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RESEARCH ARTICLE

Gut microbiota are involved in leptin-induced thermoregulation in the Mongolian gerbil (*Meriones unguiculatus*)

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ABSTRACT

Leptin is a hormone that is secreted by adipocytes and may promote energy expenditure by increasing thermogenesis. Our previous studies have shown that thermo-transient receptor potentials (thermo-TRPs) and gut microbiota are associated with thermoregulation in Mongolian gerbils, which are characterized by relative high serum leptin concentrations. Here, we tested whether leptin can stimulate non-shivering thermogenesis (NST) in Mongolian gerbils, and whether thermo-TRPs and gut microbiota are involved in leptin-induced thermogenesis. First, gerbils were given acute leptin treatment (ALT) with different doses. Results showed that ALT significantly increased the body temperature of gerbils and changed the composition of gut microbiota. Moreover, ALT groups showed a trend towards increased expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT). Then, we investigated the effect of chronic leptin treatment (CLT) on gerbils. Surprisingly, CLT did not affect gerbils' food intake and body mass, but it significantly increased the body temperature at the end. Further, CLT did not affect the expression of thermogenic markers in BAT, white adipose tissue (WAT) or skeletal muscle. However, CLT increased the expression of leptin receptors and TRPV2 in the small intestine and affected the composition of gut microbiota. Together, our data suggest leptin may increase body temperature by regulating gut microbiota. In conclusion, serum hyperleptin in Mongolian gerbils is beneficial for adapting to cold environments, and TRPV2 and gut microbiota are involved.

KEY WORDS: Leptin, Thermo-TRPs, TRPV2, Non-shivering thermogenesis

INTRODUCTION

Leptin is an adipocyte-derived hormone that controls body mass, food intake and energy expenditure (Myers et al., 2008; Zhang et al., 1994). Leptin functions by binding to the leptin receptors (LepRb, encoded by the *db* gene), which is expressed in the hypothalamus and non-neuronal tissues, including brown adipose tissue (BAT), skeletal muscle and small intestine (Chua et al., 1996, 1997; Lee et al., 1996; Tartaglia, 1997). Many studies support that leptin plays an important role in thermoregulation. It was found that leptin-deficient (ob/ob) mice display hypothermia, and supplementation of exogenous leptin increases their body temperature, metabolic rate and thermogenic capacity of BAT (Collins et al., 1996; Pelleymounter et al., 1995; Rezai-Zadeh et al., 2014; Trayhurn et al., 1977; Commins et al., 1999). Non-shivering thermogenesis (NST) in muscle is confirmed as an additional form of NST in rodents besides BAT (Bal et al., 2012, 2016; Pant et al., 2016). It has been demonstrated by studies *in vivo* and *in vitro* that leptin increases glucose and fatty acid metabolism in skeletal muscle (Ceddia et al., 2001). Moreover, leptin also interacts with other endocrine hormones, such as thyroid hormone, thyrotropin releasing hormone, etc., to jointly regulate thermogenesis and energy homeostasis (Buonfiglio et al., 2018; Hermann et al., 2006; Ukropec et al., 2006). In addition, there is a hypothesis that leptin-mediated thermoregulation as an adaptive mechanism for humans in cold climates, and high leptin levels are required to maintain body temperature (Nikanorova et al., 2020). However, whether leptin is thermogenic is still ambiguous (Fischer et al., 2020). For example, Fischer et al. (2016) found that prolonged leptin treatment does not recruit BAT in ob/ob mice. Although leptin treatment significantly induced UCP1 levels per milligram of protein, the total UCP1 per iBAT depot did not increase (Fischer et al., 2016). Further, Tang et al. (2015) found that intracerebroventricular administration leptin had no effects on basal and adaptive thermogenesis and UCP1 expression in cold-acclimated rats.

