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Effects of oral non-toxic subchronic ochratoxin A exposure on brown adipose tissue function in female mice

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ABSTRACT

Ochratoxin A (OTA) is a widespread foodborne mycotoxin known to disrupt metabolic pathways; however, its effects on brown adipose tissue (BAT) remain unclear. This study evaluated the impact of non-toxic subchronic OTA exposure on BAT function in mice. Sixteen female mice were randomly assigned to control (vehicle) or OTA-treated groups (n=8 each), receiving OTA (0.25 mg/kg body weight) by oral gavage for four weeks. BAT function was assessed in interscapular BAT by measuring UCP-1 and PGC-1α levels, and serum irisin was measured pre- and post-intervention. OTA exposure did not affect body weight or UCP-1 levels. However, PGC-1α concentrations were significantly increased, and circulating irisin remained higher in OTA-exposed animals despite an overall decline in both groups. These findings indicate that low-dose OTA exposure does not impair BAT thermogenic capacity but modulates mitochondrial regulatory pathways, suggesting an early adaptive metabolic response.

Keywords: ochratoxin A, brown adipose tissue, irisin, PGC-1α, UCP-1

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INTRODUCTION

Adipose tissue in mammals is classified into three principal types: white adipose tissue (WAT), brown adipose tissue (BAT), and beige adipose tissue. WAT primarily functions in energy storage in the form of triglycerides and contributes to metabolic homeostasis through its endocrine activity. In contrast, BAT dissipates energy as heat via action of the thermogenic protein uncoupling protein-1 (UCP-1)[1,2]. Beige adipose tissue exhibits functional characteristics of both WAT and BAT. In terms of similarity to WAT, it participates in energy storage and regulates physiological processes through the secretion of S-100 calcium-binding protein B, leptin, and other adipokines. At the same time, it also possesses the capacity to dissipate energy as heat, akin to BAT. However, compared to BAT, the thermogenic capacity of beige adipose tissue is considerably lower[3]. The presence of BAT has been observed throughout the lifespan in rodents. In humans, although BAT is known to be present in neonates and young children, it was historically not associated with adult individuals in terms of its presence or metabolic activity[4]. Studies conducted in the early 2000s led to the identification of metabolically active BAT in adult humans, thereby accelerating research focused on this tissue[5]. Preclinical and clinical studies have demonstrated that activation of BAT and/or browning of WAT is associated with increased energy expenditure. It is believed that this increase in energy expenditure may exert beneficial effects on major contemporary health problems, including obesity, disorders of glucose metabolism, dyslipidemia, and hypertension[6,7].

Existing pharmacological interventions have been shown to effectively stimulate BAT activation[8]. However, the doses of β-adrenergic receptor agonists such as Mirabegron required to achieve a significant increase in BAT activity have been reported to exert serious adverse effects on cardiovascular health[7,9]. Nutritional supplements, including vitamin A and Nicotinamide Riboside, have also been suggested to promote BAT activation. Nevertheless, the doses required to produce this effect may lead to adverse consequences for human health[10]. Furthermore, findings demonstrating efficacy *in vitro* have not yet been consistently replicated in *in vivo* studies[11]. Moreover, the use of pharmacological agents and dietary supplements for the prevention and treatment of highly prevalent conditions such as obesity and metabolic syndrome may impose substantial costs on healthcare systems, thereby limiting the practicality and sustainability of these approaches.

A series of studies conducted over the past two decades have demonstrated that the thermogenic activity of BAT can be modulated through dietary interventions[6,12]. Dietary bioactive compounds that have been shown in

clinical studies to increase the amount and activity of BAT include capsaicin, piperine, gingerol, shogaol, diallyl di- and trisulfide, allyl isothiocyanate, catechins, caffeine, flavonoids, and stilbenes[13-15]. Numerous recent review articles emphasize that diets rich in these bioactive compounds may represent a safer, more effective, and more cost-efficient strategy for the treatment of obesity and obesity-associated metabolic disorders compared with currently used therapeutic approaches[13]. Among the dietary sources of bioactive compounds that are effective in increasing the amount and activity of BAT are pepper (capsaicin and piperine), ginger (gingerol and shogaol), mustard (allyl isothiocyanate), black and green tea (catechins), coffee (caffeine), and grapes as well as other fresh or dried fruits and vegetables (flavonoids and stilbenes)[13]. Despite their content of bioactive compounds beneficial for BAT, these foods are also recognized as important dietary sources of ochratoxin A (OTA), one of the most prevalent mycotoxins in human exposure[16,17].

Mycotoxins comprise a diverse group of nearly 500 toxic secondary metabolites produced by filamentous fungi, primarily species of *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps*, and *Alternaria*. These fungi are capable of colonizing herbs, spices, and various agricultural commodities, leading to the accumulation of toxic metabolites that may pose significant risks to both human and animal health. Mycotoxin production is strongly influenced by environmental factors, including humidity, temperature, and oxygen availability. Among the mycotoxins most frequently detected in herbs, spices, and dietary supplements are aflatoxins, ochratoxin A, and fumonisins[18]. Climatic changes, including increases in temperature and humidity, can promote the growth of fungal pathogens and consequently increase the risk of mycotoxin contamination in food products[19]. In addition, studies conducted worldwide indicate that human exposure to mycotoxins may be further amplified by the globalization of the food market[20].

OTA, which is mainly produced by molds belonging to the *Aspergillus* and *Penicillium* genera, has been reported to exhibit teratogenic, immunotoxic, carcinogenic, genotoxic, neurotoxic, and hepatotoxic effects[21]. The acute toxicity of OTA varies considerably among animal species, with reported LD_{50} values ranging from 0.16 to 7.8 mg/kg in turkey poults and from groups that contained 22 to 58 mg/kg in rats and mice. Acute OTA exposure has been associated with severe histological abnormalities in multiple organs, including the intestine, kidney, spleen, and liver, as well as brain and cardiac abnormalities[22,23]. Due to its resistance to various food processing methods, including high-temperature treatments, minimizing dietary exposure remains the most effective strategy against OTA[17].

