Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Exposure to cold ameliorates glucose intolerance caused by high fat fed rats although enhancing obesity.

Shrook Elgendy [(2)]; Khaled Ezam [(1)]; Lashin Saad Lashin [(1)]; Atef Mansour [(1)].

(1) Medical physiology Department; Faculty of Medicine; Mansoura University; Egypt.

(2) Medical physiology Department; Faculty of Medicine; Damietta University; Egypt.

Submit Date : 30 Aug. 2025
Revised Date :15 Sept.. 2025
Accept Date : 26 Sept. 2025

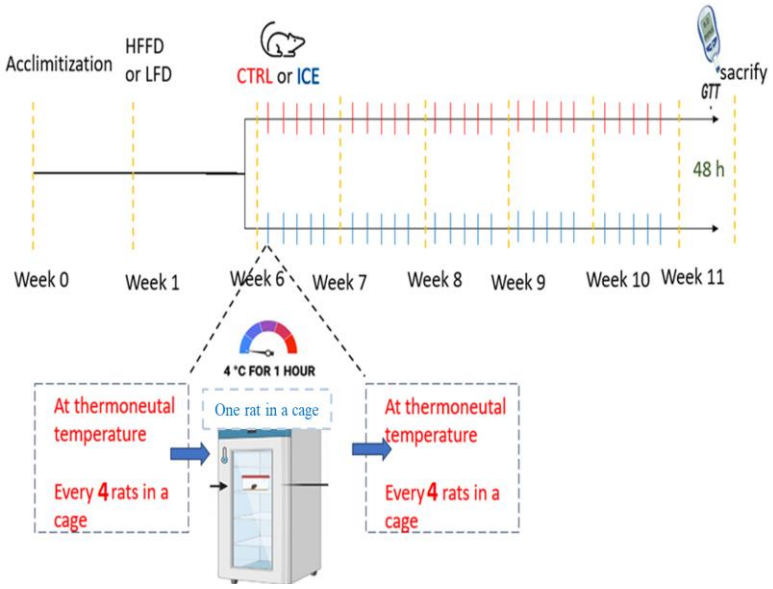
Keywords

- Intermittent cold exposure
- Obesity
- glucose homeostasis
- browning
- adipose tissue

Abstract

Background: Browning, the process by which white adipocytes acquire characteristics of brown adipocytes, is emerging as a potential therapeutic strategy to combat obesity-related metabolic dysfunction. Intermittent cold exposure (ICE) has been shown to induce browning of WAT and enhance thermogenesis, which may improve glucose and lipid metabolism. **Objective:** To evaluate the effects of intermittent cold exposure (ICE) on glucose homeostasis and adipose tissue browning in obese rats, focusing on changes in glucose tolerance, insulin sensitivity, and adipose tissue morphology. **Methods:** A 32 male Sprague-Dawley rats were divided into four groups (8 rats/group): 1) Control (normal diet), 2) Control exposed to ICE, 3) high-fat high-fructose diet (HFFD), 4) HFFD exposed to ICE. Measured parameters including; body weight, food intake, fasting glucose, insulin sensitivity (HOMA-IR), and lipid profile, were assessed. Histopathological and molecular analyses of adipose tissue were conducted to evaluate browning markers (UCP1, CD137). **Results:** Although ICE led to increased body weight in both control and obese groups, ICE significantly improved glucose homeostasis in obese rats with reduction fasting insulin and HOMA-IR. ICE significantly ameliorated dyslipidaemia in obese rats. ICE activated BAT and induced browning of WAT, as evidenced by increased UCP1 expression in interscapular fat and enhanced CD137 expression in inguinal fat. **Conclusion:** Intermittent cold exposure enhances the browning of white adipose tissue and improves glucose and lipid metabolism in obese rats.

Corresponding author: Shrook Elgendy , Email:shrookelgendy@du.edu.eg , Telephone: 01050501426



Graphical abstract or Schematic representation of the experiment.

Introduction

Obesity is a multifactorial and chronic condition characterized by excessive fat accumulation, and it has become a major risk factor for a wide range of metabolic disorders, including insulin resistance, impaired glucose homeostasis, and type 2 diabetes [1].Adipose tissue is traditionally categorized into two main types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT primarily serves as an energy reservoir by storing triglycerides, whereas BAT is specialized in energy dissipation through thermogenesis, a process that is regulated by the mitochondrial protein uncoupling protein 1 (UCP1) [2].

In addition to its classical role in energy storage, WAT is increasingly recognized as a key player in metabolic regulation, secreting a wide array of bioactive molecules known as adipokines that influence insulin sensitivity, inflammation, and systemic metabolism. However, in obesity, the excessive expansion of WAT and its associated inflammatory state lead to insulin resistance, altered glucose metabolism, and the development of metabolic diseases [3].

Interestingly, recent studies have highlighted the potential of *browning*—the process by which white adipocytes acquire characteristics of brown adipocytes—as a mechanism to counteract obesity-induced metabolic dysfunction. Browning involves the induction of UCP1 expression and other markers of thermogenic activity in WAT, which increases energy expenditure and has been linked to improvements in glucose and lipid metabolism [4; 5].

Cold exposure has long been recognized as a physiological stimulus for nonshievering thermogenesis. When exposed to cold, the body activates BAT to maintain core body temperature by enhancing thermogenic activity [6, 7].

Browning of WAT, potentially increasing energy expenditure, improving insulin sensitivity, and ameliorating disturbances in glucose homeostasis associated with obesity. Furthermore, intermittent cold exposure (ICE) has been shown to exert beneficial effects on lipid metabolism, reduce ectopic fat deposition, and promote the remodelling of adipose tissue, which could collectively improve overall metabolic health [8, 9].

In this study, we aim to investigate the effects of ICE on glucose homeostasis in obese rats, with a particular focus on the role of browning in adipose tissue. Using a rodent model of diet-induced obesity, we assess the metabolic outcomes of ICE, including changes in glucose tolerance, insulin sensitivity, and adipose tissue morphology.

2. Material and Methods:

2.1. Experimental protocol: This experimental study included 32 male Albino Sprague Dawley rats weighed 180- 200 gm. The rats were purchased from the animal house of medical experimental research centre (MERC), faculty of medicine, Mansoura University. The experimental procedures were conducted according to the guidelines of Mansoura University Animal Care and Use Committee MU-ACUC **(MED.MS.22.12.3**).

Rats will be housed in plastic cages, four animals per cage, under controlled conditions of humidity (40–70%), lighting (12 h light/dark cycle), and temperature (24°C), with free access to food and water. After acclimatization for one week the rats will be divided into 4 experimental groups (each included 8 rats) ***See graphical abstract***.