The microbiota composition in leptin-deficient mice was changed, while leptin sensitivity was enhanced in mice with depleted gut microbiota, suggesting a link between leptin and gut microbiota (Heiss et al., 2021; Ley et al., 2005). Specifically, gut epithelial leptin signaling might shape the community structure of the gut microbiome (Duggal et al., 2011; Guo et al., 2011), and regulates the composition of microbiota independently of food intake in the host (Rajala et al., 2014). Moreover, chronic oral leptin-supplemented suckling rats had lower relative abundance of *Sutterella* and a higher proportion of *Clostridium* genus (Grases-Pintó et al., 2019). Thermo-transient receptor potentials (thermo-TRPs) exhibit highly temperature-dependent gating properties, which leads to steep changes in depolarising current upon either cooling or heating (Nilius and Flockerzi, 2014). Thermo-TRPs include TRPV1 (>42°C), TRPV2 (>52°C), TRPV4 (27–34°C), TRPM2 (23–28°C), TRPM8 (10–26°C) and TRPA1 (<17°C), which are expressed in almost all tissues (Hoffstaetter et al., 2018; Nilius and Owsianik, 2011). Peripheral thermo-TRP channels that are activated can affect body temperature by modulating the autonomic system, and thermo-TRP channels in the central nervous system directly influence the detection of physiological temperature *in vivo* (Wang and Siemens, 2015). Studies have found that the expression of thermo-TRPs was significantly altered in WT mice injected with leptin (Avraham et al., 2010; Sun et al., 2017). In addition, our previous research found that one of the thermo-TRPs (TRPV1) is involved in behavioral thermoregulation in Mongolian gerbils (Wen et al., 2022). So, we are curious about how leptin affects gut microbiota and thermo-TRPs, and the roles of gut microbiota and thermo-TRPs in leptin-stimulated NST.

The Mongolian gerbil (*Meriones unguiculatus*) is a small rodent that is geographically widespread in the typical steppe, desert steppe

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or desert areas of northern China, Mongolia and the Trans-Baikal region of Russia (Wilson and Reeder, 2005), regions in which cold winter lasts for more than 5 months. The gerbils have a relatively high basal metabolic level compared with typical small desert rodents, and a wider thermoneutral zone and lower critical temperature (Pan et al., 2014; Wang et al., 2000; Zhang et al., 2016). In addition, gerbils have a special physiological characteristic of high serum leptin concentrations: the serum leptin concentration of the gerbils was close to 25 ng ml^{-1} under normal feeding conditions in the laboratory (23°C, 16 h:8 h light:dark), which is relatively higher than that of Brandt's voles (*Lasiopodomys brandti*) that are sympatrically distributed in the Inner Mongolia grasslands (Li and Wang, 2007; Li et al., 2004), and even higher than that of the rat (~10 ng ml^{-1}) (Denis et al., 2003). Furthermore, our previous studies have demonstrated that leptin may be involved in the seasonal regulation of energy balance and thermogenesis in Mongolian gerbils (Liu et al., 2012; Zhang and Wang, 2007). Therefore, the aim of the present study was to test whether leptin can stimulate NST in Mongolian gerbils, and whether thermo-TRPs and gut microbiota are involved in leptin-induced thermogenesis.

MATERIALS AND METHODS

Animals

Mongolian gerbils [*Meriones unguiculatus* (Milne-Edwards 1867)] used for this study were obtained from the laboratory breeding colony at the Institute of Zoology, Chinese Academy of Sciences, which started with animals that were originally trapped from Inner Mongolia, China. In total, 56 adult male gerbils (age 6–7 months) were housed individually in plastic cages (29×18×16 cm) with sawdust bedding in a temperature-controlled room (23±1°C) under a 16 h:8 h light:dark cycle (lights on at 04:00 h). Water and standard rodent chow (Beijing Keao Xieli Feed Co., China) were provided *ad libitum*. All procedures were approved by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (CAS).