OTA toxicity is mediated through multiple cellular and molecular mechanisms. *In vivo* studies indicate that OTA accumulates in target tissues and undergoes biotransformation by cytochrome P450 enzymes to generate toxic metabolites. This process promotes the production of reactive oxygen species (ROS), leading to oxidative stress, disruption of cellular signaling pathways, and DNA damage[24]. Consequently, OTA exposure has been associated with numerous cellular disturbances, including oxidative and nitrosative stress, apoptosis, epigenetic modifications, inhibition of protein synthesis, cell cycle arrest, lipotoxicity, mitochondrial dysfunction with ATP depletion, autophagy, and epithelial–mesenchymal transition[23].

Although the toxicological effects of ochratoxin A have been extensively investigated in organs such as the kidney, liver, and immune system, considerably less attention has been given to its potential impact on adipose tissue. Emerging evidence suggests that OTA can interfere with adipocyte differentiation and lipid metabolism by disrupting key regulatory pathways and inducing oxidative stress. Given the critical role of adipose tissue in energy homeostasis and metabolic regulation, such alterations may have important metabolic consequences. However, the potential effects of OTA exposure on BAT activity and thermogenic function remain largely unexplored. Therefore, this study aimed to investigate the effects of non-toxic subchronic OTA exposure on BAT function.

METHODOLOGY

All animal procedures were conducted in accordance with the ARRIVE guidelines and complied with the European Union Directive 2010/63/EU for animal experiments. The study protocol was approved by the local animal ethics committee (permit number 60, approval date 11.10.2023). The experiments were conducted at an institutionally accredited animal research facility.

The research protocol is summarized in Figure 1. Blood samples were collected both prior to the initiation of the intervention and after its completion. Cold exposure was performed following completion of the intervention (a day after the final OTA dose). The body weights of the mice in both groups were measured at the onset of the intervention and twice weekly throughout the intervention period using a balance with a sensitivity of 0.01g[11]. OTA and the placebo agent were administered to the study and control groups, respectively, at the predetermined dose, and route throughout the 4-week intervention period. Euthanasia of the animals was considered the endpoint of the intervention and was performed following cold exposure. The procedure was followed

by excision of the interscapular BAT. Irisin quantification from the serum samples was performed within 30 days following sample collection. Similarly, UCP-1 and PGC-1α measurements were conducted within 30 days after the acquisition of brown adipose tissue. Until the analyses were performed, serum and tissue samples were stored at -80°C.

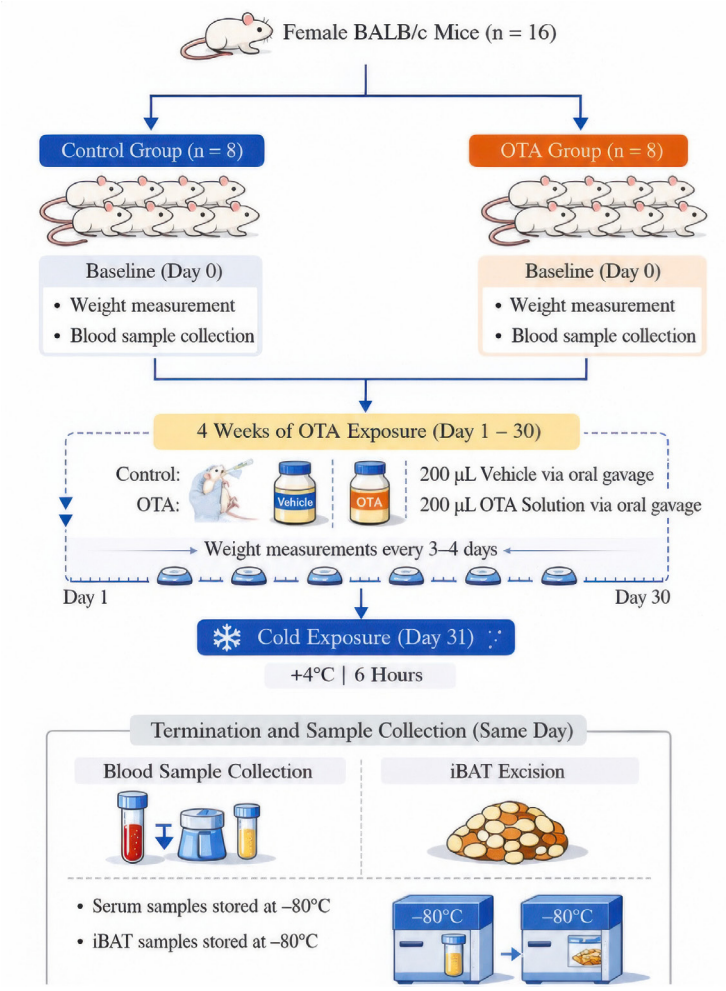


Figure 1. Experimental design. Sixteen female BALB/c mice were randomly assigned to control (n=8) and OTA-treated (n=8) groups. At baseline (Day 0), body weight was recorded, and blood samples were collected. Mice in the OTA group received OTA (0.25 mg/kg body weight) via oral gavage for 4 weeks, while control animals received an equivalent volume of vehicle. Body weight was monitored every 3–4 days throughout the exposure period. On Day 31, all animals were subjected to acute cold exposure (+4°C for 6 hours) to stimulate thermogenic activity. Animals were euthanized on the same day, and blood and interscapular brown adipose tissue (iBAT) samples were collected. Serum and tissue samples were stored at -80°C until further analysis.

Animals

The minimum sample size was calculated based on a study[25] demonstrating the statistically significant browning effect of irisin, effective on brown adipose tissue metabolism in mice (1.67 cm^3 ± 0.74 vs. 2.26 ± 0.31 cm^3), using the Power Analysis statistical software package, version 3.1 (SAS Institute, Cary, NC, USA). According to this calculation, with a 95% confidence interval, α=0.05, and 80% power, and assuming an effect size of 1.9, it was determined that 14 mice should be included in the study. Considering potential losses, the sample size was increased to 16 mice. This number was also selected in accordance with the 3R principles of refinement, reduction, and replacement of animals for research purposes, taking into account an *in vivo* study in which OTA levels in plasma and tissues, as well as certain general toxicity parameters (clinical examination and body weight), were evaluated[26].