2.2. Grouping:Rats were divided into 4 groups (8 rats each):

- **Group I:** (control group "LFD") normal rats were fed with standard laboratory chow diet and had free water access to tap water during whole experiment.
- **Group II:** (control exposed to cold "LFD/C")**:** The same as group I with intermittent cold exposure (ICE) as explained below.
- **Group III:** (obese rats receiving high fat high fructose diet (HFFD))**:** obese rats were fed with diet 45% fat (40% animal fat, 5 % in chow diet), and fructose 20% (20 gram of fructose diluted in 100 ml tap water) during whole experiment.
- **Group IV** (obese rats exposed to cold (HFFD/C))**:** obese rats exposed to ICE.

2.3. Phases of the study: The study was conducted on 2 phases.

2.3.1. The first phase: During this phase, induction of obesity was done for 16 rats (Group III and IV), and the other 16 rats (Group I and II)were maintained on standard laboratory chow diet and had free water access to tap water for six weeks (4 rats per a cage).

Rat model of obesity was induced by high fat fructose diet consists of 45g fat/ 100g diet [10], with fructose 20% in drinking water. To prepare fructose 20% drinking water, 20 g of fructose was diluted in 100 mL of tap water. The bottles were then covered with aluminium foil to prevent fermentation. The fructose drinking water was administered every day for 6 weeks induction and 5 weeks cold experiment [11].

2.3.2. The second phase was initiated on top of phase one. Intermittent cold exposure was induced for 16 rats (Group I and II).

Intermittent cold exposure protocol: ICE consisted of rats being removed from their home cages and transferred to their own designated cage (one rat per a cage) inside a refrigerator held at a constant internal temperature of 4°C. The other groups of the experiment remained unperturbed in their home cages during this time (Group I & III). After ICE protocol rats were transferred back to their home cages immediately following each bout of cold exposure. Rats were repeatedly cold exposed for 1 h/ day for 5 days/week (consecutive), over 5 weeks, and then killed. All cold exposures occurred between 09.00 and 12.00 h [10]***(figure S1 in supplementary file I)***.

2.4. Blood samples obtaining:

Retro orbital blood samples were collected from retro orbital sinus with sterile haematocrit capillary tube. During retro-orbital blood sampling a topical ophthalmic anaesthetic drops e.g. 0.5% proparacaine hydrochloride was used 5 min prior to blood sampling [12]. The collected blood samples were centrifuged at 1000 rpm for 20 minutes to separate the serum. The separated serum was stored at -20℃ for subsequent biochemical analysis ***(figure S2 in supplementary file I)***.

2.5. Oral glucose tolerance test:

Oral glucose tolerance was done at the end of the experiment before scarification. Rats were fasted for 6-8 h then received glucose (2g/kg body weight) by oral gavage (50% glucose solution). Blood samples were obtained from the retro orbital sinus of each rat and a glucometer was used to measure blood glucose levels at 0 (baseline), 60, and 120 min after administration of glucose.

2.6. Measurement of food intake and weight measurement:

The food and fluid intake for each cage (4 rats) was measured by subtracting the remaining amount in the cage from measured provided amount [11]. All rats in different experimental groups were weighed once weekly.

2.7. Animal euthanasia:

Two days after the end of experiment (5weeks of ICE), rats were fasted overnight (about 6-8 hours) then weighed before their sacrifice. "At the end of the experiment, euthanasia was performed by administering a high dose of sodium pentobarbital (60 mg/kg, intraperitoneally)[10].All animal remains were disposed of in a hygienic and ethical manner, in accordance with the guidelines of the Mansoura Experimental Research Center (MERC).

2.8. Adipose tissue samples collections:

The euthanized rat was placed on the surgery board. The dorsal and ventral external surfaces of the rat was sterilized using 70% ethanol. The external surface of the rat was sufficiently wet to minimize contamination from fur during dissection [13]**.** Then identification and isolation of three different adipose depots including interscapular, inguinal, and epididymal were performed. Finally, the specimens were excised, part of interscapular specimen was snap-frozen in liquid nitrogen and stored at −80°C and another part was preserved in 10% buffered formalin and processed in paraffin blocks and sections were made then used for histopathologic examination and immunostaining [14]***(figure S3 in supplementary file I)***.

2.9. Biochemical analysis: ***Details of biochemical analysis was presented in supplementary file (II).***

Serum insulin level was measured by ELISA technique. The kit for ELISA was purchased from Sun-Red biology and technology, China, Shanghai, **CAT. NO. 201-11-0708.** Blood glucose level during fasting and during oral glucose tolerance test was determined using Enzymatic colorimetric commercial kits (human).Homeostatic Model Assessment Index for Insulin Resistance (HOMA- IR) was calculated as follows: HOMA-IR= Fasting insulin levels (μIU/ml) × Fasting glucose levels (mg/dl) /405.

Triglyceride concentration was determined using GPO -POD Enzymatic Colorimetric Kit which was purchased from Sigma Aldrich Co. Egypt. Serum total cholesterol was determined using CHOD-POD Enzymatic Colorimetric Kit which was purchased from Sigma Aldrich Co. Egypt. High-density lipoprotein (HDL) concentration was determined using Phosphotungstic Precipitation kit which was purchased from Sigma Aldrich Co. Egypt. Low- density lipoprotein (LDL)

concentration was obtained from the following calculation (the Friedewald formula) [15]**:** LDL-cholesterol (mg/dl) = (Total cholesterol) minus (TAG/5) minus (HDL- cholesterol).

2.10. Histopathological examination of adipose tissue:

The adipose tissue was prepared for light microscopy. The fixative, which has been used, is 10% neutral buffered formalin for 24 hours. After fixation, the tissue is dehydrated by serial ascending series of ethyl alcohol 70%, 80%, 90% and 95% for 30 minutes each. Tissues were cleared in xylene for 20 minutes (two changes) then embedded in paraffin wax (three changes) at 60oC for three hours and embded in paraffin wax. The paraffin blocks were sectioned at 3-5 µm with Reichert Jung 2030 microtome (West Germany) using disposable blades mounted on glass slide and stained according to the following histological method.