Experimental design

Experiment 1 was designed to investigate the acute effect of different concentrations of leptin on thermogenesis in Mongolian gerbils. Twenty-four male gerbils were divided into four groups (n=6 for each group): the first group was injected with saline (control), the second group was injected with 0.2 mg kg^{-1} leptin (Lep-0.2), the third group was injected with 1 mg kg^{-1} leptin (Lep-1), and the fourth group was injected with 5 mg kg^{-1} leptin (Lep-5). In the leptin injection groups, 0.2 ml of saline solution containing recombinant murine leptin (PeproTech, USA) was injected by single intraperitoneal injection (i.p.). Before the experiment, rectal temperature was measured. Then, animals were injected at 09:00 h. Two hours later, rectal temperature was measured again, and the animals were then killed by CO_2 overdose. The serum, BAT, hypothalamus and cecal content samples were taken and stored at −80°C.

Experiment 2 verified the effect of chronic leptin administration on thermogenesis in gerbils, and whether thermo-TRPs and gut microbiota were involved with this. Twenty-four adult male gerbils were divided into four groups (n=6 for each group): a saline-injected group at room temperature (23°C, WS), a leptin-injected group at room temperature (23°C, WL), a saline-injected group at a cold room temperature (4°C, CS), and a leptin-injected group at a cold room temperature (4°C, CL). The gerbils were injected i.p. once daily for consecutive 14 days with 1 mg kg^{-1} leptin or with 200 ml saline. Body mass, food intake and body temperature were measured every 3 days. After the last injection, animals were killed by CO_2 overdose. The serum, hypothalamus, BAT, inguinal white adipose tissue (WAT), skeletal muscle and small intestine and cecal content samples were taken and stored at −80°C.

Measurements of body mass and food intake

Body mass and food intake of the gerbils were measured at 17:00 h every 3 days. Food intake was calculated by subtracting uneaten food mass from initial food mass and divided by 3.

Measurements of body temperature and BAT skin temperature

Body temperature (T_b) was measured during the experimental period using a temperature probe (TES 1310; ±0.1°C accuracy), which was inserted 3 cm into the rectum.

Surface body temperature was measured at an ambient temperature of 23±1°C at a distance of 40 cm from the animal using an infrared camera (FLIR E60, UK), and the temperature data were generated using FLIR Tools software (Zhang et al., 2018). The highest temperature in the BAT image and tail image and the average temperature in the body image were selected as the BAT skin temperature, tail skin temperature and shell temperature, respectively.

Measurement of thyroid hormones (T3 and T4) and leptin concentration in serum

Serum T3 and T4 concentrations were quantified via radioimmunoassay using an RIA kit (China Institute of Atomic Energy, Beijing, China) according to the instructions. The minimum detectable concentration of the assay (sensitivity) when using a 50 µl sample was 0.25 ng ml^{-1} for T3 and 3.96 ng ml^{-1} for T4. Intra- and inter-assay coefficients of variation were 2.4% and 8.8% for T3, and 4.3% and 7.6% for T4, respectively.

Serum leptin concentrations were measured using a leptin ELISA kit (ab100718, Abcam, Cambridge, UK) according to the manufacturer's instructions. Absorbance was measured at 450 nm against a blank using an ELISA reader (RayBiotech, Canada). The minimum detected concentration of the kit was 4 pg ml^{-1}. The intra- and inter-assay variations for leptin EIA were 10% and 15%, respectively.

Western blotting for measurements of thermogenic proteins and thermo-TRPs

Western blotting was performed on whole tissue lysates and were probed with the following primary antibodies: UCP1 (ab10983, Abcam), PGC1α (bs-1832R, Bioss), PPARγ (C26H12, Cell Signaling), LEPR (38530, SAB), TRPA1 (QC50165, Sigma-Aldrich), TRPV1 (66983-1-Ig, Proteintech), TRPV2 (ab272862, Abcam), TRPV4 (ab39260, Abcam), TRPM2 (QC49319, Sigma-Aldrich), TRPM8 (ab3243, Abcam), TH (AB152, Sigma-Aldrich), β-tubulin (30302ES60, Yesen) and GAPDH (A01020, Abbkine). The secondary antibodies used were either peroxidase-conjugated goat anti-rabbit IgG (33101ES60, Yesen) or peroxidase-conjugated goat anti-mice IgG (33201ES60, Yesen).