The animals were obtained from the İstanbul Medipol University Medical Research Center (MEDİTAM) and were initially assessed for appearance, food and water intake, and natural behaviors. Animals approved upon delivery were allocated to the control and study groups using a block randomization method, with 8 mice per group by a person not involved in the study. Since the study was conducted exclusively with female mice and no covariates were defined among the animals, stratified randomization was not employed.

Since disease modeling was not required for this study, adult female BALB/c mice (≥10–12 weeks old, 20–25 g) were utilized. The choice of species and strain was made in accordance with the experimental protocol and established ethical principles for the use of laboratory animals. Previous research has documented the use of BALB/c mice in studies investigating BAT[27,28]. Similarly, BALB/c mice have been used in studies investigating OTA effects(26). The C57BL/6 strain, which is frequently employed in studies evaluating BAT activity[3,11,29], was not chosen for this study primarily because the experimental protocol did not require the induction of obesity[30].

All mice were housed under a 12-hour light/12-hour dark cycle and, unless otherwise specified by the protocol, at an ambient temperature of 21°C. Mice in both groups were exposed cold (+4°C) for 6 hours on the day of study termination, one day after the final gavage administration. Mice in the control and intervention groups were kept in separate cages (8 mice per cage). Throughout the study, the animals were provided *ad libitum* access to food and water. A standard pellet diet was supplied via the institution responsible for animal care. Sterilized Beta Chip® (200 g per cage) was used as a bedding material. As cage enrichment, one cardboard tube was placed in each cage and replaced daily.

OTA

OTA (purity 99.5 ± 0.5%, Romer Labs®) standard was obtained through a commercial supplier. The OTA dose and duration of administration were determined based on the literature. To reflect the dietary exposure, a non-toxic dose of OTA (0.25 mg/kg body weight) was used, and a 28-day intervention period was chosen to model subchronic exposure. This dose falls within the range used in previous subchronic *in vivo* studies and is considered relevant for investigating the biological effects of chronic low-level dietary OTA exposure[26,31].

OTA was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution containing 100 mg/mL OTA, which was stored at 4°C. This stock solution was subsequently diluted with sterile water (10 mM Tris, pH 8) to achieve the predetermined OTA doses. OTA and the vehicle solution (sterile water with 10 mM Tris, pH 8) were administered to the mice in the study group 28 times in the morning via gavage, at a volume of 200 µL per animal[32].

To ensure that the study accurately reflected oral OTA exposure, intraperitoneal and intravenous routes were not used. The use of OTA-contaminated food or water was also considered inappropriate, as housing multiple mice per cage would prevent consistent dosing for each animal. No analgesic agents were administered prior to gavage applications. The mice were firmly restrained by hand, and gavage needle used was a 1.5-inch, 20-gauge, curved stainless-steel needle with a 2.25 mm ball tip, provided by the research institution.

Biochemical analysis

Irisin analyses were performed using serum samples collected at the beginning and at the end of the study. The collected samples were processed in accordance with the protocol recommended by the species-specific enzyme-linked immunosorbent assay (ELISA) kit (Elabscience®, Wuhan, China; detection range: 78.13–5000pg/mL; sensitivity: 46.88pg/mL) manufacturer and kept at room temperature for an hour before centrifugation for 20 minutes at 1000×g at 2-8°C. The collected supernatant was stored at -80°C until analysis. The intra-assay and inter-assay coefficient of variation (CV) values were both <10%.

Quantitative measurements of UCP-1 and PGC-1α were performed on interscapular BAT obtained from the mice. Following euthanasia and blood collection, interscapular brown adipose tissue was harvested in accordance with protocols described in the literature[33,34]. Initially, a 70% ethanol solution was sprayed onto the dorsal surface of the mice. Subsequently, the incision steps described in the protocol published by Sharon Lim and colleagues were

followed, beginning with an incision along the dorsal midline extending in the cranial direction. Bilateral interscapular brown adipose tissue was carefully isolated, and the surrounding white adipose tissue was removed. The tissue samples were stored at -80°C until the date of analysis. BAT UCP-1 and PGC1-α concentrations were measured by mouse UCP-1(Uncoupling Protein 1, Mitochondrial) ELISA kit (Elabscience®, Wuhan, China; detection range: 0.16-10 ng/mL; sensitivity: 0.1 ng/mL) and mouse Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha ELISA kit (MyBioSource, San Diego, CA, USA; detection range: 0.156-10 ng/mL; sensitivity: <0.055 ng/mL). The intra-assay and inter-assay CV values were both <10% for UCP-1, whereas for PGC-1α the intra-assay CV was <10% and the inter-assay CV was <12%.

Statistical analysis

Statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) version 27 software. During data evaluation, quantitative variables were presented using descriptive statistics, including mean, standard deviation, median, minimum, and maximum values, while qualitative variables were expressed as frequencies and percentages. Normality of data distribution was assessed using the Shapiro–Wilk test and Box Plot graphics.

For variables not conforming to a normal distribution, comparisons between two independent groups were conducted using the Mann–Whitney U test; within-group comparisons were performed using the Wilcoxon Signed-Rank test; and within-group comparisons involving three or more repeated measurements were analyzed using the Friedman test. Associations between variables were evaluated using Spearman's correlation analysis, based on data distribution characteristics. All results were interpreted within a 95% confidence interval, and statistical significance was defined as $p<0.05$.

RESULTS and DISCUSSION

The study was conducted at the institutional research center between 01.12.2025 and 31.12.2025, with a total of 16 mice, 50% (n=8) allocated to the control group and 50% (n=8) to the OTA group. During the first week of the study, one mouse in the control group (C5) died for reasons unrelated to the study procedures and was excluded from further analyses.

The body weight measurements obtained at baseline and recorded longitudinally throughout the study for the control and OTA groups are presented in Table 1 and Figure 2 (a). There were no statistically significant differences in mean body weight between the OTA and control groups at study initiation, nor at any time point during the intervention period ($p>0.05$).