2.10.1. Haematoxylin and Eosin staining:

Sections were deparaffinized in xylen for 10 minutes. Hydration was performed in descending series of ethanol (100%-90%- 80%-70%- 60%-50%) followed by distilled water. Sections were immersed into haematoxylin stain for 10 minutes and then washed in tap water for 2 minutes. Transferred to acid alcohol 1% (1 ml of conc. HCL (BDH, UK) in 100 ml of 70% ethyl alcohol) for acidification and removing the excess of haematoxylin stain. Slides were rapidly washed under running tap water for 3 minutes for removing the excess of acid alcohol and establishing the haematoxylin stain. Sections were counter-stained in aqueos eosin stain for 3 minutes and then rinsed in distilled water. Dehydration was achieved using ascending series of ethyl alcohol, 5 minutes each. Clearing in xylene (2 changes, for 5 minutes each). Mounting was done in Distyrene Plasticizer Xylen (DPX).

2.10.2. Immunohistochemistry by cd-137 marker:

Tissue sections (4 µm thick) were deparaffinized with xylene and rehydrated through graded ethanol (100-95-75%). Epitope retrieval was performed using Heat Induced Epitope Retrieval (HIER) with a pressure cooker and Cell Marque tribology. After rinsing with distilled water and phosphate-buffered saline (PBS), antigen retrieval was enhanced using citrate buffer (pH 6.0), followed by blocking endogenous peroxidase with 3% hydrogen peroxide. Sections were incubated with CD-137 polyclonal antibody (1:200) for 60 minutes, then with Ultra Vision One HRP Polymer for 15 minutes. A DAB substrate solution (Reagent B1 and B2 mixed 1:1) was applied, followed by washing with distilled water and counterstaining with hematoxylin. Dehydration occurred using increasing ethanol grades and xylene, and slides were mounted with a cover slip after applying mounting medium.

Digital image analysis:

Computer assisted digital image analysis(Digital morphometric study) was used. Slides were digitized using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 40 X objective. The result images were analyzed on Intel® Core I7® based computer using VideoTestMorphology® software (Russia) with a specific built-in routine for stain quantification. Two slides from each rat were used, 5 random field from each slide were analysed ***(Steps for digital image analysis were supplemented in supplementary file III)***.

2.11. Western blot analysis for detection of UCP1:

The ReadyPrep™ Protein Extraction Kit (Bio-Rad Inc., Catalog #163-2086) was used to extract total

protein from homogenized tissue samples. Protein concentration was determined using the Bradford Protein Assay Kit (Bio Basic Inc., SK3041) following the manufacturer's instructions. A 20 µg protein sample was combined with 2x Laemmli sample buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8), boiled at 95℃ for 5 min for denaturation, and then loaded onto polyacrylamide gels for SDS-PAGE. The gels were prepared using the TGX Stain-Free™ FastCast™ Acrylamide Kit (Bio-Rad Laboratories Inc., Cat #161-0181). Following electrophoresis, proteins were transferred to a PVDF membrane in a transfer sandwich setup using 1x transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol) at 25 V for 7 min with the Bio-Rad Trans-Blot Turbo. The membrane was blocked with tris-buffered saline with Tween 20 (TBST) and 3% bovine serum albumin (BSA) for 1 hr at room temperature. Primary antibodies (UCP1) were diluted in TBST and incubated overnight at 4℃. After washing with TBST, the membrane was incubated with HRP-conjugated secondary antibody (Goat anti-rabbit IgG-HRP, Novus Biologicals) for 1 hr at room temperature, followed by further washing. Chemiluminescent detection was performed using Clarity™ Western ECL substrate (Bio-Rad, Cat #170-5060), and protein bands were visualized with a CCD camera-based imager. Band intensity was analysed using Image Analysis Software, with beta-actin used as the control for protein normalization.

2.12. Statistical analysis:

Data was entered and analysed using IBM-SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp), and GraphPad Prism software (version 9.5.1). *Quantitative data* was initially tested for normality using Shapiro-Wilk's test (normally distributed data has p>0.050) and Q-Q-plots. The presence of significant outliers (extreme values) was tested by inspecting boxplots. Quantitative data was expressed as mean, and standard deviation (SD). The two-way mixed ANOVA test was used to ascertain the interaction effects of groups over time on the parameters studied, followed by simple main effects of group (comparing the groups at each time point) using univariate analysis. The one-way ANOVA test was used to compare a quantitative variable between the study groups. Significant results were followed by post hoc analysis (pairwise comparisons using Tukey's tests). For any of the tests used, results were considered statistically significant if p value ≤ 0.05.

3. Results:

3.1. Body weight and food intake changes:

As shown in ***table 1***, body weight of HFFD rats (groups III, IV) was significantly higher than that of LFD rats (groups I, II) over the 5 weeks. Intermittent cold exposure induced significant increase in body weight among LFD and HFFD rats (groups II, IV) by the 2nd week to the 5th week as compared to LFD and HFFD rats not exposed to cold (groups I, III). However, during the 1st week, the significant effect of ICE on body weight was among HFFD rats (group IV) ***(p= 0.014)***, meanwhile, ICE did not induce significant changes in body weight of LFD rats (group II)

Figure (1) illustrated the differences between the studied groups as regards food intake. During the 1st and 2nd weeks, Rats received HFFD and exposed to ICE (group IV) had significantly higher food intake than LFD groups (groups I, II) (p= 0.01; 0.028 in week1), (p= 0.008; 0.033 in week2). Also, rats received HFFD but were not exposed to IC (group III) had significantly higher food intake

than LFD not exposed ICE (group I) (p= 0.024 in week1, p= 0.03 in week2). However, cold exposure did not induce significant changes in food intake either among LFD or HFFD groups. During the 3rd week, similar effects were present on food intake, however, ICE induced significant increase in food intake among HFFD (group IV) than compared to HFFD rats which were not exposed to cold (group III) (p= 0.032).

By the 4th and 5th weeks, ICE induced significant increase in food intake among rats received LFD and HFFD (groups II, IV) as compared to LFD (p= 0.026, p= 0.02 successively) and HFFD (p= 0.008, p= 0.008 successively) which were not exposed to cold (groups I, III).

Table (1): Effect of ICE on body weight (g) over 5 weeks between groups.