Samples from the hypothalamus, small intestine and BAT (0.2 g) were homogenized in RIPA buffer using established methods (Bo et al., 2019). The total protein was separated by SDS-PAGE using a Mini Protean apparatus (Bio-Rad Laboratories, PA, USA) and transferred onto PVDF membranes, which were then blocked with 5% skimmed milk for 1.5 h at room temperature. Thereafter, the membranes were incubated with the primary antibodies for approximately 12 h at 4°C, followed by incubation with an appropriate horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Antibody concentrations were

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determined based on the literature and our pilot experiments. The reaction products were revealed by chemiluminescence (ECL, Yesen).

Real-time quantitative PCR (qPCR) for measurements of thermogenic and appetite-related genes

We extracted total RNA from BAT and WAT using TRIzol reagent (R401-01, Vazyme, Nanjing, China), and then 1 µg total RNA was purified and reverse-transcribed to cDNA using the HiScript® III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (R312-01/02, Vazyme, Nanjing, China). qPCR analysis was carried out as follows. The cDNA samples (1 µl) were used as a template for the subsequent PCR reaction using gene-specific primers (Table S1). The final reaction volume of 10 µl contained 5 µl of 2×Taq Pro Universal SYBR qPCR Master mix (Q712-02, Vazyme, Nanjing, China), 1 µl cDNA template, 0.2 µl of forward primer, 0.2 µl reverse primer and 3.6 µl RNase-free ddH2O. Each sample was duplicated and the mean value was the expression amount of the sample. qPCR was performed using Piko Real Software 2.2 (Piko Real 96, Thermo Fisher Scientific, Waltham, MA, USA). After an initial polymerase activation step at 95°C for 30 s, amplification was followed by 40 cycles (95°C for 10 s and 60°C for 30 s). The reaction was finished by the built-in melting curve. All samples were quantified for relative quantity of gene expression using GAPDH expression as an internal standard. Relative gene expression was determined by the comparative CT method (Liu et al., 2022b). The gene-specific primer sequences used for qPCR are listed in Table S1.

Microbiota DNA extraction

DNA from cecal contents was extracted by 2×CTAB (cetyltrimethyl ammonium bromide), phenol chloroform mixture (phenol: chloroform:isoamyl alcohol=25:24:1) and the SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, China). DNA purity and concentrations were assessed by absorbance on a Nanodrop 2000 (Thermo Fisher Scientific) by measuring the A260/A280 ratio. Only DNA with an A260/A280 ratio of 1.8–2.0 was used (Bo et al., 2019).

16S rDNA gene sequencing analysis for cecal contents

The 16S sequence paired-end dataset was joined and quality filtered using the FLASH method described by Magoč and Salzberg (2011). Sequencing was done on an Illumina HiSeq 2500. All sequence analyses were provided in the Quantitative Insights Into Microbial Ecology software suite (QIIME, version 1.9.1) according to the QIIME tutorial (http://qiime.org/) with modified methods. Sequences that did not match any entries in this reference were subsequently clustered into *de novo* operational taxonomic units (OTUs) at 97% similarity with UCLUST. The hierarchical clustering on the basis of population profiles of most common and abundant taxa was performed using UPGMA clustering (unweighted pair group method with arithmetic mean, also known as average linkage) on the distance matrix of OTU abundance.

Statistical analysis

SPSS 20.0 software was used for statistical analyses. For intra- and inter-group analyses, repeated-measures ANOVA or two-way ANOVA (leptin×cold) was applied when appropriate, followed by a Bonferroni *post hoc* test. All data are presented as means±s.e.m., and $P<0.05$ was deemed statistically significant.

For microbiota data, the OTUs that reached a nucleotide similarity level of 97% were used for α diversity (PD_whole tree). Principal coordinate analyses (PCoA) based on unweighted UniFrac distances were used to visualize the variation of bacterial structure across different groups using the vegan package in R. Significance for β-diversity analyses was checked with analysis of similarity (ANOSIM) included in the package vegan of the QIIME-incorporated version of R. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to assess differences in microbial communities using an LDA score threshold of 2 or 2.5. Differences in α diversity, and phyla and genera abundance were analyzed using two-way ANOVA with a Bonferroni *post hoc* test.

