and older adults. PloS one 2015; 10: e0120354. https://doi.org/10.1371/journal.pone.0120354

Muqaku B, Tahir A, Klepeisz P, Bileck A, Kreutz D, Mayer R L, et al. Coffee consumption modulates inflammatory processes in an individual fashion. Mol Nutr Food Res 2016; 60: 2529-2541. https://doi.org/10.1002/mnfr.201600328

Natella F, Scaccini C. Role of coffee in modulation of diabetes risk. Nutrition reviews 2012; 70: 207-217. https://doi.org/10.1111/j.1753-4887.2012.00470.x

Norheim F, Langleite T M, Hjorth M, Holen T, Kielland A, Stadheim H K, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FASEB J 2014; 281: 739-749. https://doi.org/10.1111/febs.12619

Ojuka E O, Jones T E, Han D H, Chen M, Holloszy J O. Raising Ca2+ in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. FASEB J 2003; 17: 675-681. https://doi.org/10.1096/fj.02-0951com

Paiva C, Beserra B, Reis C, Dorea J, Da Costa T, Amato A. Consumption of coffee or caffeine and serum concentration of inflammatory markers: A systematic review. Crit Rev Food Sci Nutr 2019; 59: 652-663. https://doi.org/10.1080/10408398.2017.1386159

Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nature medicine 2012; 18: 1279-1285. https://doi.org/10.1038/nm.2851

Pérez-Sotelo D, Roca-Rivada A, Larrosa-García M, Castelao C, Baamonde I, Baltar J, et al. Visceral and subcutaneous adipose tissue express and secrete functional alpha2hsglycoprotein (fetuin a) especially in obesity. Endocrine 2017; 55: 435-446. https://doi.org/10.1007/s12020-016-1132-1

Peter P R, Park K H, Huh J Y, Wedick N M, Mantzoros C S. Circulating irisin levels are not affected by coffee intake: a randomized controlled trial. PLoS One 2014; 9: e94463. https://doi.org/10.1371/journal.pone.0094463

Porter C, Herndon D N, Chondronikola M, Chao T, Annamalai P, Bhattarai N, et al. Human and mouse brown adipose tissue mitochondria have comparable UCP1 function. Cell metabolism 2016; 24: 246-255. https://doi.org/10.1016/j.cmet.2016.07.004

Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, et al. Evidence against a beneficial effect of irisin in humans. PloS one 2013; 8: e73680. https://doi.org/10.1371/journal.pone.0073680

Roca-Rivada A, Castelao C, Senin L L, Landrove M O, Baltar J, Crujeiras A B, et al. FNDC5/irisin is not only a myokine but also an adipokine. PloS one 2013; 8: e60563. https://doi.org/10.1371/journal.pone.0060563

Stepto N K, Benziane B, Wadley G D, Chibalin A V, Canny B J, Eynon N, et al. Short-term intensified cycle training alters acute and chronic responses of PGC1α and Cytochrome C oxidase IV to exercise in human skeletal muscle. PLOS one 2012; 7: e53080. https://doi.org/10.1371/journal.pone.0053080

Timmons J A, Baar K, Davidsen P K, Atherton P J. Is irisin a human exercise gene? Nature 2012; 488: 9-10. https://doi.org/10.1038/nature11364

Tsuchiya Y, Ando D, Goto K, Kiuchi M, Yamakita M, Koyama K. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. Tohoku J Exp Med 2014; 233: 135-140. https://doi.org/10.1620/tjem.233.135

Tunc T, Aydemir G, Karaoglu A, Cekmez F, Kul M, Aydinoz S, et al. Toll-like receptor levels and caffeine responsiveness in rat pups during perinatal period. Regulatory peptides 2013; 182: 41-44. https://doi.org/10.1016/j.regpep.2012.12.016

Tupone D, Madden C J, Morrison S F. Central activation of the A1 adenosine receptor (A1AR) induces a hypothermic, torpor-like state in the rat. Journal of Neuroscience 2013; 33: 14512-14525. https://doi.org/10.1523/JNEUROSCI.1980-13.2013

Van Schaik L, Kettle C, Green R, Irving H R, Rathner J A. Effects of Caffeine on Brown Adipose Tissue Thermogenesis and Metabolic Homeostasis: A Review. Frontiers in Neuroscience 2021: 54. https://doi.org/10.3389/fnins.2021.621356

Vargas-Pozada E E, Ramos-Tovar E, Rodriguez-Callejas J D, Cardoso-Lezama I, Galindo-Gómez S, Talamás-Lara D, et al. Caffeine inhibits NLRP3 inflammasome activation by downregulating TLR4/MAPK/NF-κB signaling pathway in an experimental NASH model. Int J Mol Sci 2022; 23: 9954. https://doi.org/10.3390/ijms23179954

Vettor R, Di Vincenzo A, Maffei P, Rossato M. Regulation of energy intake and mechanisms of metabolic adaptation or maladaptation after caloric restriction. Rev Endocr Metab Disord 2020; 21: 399-409. https://doi.org/10.1007/s11154-020-09565-6

Villarroya F, Cereijo R, Gavaldà-Navarro A, Villarroya J, Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. J Intern Med 2018; 284: 492-504. https://doi.org/10.1111/joim.12803

Yamada A K, Pimentel G D, Pickering C, Cordeiro A V, Silva V R. Effect of Caffeine on mitochondrial biogenesis in the skeletal muscle-a narrative review. Clinical nutrition ESPEN 2022. https://doi.org/10.1016/j.clnesp.2022.09.001

Zhang Q, Ye R, Zhang Y-Y, Fan C-C, Wang J, Wang S, et al. Brown adipose tissue and novel management strategies for polycystic ovary syndrome therapy. Frontiers in Endocrinology 2022; 13: 847249. https://doi.org/10.3389/fendo.2022.847249