	Group I	Group II	Group III	Group IV	F/ η^2	P
Week 1	199.3±12.9	209.9±7.3	268.6±4.7 **a, b**	283.0±8.2 **a, b, c**	13.5/0.59	***<.001***
Week 2	208.5±12.9	222.1±7.3 **a**	284.5±4.2 **a, b**	299.3±8.2 **a, b, c**	13.5/0.59	***<.001***
Week 3	219.5±12.9	234.2±7.3 **a**	295.4±5.5 **a, b**	316.1±8.2 **a, b, c**	13.5/0.59	***<.001***
Week 4	230.5±12.9	246.8±7.3 **a**	302.4±9.0 **a, b**	333.6±8.2 **a, b, c**	13.5/0.59	***<.001***
Week5	238.5±12.9	259.9±7.3 **a**	315.0±10.7 **a, b**	351.8±8.2 **a, b, c**	13.5/0.59	***<.001***

Data is presented as mean ± SD. Tests of significance was one-way ANOVA (P); (a, b, c) post- hoc analysis; a: p value against group I < 0.05; b: p value against group II < 0.05; c: p value against group III< 0.05; **η^2: partial Eta squared was used to calculate effect size.**

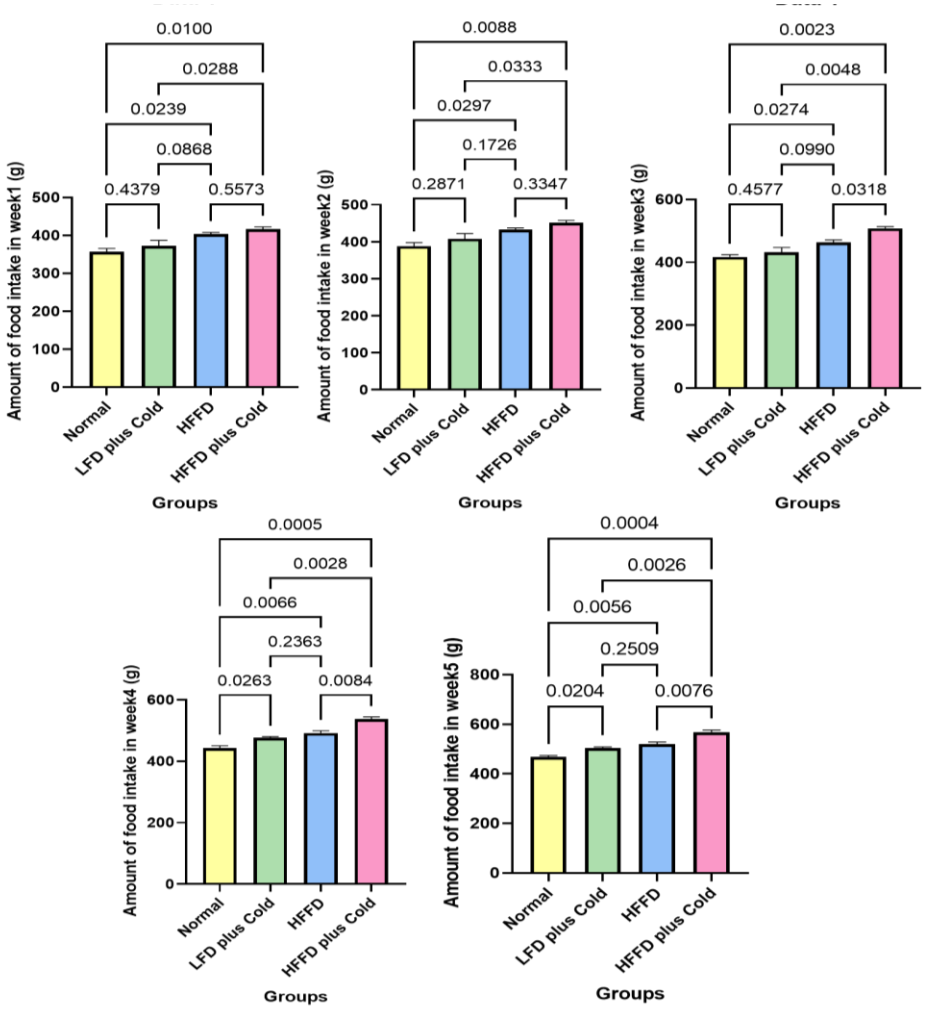


Figure (1): Multiple Tukey's pairwise comparisons for amount of food (g) over 5 weeks between groups.

3.2. Biochemical parameters:

3.2.1. Fasting blood glucose and oral glucose tolerance test results:

As shown in ***table 2,*** Fasting blood glucose, 1-hour and 2- hour post prandial blood glucose level showed statistically significant differences between the studied groups as high fat high fructose diet groups (HFFD) (groups III, IV) had significantly higher blood glucose levels than low fat diet groups (LFD) (groups I, II) ***(p< 0.001)***. Exposure to intermittent cold resulted in significant reduction of glucose level (either fasting or post- prandial) among HFFD- ICE group (group IV) as compared to HFFD group not exposed to cold (group III) ***(p= 0.0052; 0.0052; 0.0061 respectively) (figure 2)***.

3.2.2. Fasting insulin and HOMA IR differences between the studied groups:

Table 2 showed that fasting insulin and HOMA-IR significantly differed between the studied groups with large effect size (η^2= 0.64) as fasting insulin and HOMA- IR were significantly higher among HFFD groups (group III, IV) as compared to LFD groups (groups I, II). Exposure of LFD rats to intermittent cold (group II) did not induce significant changes in fasting insulin (p= 0.99) or HOMA- IR (p= 0.99) as compared to LFD rats which were not exposed to cold (group I). Meanwhile, Exposure of HFFD rats to ICE (group IV) induced significant reduction of fasting insulin ***(p= 0.008)*** HOMA- IR ***(p= 0.0052)*** as compared to HFFD rats which were not exposed to cold (group III) ***(figure 2)***.

Table (2): Effect of ICE on Fasting blood glucose level (FBG), One-hour Glucose level, Two-hour glucose level (mg/dl) between groups.

	Group I	Group II	Group III	Group IV	F/ η^2	P
FBG (mg/dl)	93.6±7.2	92.4±8.1	170.8±12.3 **a, b**	148.8±17.1 **a, b, c**	14.5/0.61	***<.001***
1-h PPBG (mg/dl)	125.8±6.3	123.6±12.6	263.4±39.3 **a, b**	211.1±26.6 **a, b, c**	13.2/0.59	***<.001***
2-h PPBG (mg/dl)	114.1±4.9	105.9±6.8	217.1±23.8 **a, b**	174.3±20.4 **a, b, c**	13.13/0.58	***<.001***
Fasting insulin (microIU/ml)	5.4±0.3	5.4±0.6	8.2±0.8 **a, b**	6.8±0.7 **a, b, c**	16.5/0.64	***<.001***
HOMA-IR	1.25±0.11	1.23±0.19	3.44±0.41 **a, b**	2.48±0.31 **a, b, c**	16.5/0.64	***<.001***
Total Cholesterol (mg/dl)	83.8±11.4	79.0±12.3	155.6±8.4 **a, b**	131.5±5.8 **a, b, c**	14.5/0.61	***.009***
Serum TG (mg/dl)	74.1±4.0	69.5±5.6	129.6±19.7 **a, b**	100.1±8.1 **a, b, c**	15/0.62	***<.001***
LDL-C (mg/dl)	25.5±6.7	22.9±6.9	79.4±3.0 **a, b**	62.5±3.1 **a, b, c**	13.5/0.59	***.002***
HDL-C (mg/dl)	45.9±5.2	48.6±2.8	29.9±3.1 **a, b**	37.0±3.3 **a, b, c**	15/0.62	***<.001***
UCP1 interscapular	1.57±0.31	2.01±0.27 **a**	.15±0.07 **a, b**	1.64±0.13 **b, c**	14/0.6	***<.001***
CD_137 Epididymal	102.11±1.67	104.49±3.01	103.10±1.11	105.03±2.67	2/0.18	0.06
CD_137 Inguinal	1219.00±21.78	2764139.56±12130.46 **a**	1445.70±17.24 **b**	2657271.07±14726.70 **a, b, c**	15/0.62	***<.001***