RESULTS

Acute leptin treatment with high concentration significantly affects the thermogenesis and composition of gut microbiota of gerbils

Plasma leptin of three groups with different dosage increased significantly following leptin administration ($F_{3,23}=30.713$, $P<0.001$; Fig. 1A). We detected the expression of hypothalamic agouti related peptide (*AGRP*), neuropeptide Y (*NPY*), pro-opiomelanocortin (*POMC*), and cocaine and amphetamine regulated transcript peptide (*CART*) at mRNA levels, which are important mediators of leptin action. Acute leptin treatment had no significant effect on the expression of *AGRP*, *NPY* and *POMC* in the hypothalamus of Mongolian gerbils, but significantly decreased the expression of *CART* ($F_{3,23}=4.589$, $P=0.013$; Fig. 1C).

We compared the T_b before treatment and after injection for 2 h. The results showed that there was no significant difference in T_b between initial value and after 0.2 mg kg^{-1} leptin administration ($t=-0.954$, d.f.=5, $P=0.384$; Fig. 1C). However, the 1 mg kg^{-1} ($t=-3.757$, d.f.=5, $P=0.013$; Fig. 1F) and 5 mg kg^{-1} ($t=-4.715$, d.f.=5, $P=0.005$) acute leptin treatments significantly increased gerbil T_b. Further, shell temperature ($F_{3,23}=4.446$, $P=0.015$; Fig. 1G) and BAT skin temperature ($F_{3,23}=5.508$, $P=0.006$; Fig. 1H) were significantly affected by leptin, and there was no difference in tail skin temperature. Specifically, gerbils administrated with 1 mg kg^{-1} ($P=0.011$) and 5 mg kg^{-1} ($P=0.022$) had higher BAT skin temperature than the control group. However, UCP1 protein expression in BAT was slightly increased by leptin, but there was no statistically significant difference (Fig. 1J).

We further analyzed 16S rRNA gene sequencing to explore whether gut microbiota are involved. The α diversity of gerbil's cecal microbiota did not differ in the acute leptin-treated gerbils (Fig. 2A). For β diversity, analysis based on unweighted UniFrac distance (ANOSIM, $R=0.133$, $P=0.015$; Fig. 2B) showed that the cecal microbiota structure of the Lep-5 group was separated from the control. At a threshold of 97%, the LEfSe method with an LDA score >2 identified the different microbial communities among groups (Fig. 2C). *Firmicutes* and *Bacteroidetes* were the most abundant phyla in all samples (Fig. 2D,E), and significant differences were observed at the phylum level in *Actinobacteria* ($F_{3,23}=3.358$, $P=0.039$; Fig. 2G). The *Actinobacteria* phylum was decreased by leptin administration. At the genus level, acute leptin administration significantly increased the abundance of *Peptococcus* ($F_{3,23}=4.509$, $P=0.014$; Fig. 2H) and decreased the relative abundance of *Sphingomonas* ($F_{3,23}=7.801$, $P=0.007$; Fig. 2I).

Chronic leptin administration does not stimulate the NST of BAT, WAT and skeletal muscle in gerbils

Chronic leptin treatment significantly increased serum leptin concentration ($F_{1,20}=121.367$, $P<0.001$; Fig. 3E), whereas cold induced a reduction ($F_{1,20}=18.538$, $P<0.001$; Fig. 3E). Of note, chronic leptin treatment had no effect on gerbil body mass, food