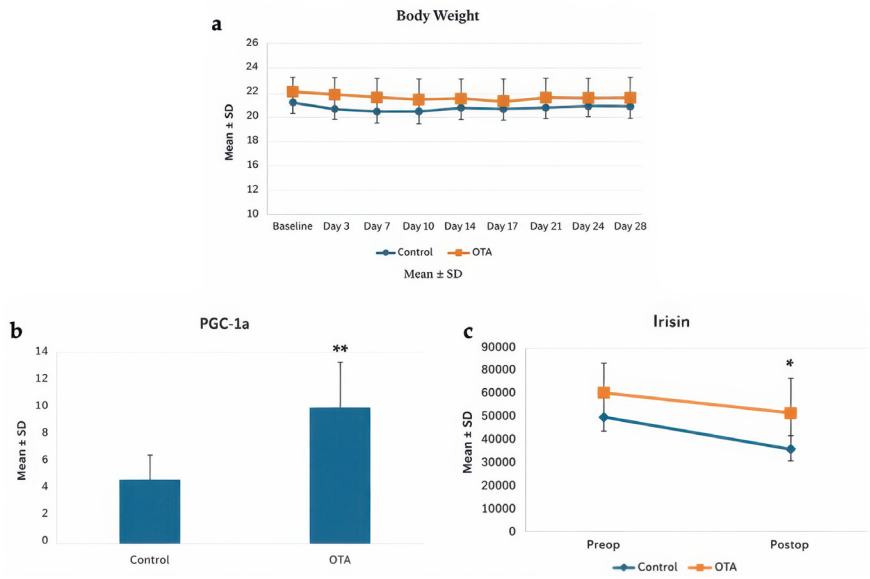


Figure 2. Effects of OTA exposure on body weight and thermogenesis-related biomarkers. Changes in body weight (g) from baseline to day 28 in the control and OTA groups (**a**), comparison of PGC-1α concentrations (ng/mL) between groups (**b**), and pre- and post-intervention serum irisin concentrations (pg/mL) in the control and OTA groups (**c**). Data are presented as mean ± SD. *p<0.05 and **p<0.01

Table 1. Body weight (g) comparison between OTA and control groups

Time Point	Total (n=15)	Control (n=7)	OTA (n=8)	p[a]
Baseline	21.85 ± 1.40	21.54 ± 1.37	22.16 ± 1.44	0.574
	21.7 (19.5–24.5)	21.6 (19.5–23.5)	22.3 (20.3–24.5)	
Day 3	21.40 ± 1.93	20.61 ± 1.82	22.09 ± 1.86	0.189
	21.2 (18.9–25.1)	19.7 (18.9–23.3)	21.6 (19.4–25.1)	
Day 7	21.14 ± 1.79	20.44 ± 1.50	21.76 ± 1.89	0.189
	20.7 (18.5–24.6)	19.8 (18.6–22.6)	21.8 (18.5–24.6)	
Day 10	21.06 ± 1.69	20.43 ± 1.46	21.60 ± 1.78	0.281
	21.0 (18.5–24.3)	20.2 (18.6–22.6)	21.7 (18.5–24.3)	
Day 14	21.38 ± 1.62	21.02 ± 1.40	21.70 ± 1.82	0.536
	21.2 (18.9–24.2)	20.7 (19.2–23.1)	22.1 (18.9–24.2)	
Day 17	21.00 ± 1.52	20.71 ± 1.45	21.25 ± 1.63	0.613
	20.9 (18.6–23.4)	20.4 (18.6–22.9)	21.6 (18.6–23.4)	
Day 21	21.32 ± 1.62	20.86 ± 1.39	21.73 ± 1.79	0.397
	20.9 (18.7–24.1)	20.8 (18.9–23.0)	22.1 (18.7–24.1)	
Day 24	21.37 ± 1.30	21.07 ± 1.03	21.64 ± 1.51	0.613
	21.2 (19.3–23.5)	20.8 (19.5–22.4)	22.0 (19.3–23.5)	
Day 28	21.39 ± 1.56	21.02 ± 1.30	21.71 ± 1.78	0.536
	21.1 (18.8 – 24.1)	21.0 (18.9–22.7)	22.0 (18.8–24.1)	

Values are expressed as mean ± standard deviation (SD) and median (min-max).
[a] Mann–Whitney U Test

The magnitude of body weight change observed in the OTA group from baseline to day 3 and day 7 was significantly lower than that of the control group ($p=0.044$ and $p=0.021$, respectively; $p<0.05$). However, after day 10, the difference in weight change between the OTA and control groups, relative to baseline, diminished and was no longer statistically significant ($p>0.05$). These findings are presented in Table 2.

Table 2. Changes in body weight (g) relative to baseline in the control and OTA groups

Body Weight Change	Total (n=15)	Control (n=7)	OTA (n=8)	p[a]
Baseline–Day 3	–0.46 ± 0.90	–0.91 ± 0.92	–0.07 ± 0.71	0.044*
Baseline–Day 7	–0.72 ± 0.66	–1.08 ± 0.48	–0.40 ± 0.65	0.021*
Baseline–Day 10	–0.80 ± 0.58	–1.09 ± 0.38	–0.56 ± 0.63	0.094
Baseline–Day 14	–0.48 ± 0.49	–0.50 ± 0.39	–0.46 ± 0.60	0.613
Baseline–Day 17	–0.86 ± 0.49	–0.81 ± 0.38	–0.91 ± 0.60	0.397
Baseline–Day 21	–0.54 ± 0.55	–0.66 ± 0.35	–0.43 ± 0.69	0.281
Baseline–Day 24	–0.48 ± 0.59	–0.45 ± 0.64	–0.51 ± 0.59	0.955
Baseline–Day 28	–0.47 ± 0.61	–0.50 ± 0.41	–0.45 ± 0.78	0.694

Values are expressed as mean ± standard deviation (SD).
[a] Mann–Whitney U Test
*$p<0.05$

In order to assess the impact of oral, non-toxic, subchronic OTA exposure on brown adipose tissue function, UCP-1 and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) levels were quantified in brown adipose tissue samples. After 4 weeks of OTA exposure, no statistically significant difference was observed in interscapular brown adipose tissue UCP-1 concentrations between the control and OTA groups ($p>0.05$). Conversely, PGC-1α concentrations in the OTA group were significantly higher compared to those in the control group ($p=0.007$; $p<0.01$) (Table 3 and Figure 2[b]).