Data is presented as mean ± SD. Test of significance was one-way ANOVA (P); (a, b, c) post- hoc analysis; a: p value against group I < 0.05; b: p value against group II < 0.05; c: p value against group III< 0.05; **η^2: partial Eta squared was used to calculate effect size; FBG: Fasting blood glucose; PPBG: Post- prandial blood glucose. TG: Triglycerides; LDL- C: Low density lipoprotein- cholesterol; HDL-C: High density lipoprotein- cholesterol.** UCP: uncoupling protein; CD: Cluster of differentiation.

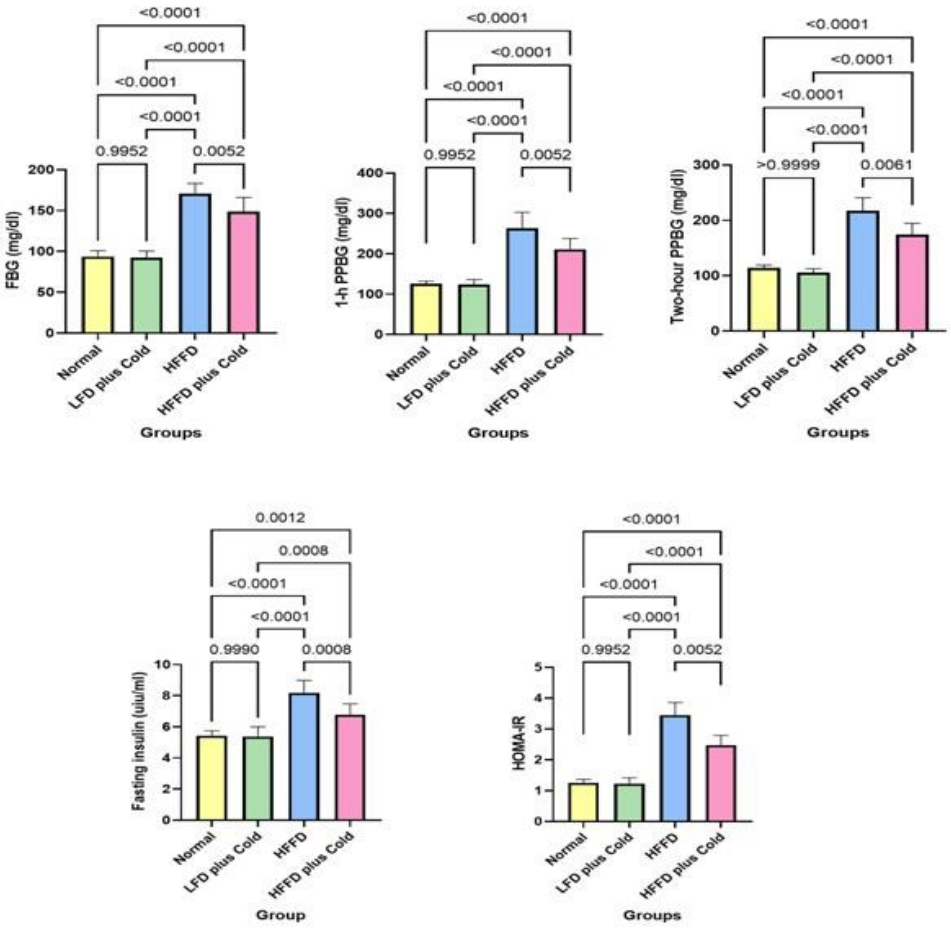


Figure (2): Multiple Tukey's pairwise comparisons for FBG, 1 -h PPBG, 2-h PPBG for fasting insulin, HOMA-IR between groups.

3.2.3. Lipid profile differences between the studied groups:

As reported in ***table 2,*** all studied lipid profile parameters showed statistically significant differences between the studied groups with large effect size. Total cholesterol, triglyceride and LDL- C were all expressed at significantly higher levels among HFFD rats (groups III, IV) as compared to LFD rats (groups I, II). HDL- C was significantly lower levels among HFFD rats (groups III, IV) as compared to LFD rats (groups I, II). LFD rats which were exposed to cold did not show significant changes in all studied lipid profile parameters (cholesterol, triglycerides, LDL, HDL p= 0.76; 0.84; 0.73; 0.47) as compared to LFD rats which were not exposed to cold. On the other side, ICE resulted in significant improvement of all lipid profile parameters among HFFD rats (group IV) as compared to HFFD rats which were not exposed to cold as total cholesterol, triglycerides and LDL were significantly lower ***(p= 0.0002; < 0.001; < 0.001 respectively)*** and HDL was significantly higher ***(p= 0.0038)*** among group IV than group III.

3.3. Adipose tissue histopathological examination:

3.3.1. Histopathological assessment of adipose tissue by haematoxylin and eosin:

Figure (3a) shows sections of epididymal adipose tissue stained with hematoxylin and eosin in the studied groups. The control normal group (I) showing unilocular/large lipid droplet, with flat peripheral nuclei. In HFFD exposed group (III), there was a noticeable increase in the size of unilocular adipocyte in comparison with normal

group. On contrary, LFD exposed to cold group (II) and HFFD and cold exposed group (IV) showing smaller size adipocytes compared to normal and HFFD group. **Figure (3b)**shows sections of inguinal adipose tissue stained with hematoxylin and eosin. The same as epididymal adipose tissue in normal (I) and HFFD (III) groups. As regards LFD exposed to cold group (II) showing scattered beige adipocyte in between white adipocytes. The morphology of beige adipocyte shows more abundant spherical nucleus, with more cytoplasm vacuolation. also, HFFD and cold exposed group (IV), the inguinal showing apparent decreased size of the fat cells compared to HFFD and relative apparently increases number of nuclei in inguinal field (beiging).