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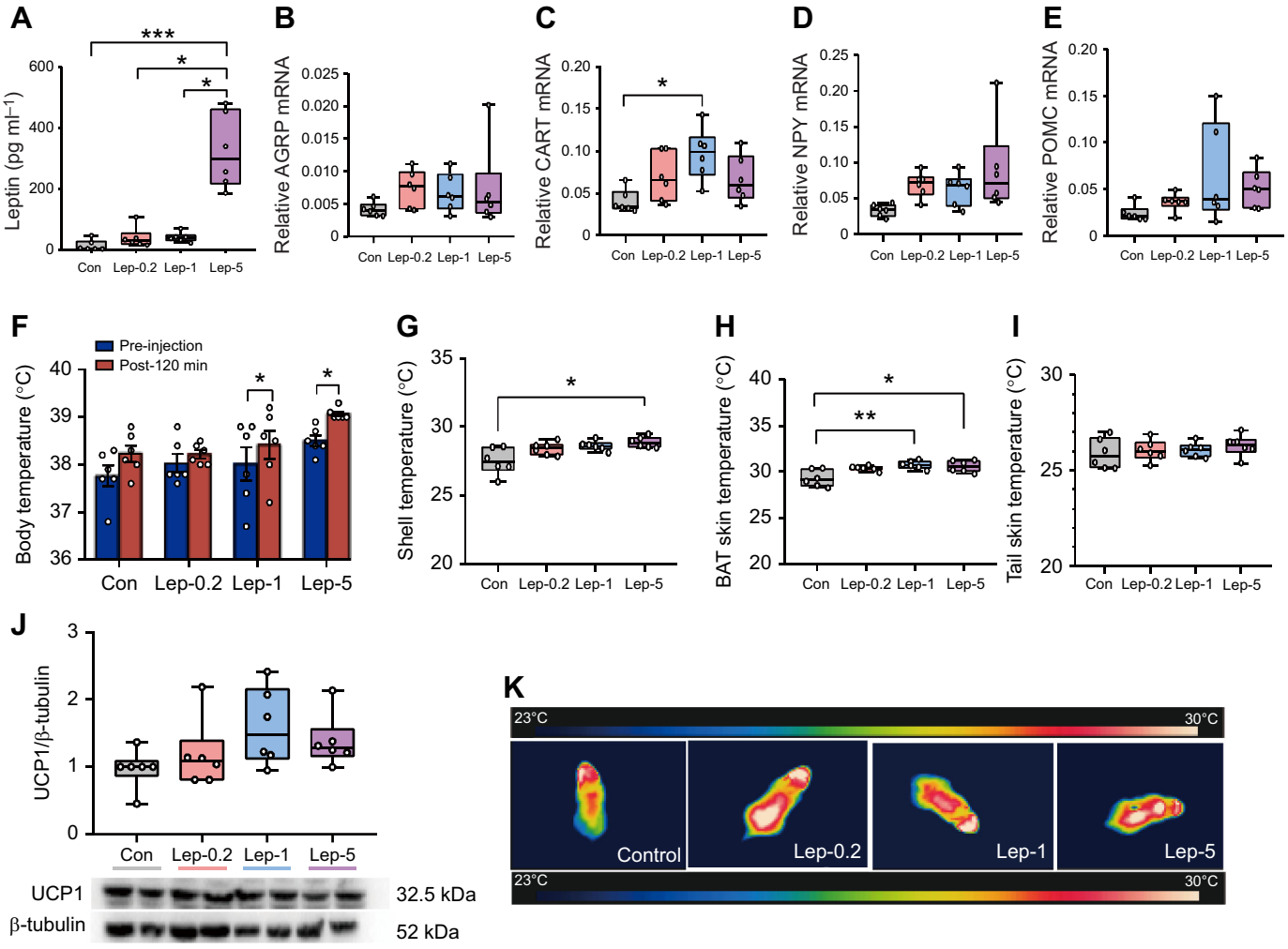

Fig. 1. The effects of acute leptin treatment on serum leptin level, appetite neuropeptide gene expression and thermogenesis in Mongolian gerbils. (A) Serum leptin concentration after acute leptin injection. (B–E) Effects of acute leptin on appetite related peptide gene expression in the hypothalamus. (F) Rectal temperature before and 2 h after injection. (G–I) Infrared skin temperature 2 h after injection. (J) UCP1 protein expression in BAT. (K) Photos of gerbil infrared imaging after injection. Control, gerbils with 200 µl saline intraperitoneal injection; Lep-0.2, gerbils with 0.2 mg kg^{-1} leptin intraperitoneal injection; Lep-1, gerbils with 1 mg kg^{-1} leptin intraperitoneal injection; Lep-5, gerbils with 5 mg kg^{-1} leptin intraperitoneal injection. Different letters indicate significant differences (*P<0.05, **P<0.01, ***P<0.001; n=6 for each group).