Table 3. Comparison of UCP-1 and PGC-1α concentrations between the control and OTA groups

Parameter	Total (n=15)	Control (n=7)	OTA (n=8)	p[a]
UCP-1 (ng/mL)	15.08 ± 3.82	16.10 ± 1.13	14.06 ± 5.25	0.574
	15.8 (1.3-18)	16 (14.6-18)	15.6 (1.3-17.1)	
PGC-1 (ng/mL)	7.24 ± 3.52	4.96 ± 1.69	9.53 ± 3.43	0.007**
	6.6 (3-14.6)	4.5 (3-7.6)	9.3 (4.7-14.6)	

Values are expressed as mean ± standard deviation (SD) and median (min-max).
[a] Mann–Whitney U test.
**p<0.01

Serum irisin concentrations before and after the study, along with the corresponding changes, for mice in the OTA and control groups are presented in Table 4 and Figure 2 (c). Analysis indicated that, at baseline, serum irisin concentrations were comparable between the groups, with no significant differences observed (p>0.05). During the study, serum irisin concentrations decreased significantly in both groups (control group: 17.590,63 ± 7.974,92 pg/mL, p=0.012; OTA group: 11.225,92 ± 10.464,82 pg/mL, p=0.028; p<0.05). However, after 4 weeks of OTA exposure, serum irisin concentrations in the study group were significantly higher than those in the control group (p=0.040; p<0.05).

Table 4. Comparison of pre- and post-intervention irisin concentrations and their changes between the control and OTA groups

Parameter	Total (n=15)	Control (n=7)	OTA (n=8)	[a]p
Preoperative irisin (pg/mL)	58,380.96 ± 13,731.04 57,247.2 (39,435–88,199.6)	52,503.98 ± 9,059.12 53,465.4 (39,435–63,141.6)	64,257.94 ± 15,588.57 58,874.6 (40,119.1–88,199.6)	0.279
Postoperative irisin (pg/mL)	43,940.28 ± 17,425.05 40,119.1 (21,825.7–86,891.3)	34,913.34 ± 7,198.40 34,660.6 (25,116.9–47,291.1)	54,256.78 ± 20,376.62 50,087.6 (21,825.7–86,891.3)	0.040*
Δ Change	[c]p	**0,012***	**0,028***	
Preop-postop irisin concentration	-14620,43 ± 9462,35	-17590,63 ± 7974,92	-11225,92 ± 10464,82	**0,152**

Values are expressed as mean ± standard deviation (SD) and median (min-max).
[a] Mann Whitney U Test
[c] Wilcoxon Signed Rank Test
*p<0.05

Irisin is a myokine that regulates the expression of molecules such as UCP-1 and PGC-1α and plays an important role in BAT activation as well as the browning of WAT[35]. Due to its identical structure in mice and humans, irisin has been widely used as a biomarker in numerous preclinical and clinical studies evaluating BAT activity[36,37]. The present study demonstrated that serum irisin concentrations significantly decreased during the experimental period in both the control and OTA-exposed groups; however, after four weeks, circulating irisin levels remained significantly higher in the OTA group compared with controls. To our knowledge, studies directly examining the relationship between OTA exposure and circulating irisin concentrations are limited. However, previous experimental studies have shown that OTA exposure can influence metabolic pathways involved in energy homeostasis. For example, Jennings et al. investigated the molecular effects of OTA using multiple *in vitro* renal proximal tubular cell models derived from both human and rat cells, as well as a rat *in vivo* renal cortex model. In that study, cultured human and rat kidney epithelial cells were exposed to OTA at concentrations ranging from approximately 0.3–5 μM for 1–3 days, while the *in vivo* component involved male Wistar rats receiving 3 mg/kg/day OTA by oral gavage for up to 7 days. Transcriptomic analysis revealed that OTA exposure altered numerous cellular pathways associated with cytoskeletal organization, transcription, and cellular stress responses, indicating disruption of cellular metabolic regulation and signaling networks[38]. Similarly, Zhang et al. demonstrated that OTA exposure at micromolar concentrations (0.2–10 μM for 24–72 hours) induces oxidative stress, mitochondrial dysfunction, and apoptosis in cultured kidney epithelial cells[39]. Although these studies did not specifically evaluate irisin, their findings support the possibility that OTA exposure may influence metabolic regulatory pathways linked to myokine secretion.

The relatively higher circulating irisin concentrations observed in the OTA-exposed animals may reflect a compensatory metabolic response. Increased irisin secretion has been reported under conditions associated with metabolic stress or altered energy balance, potentially representing an adaptive mechanism to maintain metabolic homeostasis. In such situations, elevated irisin levels may stimulate thermogenic pathways in adipose tissue to counteract metabolic disturbances[40].

Another possible explanation relates to the endocrine-disrupting properties of OTA. Mycotoxins, including OTA, have been reported to interfere with metabolic and hormonal signaling pathways and are increasingly recognized as metabolic disruptors that affect key metabolic organs and regulatory

mechanisms[41]. Such disruptions may indirectly influence myokine secretion and endocrine communication in adipose tissue. Skeletal muscle–derived myokines, including irisin, play an important role in regulating thermogenesis and systemic energy homeostasis through interactions with adipose tissue. Therefore, disturbances in this muscle–adipose tissue signaling network may contribute to the altered circulating irisin concentrations observed in the OTA-exposed animals[42].

Taken together, these findings suggest that subchronic OTA exposure may influence irisin-related metabolic pathways, potentially reflecting adaptive responses within the muscle–adipose tissue endocrine axis. Nevertheless, given the multifactorial regulation of irisin secretion, further studies incorporating molecular analyses and direct assessments of brown adipose tissue thermogenic activity are required to clarify the mechanisms underlying these changes.