While **figure (3c)**shows sections of interscapular adipose tissue stained with hematoxylin and eosin. The control normal group (I) showing multilocular brown adipocyte (cytoplasm more abundant and vacuolated, the nuclei are numerous and more centrally situated). On contrary HFFD (III) showing whitening (large lipid droplet, nucleus became at the periphery in some adipocyte). LFD exposed to cold group (II) and HFFD and cold exposed group (IV) demonstrating more density (more nucleus, more vacuolation) compared to those non exposed to cold.

3.3.2. Immune histochemical staining and western blot analysis of adipose tissue:

As shown in ***table 2,*** interscapular fat expression of UCP1-1 showed statistically significant differences between the studied groups as rats received HFFD and were not exposed to cold had significantly lower UCP-1 expression than LFD rats, either exposed to cold ***(p< 0.001)*** or not exposed to IC ***(p< 0.001)***. Exposure of LFD and HFFD rats to ICE (group II, IV) resulted in significant increase of UCP-1 expression as compared to LFD and HFFD rats which were not expressed to cold ***(p= 0.0021; < 0.001)***. HFFD rats exposed to ICE showed comparable interscapular UCP-1 expression to LFD rats not exposed to cold (p= 0.9). Epididymal fat expression of CD- 137 did not show significant differences between the studied groups. However, inguinal fat expression of CD- 137 was significantly increased after exposure to intermittent cold in both LFD and HFFD rats (groups II, IV) ***(p< 0.001; < 0.001) (figures 4a, b)***.

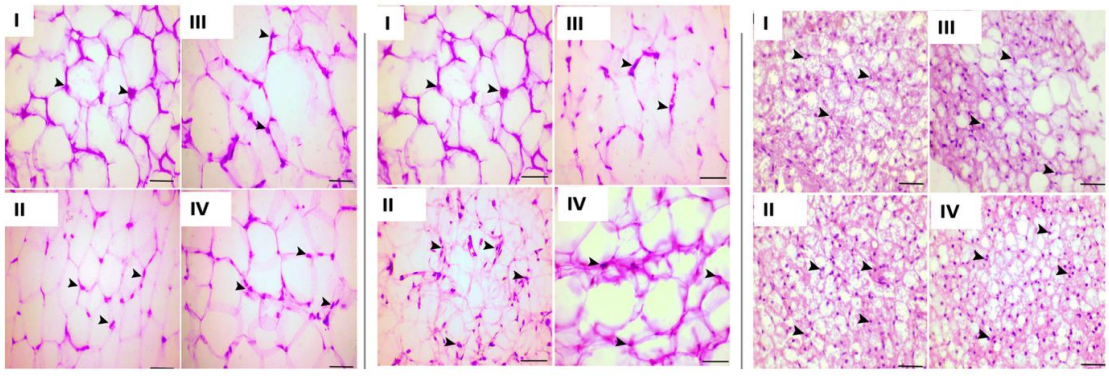


Figure 3a Figure 3b Figure 3c

Figure (3): (a): Sections of epididymal adipose tissue stained with hematoxylin and eosin in the studied groups. (b): Sections of inguinal adipose tissue stained with hematoxylin and eosin in the studied groups. (c): Sections of interscapular adipose tissue stained with hematoxylin and eosin in the studied groups.

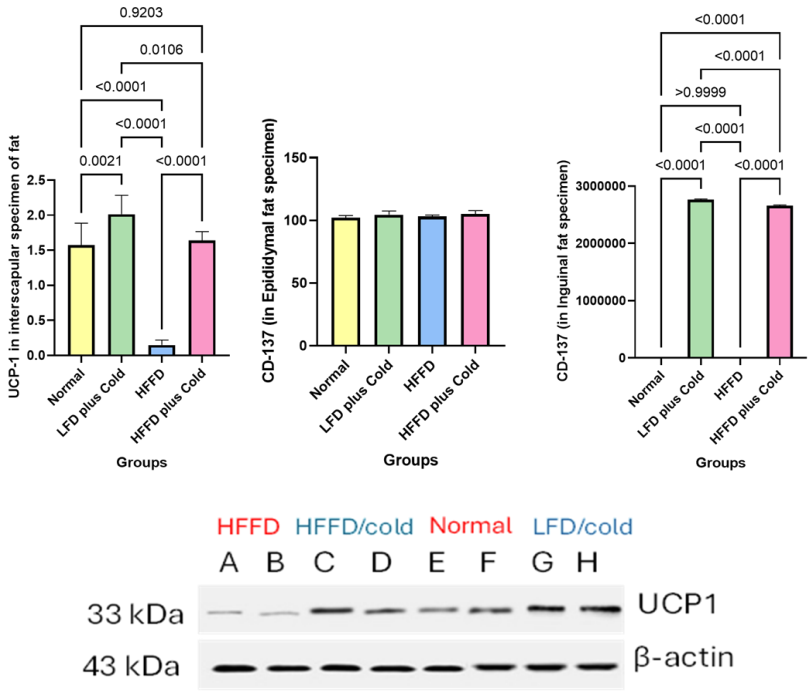


Figure (4): (a) Multiple Tukey's pairwise comparisons for UCP1 expression in interscapular specimen of fat, CD_137 in epididymal specimen of fat & CD_137 in Inguinal specimen of fat between groups. (b) shows protein bands of UCP1 expression in the four groups. A and B represent HFFD group, C and D represent HFFD plus cold group, E and F represent LFD group, G and H represent LFDplus cold group

4. Discussion:

The present study aimed to confirm the transformation of white adipose tissue into brown or beige adipose tissue following intermittent cold exposure and to investigate the role of this brown adipose tissue in ameliorating disturbed glucose homeostasis in obese rat model fed high fat and fructose diet. Also, the study targeted to evaluate the effect of browning on food intake and body weight.

Effect of intermittent cold on body weight, food intake:

In this study, intermittent cold exposure has a positive effect on rats' body weight and food intake over time regardless their diet. There is a significant change in body weight of high-fat diet group exposed to cold compared to those not exposed to cold all over the duration of the study. Also, low-fat diet rats exposed to cold are significantly higher in weight compared to low fat diet group during the period of the study except in the first week. Hyperphagia was significant in high and low-fat diet group exposed to cold compared to those not exposed to cold (in week 3, 4, 5 and in week 4,5respectively). These findings suggest that while cold-induced BAT activation increases energy expenditure, the associated increase in food intake more than this increase in energy expenditure leading to weight gain. The study did not assess energy expenditure directly, but this aligns with **McKie et al.,** who demonstrated that ICE induced weight gain was energy intake dependent [10]. Cold-induced hyperphagia may be a central response to cold stress. Zhu et al. reported that chronic cold exposure in mice fed a high-fat diet led to greater weight gain compared to those kept at room temperature, potentially due to activation of the amygdala. This activation may stimulate orexigenic neuropeptides, which promote increased food intake and fat accumulation [16]. In

contrast, **Deem et al.,** observed cold-induced hyperphagia without weight gain, attributing it to a balance between energy intake and expenditure [17].