intake and body temperature (Fig. 3A–C). However, the last measured body temperature was significantly increased by leptin ($F_{1,20}$=121.367, P<0.001; Fig. 3D). We observed serum T3 levels were not affected by leptin and cold in gerbils. Serum T4 ($F_{1,20}$=13.586, P=0.001) levels and the T3/T4 ratio ($F_{1,20}$=12.362, P=0.002) were significantly affected by cold, but not leptin (Fig. 3G,H). Furthermore, we measured the expression of leptin receptors (LepR) in different tissues and found that cold temperature significantly decreased LepR in BAT ($F_{1,20}$=5.675, P=0.027; Fig. 3J), and leptin significantly increased LepR expression in small intestine ($F_{1,20}$=5.986, P=0.024; Fig. 3K), but there were no significant differences in LepR in the hypothalamus and skeletal muscle.

Western blot analysis revealed that chronic leptin treatment did not affect the expression of thermogenic genes and proteins, including uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor γ (PPARγ) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (Fig. 4A–C). Cold temperature significantly increased the expression of UCP1 protein ($F_{1,20}$=23.329, P<0.001; Fig. 4A) and significantly decreased the expression of PPARγ protein ($F_{1,20}$=8.217, P=0.01; Fig. 4B) of BAT. Besides, we also measured the above thermogenic genes in WAT, and found that leptin did not affect the expression of these thermogenic genes in WAT (Fig. 4D–H). Furthermore, we also measured the NST in skeletal muscle and found that leptin did not affect UCP1, UCP3 and SERCA1 (Fig. 4I–K) protein expression in skeletal muscle.

TRPV2 and gut microbiota may be involved in regulating chronic leptin-induced elevation of body temperature

TRPV1, TRPV2, TRPV4, TRPM2, TRPM8 and TRPA1 were all present in the hypothalamus, BAT and small intestine of Mongolian gerbils. In the hypothalamus, there was a significant interaction between leptin treatment and cold on TRPV4 ($F_{1,20}$=5.517, P=0.034; Fig. 5C). Cold increased the expression of TRPV4 ($F_{1,20}$=4.553, P=0.045; Fig. 5I) and decreased the expression of TRPM2 ($F_{1,20}$=15.617, P<0.001; Fig. 5J) in BAT, whereas leptin had no effect on BAT thermo-TRPs. We detected the expression of thermo-TRPs in the small intestine as well. Our results indicated that leptin increased expression of TRPV2 in the small intestine