UCP-1 (thermogenin) is a biochemical marker widely used to assess the thermogenic activity of BAT[43,44]. Similarly, PGC-1α plays a critical role in BAT thermogenic activity, mitochondrial biogenesis, and the transdifferentiation of WAT into BAT, and is therefore frequently used in preclinical and clinical studies evaluating BAT function[11,43,45]. In the present study, PGC-1α concentrations were significantly increased following four weeks of OTA exposure, whereas UCP-1 concentrations did not differ between the OTA and control groups. This divergence between the two markers may indicate that OTA exposure influenced upstream regulatory pathways associated with mitochondrial metabolism without fully activating BAT thermogenic output. Although PGC-1α participates in the regulation of thermogenic gene networks, its activation does not necessarily result in immediate increases in UCP-1 expression. Instead, PGC-1α can enhance mitochondrial biogenesis and oxidative metabolism independently of downstream thermogenic protein induction, thereby supporting cellular energy metabolism under conditions of metabolic challenge. Recent studies have highlighted that PGC-1α functions as a central regulator of mitochondrial metabolic adaptation and energy homeostasis in adipose tissue and other metabolically active tissues[46]. In this context, the elevated PGC-1α levels observed in the OTA-exposed animals may reflect mitochondrial metabolic adaptation rather than direct activation of thermogenesis.

In addition, the absence of significant changes in UCP-1 concentrations in both the control and OTA groups may also be related to the characteristics of the cold exposure protocol applied in the present study. Activation of UCP-1–

mediated thermogenesis typically requires sustained sympathetic stimulation and prolonged cold exposure to induce robust transcriptional and translational responses in brown adipose tissue. Experimental cold-acclimation models commonly expose mice to temperatures around 4°C for extended periods ranging from one to several weeks, which promotes pronounced thermogenic activation and increased mitochondrial content in adipose tissue. For example, Lim et al. reported that one week of exposure to 4°C is sufficient to initiate brown adipose tissue activation, whereas more pronounced metabolic and morphological adaptations become evident after 4–5 weeks of continuous cold exposure[34]. In contrast, the cold exposure applied in the present study was intentionally brief and performed only at the end of the intervention period. Short-term cold exposure can activate BAT metabolically without necessarily producing measurable increases in UCP-1 expression levels, particularly when the stimulus duration is limited[47]. Furthermore, experimental guidelines for cold-acclimation studies indicate that certain mouse strains may exhibit increased sensitivity to cold exposure and may even experience mortality under prolonged cold conditions, especially without adequate adaptation periods[34]. Therefore, the duration of cold exposure in the present study was deliberately kept short to minimize potential risks of animal loss. Consequently, the relatively brief cold exposure protocol may not have been sufficient to induce detectable changes in UCP-1 concentrations in either experimental group.

Another factor that may have contributed to the observed findings is the relatively low dose of OTA used in the present study. Previous experimental studies have shown that OTA exposure can disrupt metabolic regulatory pathways *in vivo*. For example, Zheng et al. reported that chronic oral administration of OTA (1 mg/kg body weight for 12 weeks) in C57BL/6 mice promoted hepatic lipid accumulation and activated the PPARγ–CD36 signaling axis, leading to the development of hepatic steatosis[48]. These findings indicate that OTA can modulate key metabolic regulators involved in lipid metabolism and energy homeostasis. In contrast to this long-term high-dose exposure model, the present study employed a lower dose (0.25 mg/kg) and shorter exposure period (4 weeks), which may explain why pronounced metabolic pathology was not observed. According to the study protocol, OTA exposure was designed as a low-dose subchronic exposure, which may not have been sufficient to induce pronounced metabolic or thermogenic alterations in brown adipose tissue. The biological effects of OTA are known to be strongly dose- and duration-dependent, with higher or chronic exposure levels producing more pronounced metabolic disturbances, mitochondrial dysfunction, and tissue damage[49]. At lower exposure levels, organisms may instead activate

adaptive metabolic responses to maintain cellular homeostasis, particularly in metabolically active tissues[50]. Taken together, the increase in PGC-1α observed in the OTA-exposed animals may represent an adaptive mitochondrial regulatory response rather than a direct toxic effect on BAT thermogenic activity. The absence of significant changes in UCP-1 concentrations may therefore reflect the relatively mild physiological impact of the low-dose OTA exposure applied in the present study.

A number of limitations should be considered when interpreting the findings of this study. First, only female mice were included; therefore, potential sex-specific differences in metabolic responses to ochratoxin A exposure cannot be excluded. Second, the functional assessment of brown adipose tissue was based on a limited number of biochemical parameters. Although these markers provide useful information regarding BAT activity, additional approaches—such as the evaluation of a broader panel of metabolic markers or the use of imaging-based techniques—could offer a more comprehensive assessment of BAT function. Finally, the cold exposure and cold tolerance interventions were relatively short in duration, which may limit the ability to fully evaluate adaptive thermogenic responses.

In conclusion, under the conditions of this study, non-toxic subchronic OTA exposure appears to promote mild metabolic or mitochondrial adaptive responses rather than negatively affecting brown adipose tissue thermogenic capacity. However, considering the complex regulation of BAT function and the dose-dependent nature of OTA toxicity, further research involving different exposure levels, longer intervention periods, and more comprehensive assessments of BAT activity is required to better understand the potential metabolic effects of OTA exposure.

STATEMENT OF ETHICS

The study protocol was reviewed and approved by the İstanbul Medipol University Animal Experiments Local Ethics Committee (Approval No: 60; 11.10.2023). All animal procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHOR CONTRIBUTIONS

Study design, CZ and GÜ; acquisition of data, CZ and BC; analysis of data, CZ, FB, and BC; drafting of the manuscript, CZ; critical revision of the manuscript, GÜ and GZO; statistical analysis, CZ and FB; technical or financial support, GÜ, BC, FB, and GZO; supervision, GÜ.