Effect of intermittent cold on metabolic parameters (Glucose homeostasis):

This study finds that the increase in weight gain and fat mass seen in cold-exposed rats is not associated with impaired metabolic parameters (i.e., fasting glucose and lipids). Moreover, intermittent cooling ameliorates the disturbance with a high-fat diet. Intermittent cold exposure seems to drive a phenotypically healthy expansion of adipose tissue. Intermittent cold exposure (ICE) significantly reduced fasting and postprandial glucose, insulin, and HOMA-IR in high-fat diet rats, while similar trends were observed in low-fat diet rats, though not statistically significant. The glucose-lowering effect was linked to activated thermogenic adipocytes.

These results align with **McKie et al.,** who found that ICE improves glucose tolerance, independent of diet and this could be due to potentiation of pancreatic glucose-stimulated insulin secretion [10]. **Becher et al.,**also showed that individuals with brown adipose tissue (BAT) had lower rates of type 2 diabetes, particularly those with higher BMI, compared to others without detectable BAT [18]. In humans, **Blondin et al.,** reported a reduction in fasting insulin levels after 4 weeks of cold exposure [19], and **Herz et al.,** found improved insulin sensitivity and browning of adipose tissue in 35% of participants [20]. However, **Mihalopoulos et al.,** found no significant links between BAT activity and metabolic parameters [21], and **Zhu et al.,** observed increased serum glucose levels and slower glucose clearance in cold-exposed mice, attributing this to stress-induced neuropeptide Y (NPY) release, which may promote adipogenesis and worsen glucose intolerance [16].

Effect of intermittent cold on metabolic parameters (Lipid profile):

In the present study, high- fat diet induced impairment of lipid profile as compared to low- fat diet rats as serum cholesterol, triglycerides, LDL were significantly elevated, and HDL was significantly reduced in high- fat diet rats as compared to low- fat diet rats. The effect of intermittent cold on lipid profile derangement was obvious in the present study as cholesterol, triglycerides, LDL significantly decreased, and HDL significantly increased after intermittent cold in rats received high- fat diet as compared to rats received high- fat diet without cooling. The lipid-lowering effect of cooling could be attributed to activated thermogenic adipocytes. This is concomitant with a previous study which showed that obese patients with high BAT exhibited significantly lower rates of dyslipidemia (18.9%) as compared to low BAT group. He reported improvements in TG and HDL [18]. In addition, **Mihalopoulos et al.,** found that cold-activated BAT volume correlated with triglycerides (inversely) [21]. Similarly, **Berbée et al.,** found that BAT activation consistently reduced plasma total cholesterol levels [22].

Effect of intermittent cold on thermogenic adipocytes:

In the current study, Hematoxylin and eosin staining of interscapular and inguinal adipose tissue specimen demonstrated appearance of beige adipocyte with intermittent cold exposure (multilocular adipocyte with spherical nucleus).

This comes in line with **Sanchez-Gurmaches et al.,** work and **Zhang et al.,**research [23, 24]. Also, the present study confirms BAT activation and browning process through measurement of UCP1 protein expression level using western blot analysis and immunohistochemical examination against CD137 a marker for beige fat. The results demonstrated a significant change in expression of UCP1 in interscapular fat which is a marker for brown adipose tissue after intermittent cold exposure in both high-fat diet and low-fat diet group. On the other hand, UCP1 expression is significantly decreased in high fat diet group compared to normal group. In addition, there is a significant change in expression of CD137 which is a marker for beige fat in inguinal fat (subcutaneous WAT, sWAT) after intermittent cold exposure in both high-fat diet and low-fat diet group with obvious increase in low-fat group exposed to cold than high fat group exposed to cold. Also, the present work shows that CD137 expression in epididymal fat (visceral WAT, vWAT) does not show any significant difference between the 4 groups.

In agreement with present study, the study of **Wang et al.,** who demonstrated that ICE upregulated BAT activity and UCP1 levels [25]. Similarly in the study of **Presby et al.,** the level of UCP1 protein expression was significantly higher in BAT with ICE [26]. Moreover **Liang et al.,** showed that acute cold stimulation induced the formation of beige adipocytes in mouse inguinal fat with an increase of the beige adipocyte marker, CD137 [27]. Furthermore, **Yoo et al.,** report presence of more beige cells in inguinal region expressing more Ucp1 with ICE treatment [28].

On contrary to the present study, **Kim et al.,** observed that fish oil intake can induce significantly increase in UCP1 expression in classical brown and beige adipocytes via the SNS. It seems that the source of dietary fat and thus its fatty acid composition is the cause of the conflict with this present study [29]. In discordance with the present study also **Zhang et al.,** suggests that after two weeks of continuous cold exposure, browning is induced in periovarian adipose tissue (vWAT) [30].

5. Limitations:

The study had some limitations. One of these limitations that the study did not explore the energy expenditure and total body fat distribution thus, we were not able to have sufficient explanation for increased body weight after cooling. Another limitation that the study did not explore the acute effect of cooling (within hours).

6. Conclusion:

Intermittent cold exposure enhanced the browning of white adipose tissue, as indicated by increased expression of beige adipose tissue markers (CD137) in inguinal fat depots and increase activity of brown adipose tissue indicated by uncoupling protein- 1 (UCP1) in interscapular fat depots. This activation of thermogenic adipocyte had many beneficial aspects on body metabolism. Intermittent cold exposure significantly improved glucose homeostasis in obese rats by enhancing oral glucose tolerance and reducing fasting insulin levels. Also, Obesity-induced dyslipidaemia, characterized by elevated cholesterol, triglycerides, and LDL levels, alongside reduced HDL, was significantly ameliorated by intermittent cold exposure. It is notable that intermittent cold exposure effectively induced browning in

subcutaneous fat depots with minimal impact on visceral adipose tissue (epididymal fat) in terms of browning marker expression.