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REFERENCES

1. Wang W, Seale P. Control of brown and beige fat development. Nat Rev Mol Cell Biol, 2016;17(11):691–702. Doi: 10.1038/nrm.2016.96

2. Hachemi I, U-Din M. Brown adipose tissue: activation and metabolism in humans. Endocrinol Metab, 2023;38(2):214–222. Doi: 10.3803/enm.2023.1659

3. Wu M, Junker D, Branca RT, Karampinos DC. Magnetic resonance imaging techniques for brown adipose tissue detection. Front Endocrinol, 2020;11:421. Doi: 10.3389/fendo.2020.00421

4. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev, 2004;84(1):277–359. Doi: 10.1152/physrev.00015.2003

5. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab, 2007;293(2):e444–e452. Doi: 10.1152/ajpendo.00691.2006

6. Suchacki KJ, Stimson RH. Nutritional regulation of human brown adipose tissue. Nutrients, 2021;13(6):1748. Doi: 10.3390/nu13061748

7. Marlatt KL, Ravussin E. Brown adipose tissue: an update on recent findings. Curr Obes Rep, 2017;6(4):389–396. Doi: 10.1007/S13679-017-0283-6

8. Infante M, Armani A, Mammi C, Fabbri A, Caprio M. Impact of adrenal steroids on regulation of adipose tissue. Compr Physiol, 2017;7(4):1425–1447. Doi: 10.1002/cphy.c160037

9. Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elía E, Kessler SH, Kahn PA, et al. Activation of human brown adipose tissue by a β3-adrenergic receptor agonist. Cell Metab, 2015;21(1):33–38. Doi: 10.1016/j.cmet.2014.12.009

10. Villarroya F, Vidal-Puig A. Beyond the sympathetic tone: the new brown fat activators. Cell Metab, 2013;17(5):638–643. Doi: 10.1016/j.cmet.2013.02.020

11. Nascimento EBM, Moonen MPB, Remie CME, Gariani K, Jörgensen JA, Schaart G, et al. Nicotinamide riboside enhances *in vitro* beta-adrenergic brown adipose tissue activity in humans. J Clin Endocrinol Metab, 2021;106(5):1437–1447. Doi: 10.1210/clinem/dgaa960

12. Bonet ML, Mercader J, Palou A. A nutritional perspective on UCP1-dependent thermogenesis. Biochimie, 2017;134:99–117. Doi: 10.1016/j.biochi.2016.12.014

13. Armani A, Feraco A, Camajani E, Gorini S, Lombardo M, Caprio M. Nutraceuticals in brown adipose tissue activation. Cells, 2022;11(24):3996. Doi: 10.3390/cells11243996

14. Moreno-Aliaga MJ, Villarroya F. Nutritional and metabolic regulation of brown and beige adipose tissues. J Physiol Biochem, 2020;76(2):181–184. Doi: 10.1007/s13105-020-00745-1

15. Virtanen KA. Activation of human brown adipose tissue (BAT): focus on nutrition and eating. In: Pfeifer A, Klingenspor M, Herzig S, editors. Brown Adipose Tissue. Handbook of experimental pharmacology, Vol. 251 [Internet]. Springer Cham; 2019. p. 349–359.

16. Li X, Ma W, Ma Z, Zhang Q, Li H. The occurrence and contamination level of ochratoxin A in plant and animal-derived food commodities. Molecules, 2021;26(22):6928. Doi: 10.3390/molecules26226928

17. Wang L, Hua X, Shi J, Jing N, Ji T, Lv B, et al. Ochratoxin A: occurrence and recent advances in detoxification. Toxicon, 2022;210:11–18. Doi: 10.1016/j.toxicon.2022.02.010

18. Kanabus J, Bryła M, Leśnowolska-Wnuczek K, Waśkiewicz A, Twarużek M. Mycotoxins occurrence in herbs, spices, dietary supplements, and their exposure assessment. Toxins (Basel), 2025;18(1):20. Doi: 10.3390/toxins18010020

19. Casu A, Camardo Leggieri M, Toscano P, Battilani P. Changing climate, shifting mycotoxins: a comprehensive review of climate change impact on mycotoxin contamination. Compr Rev Food Sci Food Saf, 2024;23(2):e13323. Doi: 10.1111/1541-4337.13323

20. Niaz W, Iqbal SZ, Ahmad K, Majid A, Haider W, Li X. Mycotoxins: a comprehensive review of its global trends in major cereals, advancements in chromatographic detections and future prospectives. Food Chem X, 2025;27:102350. Doi: 10.1016/j.fochx.2025.102350

21. Shen Y, Ma N, Liu S, Xu G, Zhang J. Mycotoxins and secondary metabolites from fruit postharvest pathogenic *Penicillium* species. Food Chem, 2025;488:144880. Doi: 10.1016/j.foodchem.2025.144880

22. Więckowska M, Szelenberger R, Niemcewicz M, Harmata P, Poplawski T, Bijak M. Ochratoxin A—the current knowledge concerning hepatotoxicity, mode of action and possible prevention. Molecules, 2023;28(18):6617. Doi: 10.3390/molecules28186617

23. Khoi C-S, Chen J-H, Lin T-Y, Chiang C-K, Hung K-Y. Ochratoxin A-induced nephrotoxicity: up-to-date evidence. Int J Mol Sci, 2021;22(20):11237. Doi: 10.3390/ijms222011237

24. Hassan R, González D, Hobloss Z, Brackhagen L, Myllys M, Friebel A, et al. Inhibition of cytochrome P450 enhances the nephro- and hepatotoxicity of ochratoxin A. Arch Toxicol, 2022;96(12):3349–3361. Doi: 10.1007/s00204-022-03395-y

25. Chen Y, Ding J, Zhao Y, Ju S, Mao H, Peng X-G. Irisin induces white adipose tissue browning in mice as assessed by magnetic resonance imaging. Exp Biol Med (Maywood), 2021;246(14):1597–1606. Doi: 10.1177/15353702211006049

26. Izco M, Vettorazzi A, Forcen R, Blesa J, de Toro M, Alvarez-Herrera N, et al. Oral subchronic exposure to the mycotoxin ochratoxin A induces key pathological features of Parkinson's disease in mice six months after the end of the treatment. Food Chem Toxicol, 2021;152:112164. Doi: 10.1016/j.fct.2021.112164

27. Wang H, Wang M, Chansaenpak K, Liu Y, Yuan H, Xie J, et al. A novel PET probe for brown adipose tissue imaging in rodents. Mol Imaging Biol, 2020;22(3):675–684. Doi: 10.1007/s11307-019-01426-2

28. Wu C-Z, Chang L-C, Cheng C-W, Fang T-C, Lin Y-F, Pei D, et al. Reduced expression of urokinase plasminogen activator in brown adipose tissue of obese mouse models. Int J Mol Sci, 2021;22(7):3407. Doi: 10.3390/ijms22073407