7. References:

1. **Riaz T, Akram M, Laila U, Zainab R, Khalil MT, Iftikhar M, Ozdemir FA, Sołowski G, Altable M, Sfera A, Ibrahim HK.** Causes, risks factors and medical consequences of obesity. Int Arch Integr Med. 2023 Aug 1;10(8):39–48.
2. **Das S, Mukhuty A, Mullen GP, Rudolph MC. Adipocyte Mitochondria**: Deciphering Energetic Functions across Fat Depots in Obesity and Type 2 Diabetes. *Int J Mol Sci*. **25**(12):6681; 2024.
3. **Liu Y, Qian SW, Tang Y, Tang QQ.** The secretory function of adipose tissues in metabolic regulation. *Life Metab*. **3**(2):loae003; 2024.
4. **Schirinzi V, Poli C, Berteotti C, Leone A. Browning of adipocytes**: A potential therapeutic approach to obesity. *Nutrients*. **15**(9):2229; 2023.
5. **Machado SA, Pasquarelli-do-Nascimento G, Da Silva DS, Farias GR, de Oliveira Santos I, Baptista LB, et al.** Browning of the white adipose tissue regulation: New insights into nutritional and metabolic relevance in health and diseases. *NutrMetab*. **19**(1):61; 2022.
6. **Scheel AK, Espelage L, Chadt A.** Many ways to Rome: exercise, cold exposure and diet—do they all affect BAT activation and WAT browning in the same manner? *Int J Mol Sci*. **23**(9):4759; 2022.
7. **Li FXZ, Xu F, Li CC, Lei LM, Shan SK, Zheng MH, et al.** Cold Exposure Alleviates T2DM Through Plasma-Derived Extracellular Vesicles. *Int J Nanomedicine*. **19**:10077-95; 2024.
8. **Scherer T, Sakamoto K, Buettner C.** Brain insulin signalling in metabolic homeostasis and disease. *Nat Rev Endocrinol*. **17**(8):468-83; 2021.
9. **Ivanova YM, Blondin DP.** Examining the benefits of cold exposure as a therapeutic strategy for obesity and type 2 diabetes. *J Appl Physiol*. **130**(5):1448-59; 2021.
10. **McKie GL, Shamshoum H, Hunt KL, Thorpe HH, Dibe HA, Khokhar JY, et al.** Intermittent cold exposure improves glucose homeostasis despite exacerbating diet-induced obesity in mice housed at thermoneutrality. *J Physiol*. **600**(4):829-45; 2022.
11. **Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Mohd Yusof MR, Haji Suhaimi F.** The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. *Biomed Res Int*. **2014**;2014:263897.
12. **Hoggatt J, Hoggatt AF, Tate TA, Fortman J, Pelus LM.** Bleeding the laboratory mouse: Not all methods are equal. *Exp Hematol*. **44**(2):132-7; 2016.
13. **Mann A, Thompson A, Robbins N, Blomkalns AL.** Localization, identification, and excision of murine adipose depots. Journal of visualized experiments: JoVE. 2014 Dec 4(94):52174.

14. **Cho SW, Kim I, Kim SH, Rhie JW, Choi CY, Kim BS.** Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. *BiochemBiophys Res Commun*. **345**(2):588-94; 2006.
15. **Romaszko J, Gromadziński L, Buciński A.** Friedewald formula may be used to calculate non-HDL-C from LDL-C and TG. *Front Med*. **10**:1247126; 2023.
16. **Zhu P, Zhang ZH, Huang XF, Shi YC, Khandekar N, Yang HQ, et al.** Cold exposure promotes obesity and impairs glucose homeostasis in mice subjected to a high fat diet. Mol Med Rep. 18(4):3923-31; 2018.
17. **Deem JD, Faber CL, Pedersen C, Phan BA, Larsen SA, Ogimoto K, et al.** Cold-induced hyperphagia requires AgRP neuron activation in mice. Elife. 9:e58764; 2020.
18. **Becher T, Palanisamy S, Kramer DJ, Eljalby M, Marx SJ, Wibmer AG, et al.** Brown adipose tissue is associated with cardiometabolic health. Nat Med. 27(1):58-65; 2021.
19. **Blondin DP, Daoud A, Taylor T, Tingelstad HC, Bézaire V, Richard D, et al.** Four-week cold acclimation in adult humans shifts uncoupling thermogenesis from skeletal muscles to brown adipose tissue. J Physiol. 595(6):2099-113; 2017.
20. **Herz CT, Kulterer OC, Prager M, Schmöltzer C, Langer FB, Prager G, et al.** Active brown adipose tissue is associated with a healthier metabolic phenotype in obesity. Diabetes. 71(1):93-103; 2022.
21. **Mihalopoulos NL, Yap JT, Beardmore B, Holubkov R, Nanjee MN, Hoffman JM.** Cold-Activated Brown Adipose Tissue is Associated with Less Cardiometabolic Dysfunction in Young Adults with Obesity. Obesity. 28(5):916-23; 2020.
22. **Berbée JF, Boon MR, Khedoe PPS, Bartelt A, Schlein C, Worthmann A, et al.** Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat Commun. 6(1):6356; 2015.
23. **Sanchez-Gurmaches J, Hung CM, Guertin DA.** Emerging complexities in adipocyte origins and identity. Trends Cell Biol. 26(5):313-26; 2016.
24. **Zhang F, Hao G, Shao M, Nham K, An Y, Wang Q, et al.** An adipose tissue atlas: an image-guided identification of human-like BAT and beige depots in rodents. Cell Metab. 27(1):252-62; 2018.
25. **Wang TY, Liu C, Wang A, Sun Q.** Intermittent cold exposure improves glucose homeostasis associated with brown and white adipose tissues in mice. Life Sci. 139:153-9; 2015.
26. **Presby DM, Jackman MR, Rudolph MC, Sherk VD, Foright RM, Houck JA, et al.** Compensation for cold-induced thermogenesis during weight loss maintenance and regain. Am J Physiol Endocrinol Metab. 316(5):E977-86; 2019.

27. **Liang X, Pan J, Cao C, Zhang L, Zhao Y, Fan Y, et al.** Transcriptional response of subcutaneous white adipose tissue to acute cold exposure in mice. Int J Mol Sci. 20(16):3968; 2019.
28. **Yoo HS, Qiao L, Bosco C, Leong LH, Lytle N, Feng GS, et al.** Intermittent cold exposure enhances fat accumulation in mice. PLoS One. 9(5):e96432; 2014.
29. **Kim M, Goto T, Yu R, Uchida K, Tominaga M, Kano Y, et al.** Fish oil intake induces UCP1 upregulation in brown and white adipose tissue via the sympathetic nervous system. Sci Rep. 5(1):18013; 2015.
30. **Zhang L, An G, Wu S, Wang J, Yang D, Zhang Y, et al.** Long-term intermittent cold exposure affects peri-ovarian adipose tissue and ovarian microenvironment in rats. J Ovarian Res. 14(1):107; 2021.