29. Du J, He Z, Xu M, Qu X, Cui J, Zhang S, et al. Brown adipose tissue rescues bone loss induced by cold exposure. Front Endocrinol, 2022;12:778019. Doi: 10.3389/fendo.2021.778019

30. Kou G, Yao S, Ullah A, Fang S, Guo E, Bo Y. Polystyrene microplastics impair brown and beige adipocyte function via the gut microbiota-adipose tissue crosstalk in high-fat diet mice. J Hazard Mater, 2025;492:138225. Doi: 10.1016/j.jhazmat.2025.138225

31. Hou L, Le G, Lin Z, Qian G, Gan F, Gu C, et al. Nontoxic concentration of ochratoxin A decreases the dosage of cyclosporine A to induce chronic nephropathy model via autophagy mediated by toll-like receptor 4. Cell Death Dis, 2020;11:153. Doi: 10.1038/s41419-020-2353-z

32. Ferenczi S, Kuti D, Cserháti M, Krifaton C, Szoboszlay S, Kukolya J, et al. Effects of single and repeated oral doses of ochratoxin A on the lipid peroxidation and antioxidant defense systems in mouse kidneys. Toxins (Basel), 2020;12(11):732. Doi: 10.3390/toxins12110732

33. Diaz Marin R, Crespo-Garcia S, Wilson AM, Sapieha P. RELi protocol: optimization for protein extraction from white, brown and beige adipose tissues. MethodsX, 2019;6:918–928. Doi: 10.1016/j.mex.2019.04.010

34. Lim S, Honek J, Xue Y, Seki T, Cao Z, Andersson P, et al. Cold-induced activation of brown adipose tissue and adipose angiogenesis in mice. Nat Protoc, 2012;7(3):606–615. Doi: 10.1038/nprot.2012.013

35. Jeremic N, Chaturvedi P, Tyagi SC. Browning of white fat: novel insight into factors, mechanisms, and therapeutics. J Cell Physiol, 2017;232(1):61–68. Doi: 10.1002/jcp.25450

36. Zheng Y, He J, Yang D, Yuan M, Liu S, Dai F, et al. Irisin reduces the abnormal reproductive and metabolic phenotypes of PCOS by regulating the activity of brown adipose tissue in mice. Biol Reprod, 2022;107(4):1046–1058. Doi: 10.1093/biolre/ioac125

37. Pang M, Yang J, Rao J, Wang H, Zhang J, Wang S, et al. Time-dependent changes in increased levels of plasma irisin and muscle PGC-1α and FNDC5 after exercise in mice. Tohoku J Exp Med, 2018;244(2):93–103. Doi: 10.1620/tjem.244.93

38. Jennings P, Weiland C, Limonciel A, Bloch KM, Radford R, Aschauer L, et al. Transcriptomic alterations induced by ochratoxin A in rat and human renal proximal tubular *in vitro* models and comparison to a rat *in vivo* model. Arch Toxicol, 2012;86(4):571–589. Doi: 10.1007/s00204-011-0780-4

39. Zhang Q, Chen W, Zhang B, Li C, Zhang X, Wang Q, et al. Central role of TRAP1 in the ameliorative effect of oleanolic acid on the mitochondrial-mediated and endoplasmic reticulum stress-excitated apoptosis induced by ochratoxin A. Toxicology, 2021;450:152681. Doi: 10.1016/j.tox.2021.152681

40. Leustean L, Preda C, Teodoriu L, Mihalache L, Arhire L, Ungureanu M-C. Role of irisin in endocrine and metabolic disorders—possible new therapeutic agent? Appl Sci, 2021;11(12):5579. Doi: 10.3390/app11125579

41. Heikkinen T, Küblbeck J, Rysä J. Metabolic disruption by mycotoxins: focus on metabolic endpoints steatosis, adipogenesis and glucose metabolism *in vivo* and *in vitro*. Arch Toxicol, 2025;99(5):1749–1767. Doi: 10.1007/s00204-025-03957-w

42. Guo W, Peng J, Su J, Xia J, Deng W, Li P, et al. The role and underlying mechanisms of irisin in exercise-mediated cardiovascular protection. PeerJ, 2024;12:e18413. Doi: 10.7717/peerj.18413

43. Zhang Q, Lian J, He M, Deng C, Wang H, Huang X-F. Olanzapine reduced brown adipose tissue thermogenesis and locomotor activity in female rats. Prog Neuropsychopharmacol Biol Psychiatry, 2014;51:172–180. Doi: 10.1016/j.pnpbp.2014.02.003

44. Okamatsu-Ogura Y, Kuroda M, Tsutsumi R, Tsubota A, Saito M, Kimura K, et al. UCP1-dependent and UCP1-independent metabolic changes induced by acute cold exposure in brown adipose tissue of mice. Metabolism, 2020;113:154396. Doi: 10.1016/j.metabol.2020.154396

45. Seale P. Transcriptional regulatory circuits controlling brown fat development and activation. Diabetes, 2015;64(7):2369–2375. Doi: 10.2337/db15-0203

46. Cao L, Li Y, Smirnov A, Voshtani R, Wang T, Shao C, et al. PGC-1α: key regulator of mitochondrial biogenesis and cellular differentiation in metabolic and regenerative tissues. Cell Biosci, 2025;16(1):9. Doi: 10.1186/s13578-025-01519-2

47. Yoneshiro T, Matsushita M, Sakai J, Saito M. Brown fat thermogenesis and cold adaptation in humans. J Physiol Anthropol, 2025;44(1):11. Doi: 10.1186/s40101-025-00391-w

48. Zheng Q-W, Ding X-F, Cao H-J, Ni Q-Z, Zhu B, Ma N, et al. Ochratoxin A induces steatosis via PPARγ-CD36 axis. Toxins, 2021;13(11):802. Doi: 10.3390/toxins13110802

49. Schrenk D, Bodin L, Chipman JK, Del Mazo J, Grasl-Kraupp B, Hogstrand C, et al. Risk assessment of ochratoxin A in food. EFSA J, 2020;18(5):e06113. Doi: 10.2903/j.efsa.2020.6113

50. Malir F, Ostry V, Pfohl-Leszkowicz A, Malir J, Toman J. Ochratoxin A: 50 years of research. Toxins, 2016;8(7):191. Doi: 10.3390/toxins8070191