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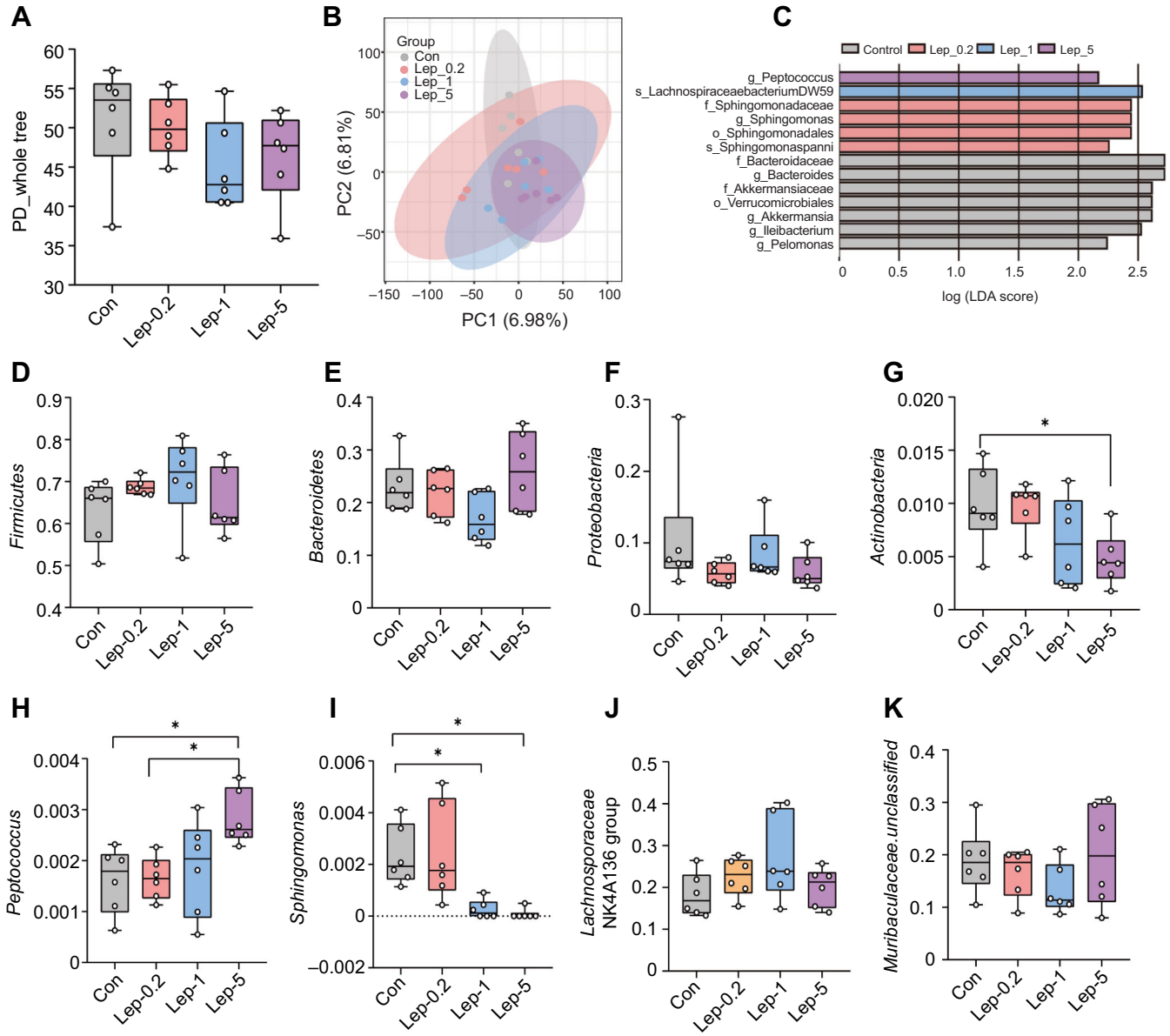

Fig. 2. The effects of acute leptin treatment on gut microbiota in Mongolian gerbils. Alpha diversity (PD_whole tree) of bacterial communities across groups. NMDS plot based on unweighted UniFrac distance metrics representing the differences in fecal microbial community structure in different groups (ANOSIM). (C) Differential bacterial taxonomy selected by LEfSe analysis with LDA score >2 in the cecal microbiota community of the four groups with different injection concentrations. (D–G) Relative abundance at the phylum level of acute leptin administration. (H–K) Relative abundance at the genus level of acute leptin administration. Control, gerbils with 200 µl saline intraperitoneal injection; Lep-0.2, gerbils with 0.2 mg kg^{-1} leptin intraperitoneal injection; Lep-1, gerbils with 1 mg kg^{-1} leptin intraperitoneal injection; Lep-5, gerbils with 5 mg kg^{-1} leptin intraperitoneal injection. *P<0.05, significant difference between groups, n=6 for each group.

($F_{1,20}$=4.918, P=0.04; Fig. 5N), and the expression of TRPV1 was decreased ($F_{1,20}$=4.558, P=0.045; Fig. 5M) and TRPV4 was increased ($F_{1,20}$=4.591, P=0.045; Fig. 5O) at cold temperature. Pearson correlation analysis was conducted on relevant data in the hypothalamus, BAT and small intestine. The protein content of TRPV1, TRPV2 and TRPM2 in the small intestine was significantly positively correlated with serum leptin concentration (TRPV1: r=0.415, P=0.044; TRPV2: r=0.453, P=0.026; TRPM2: r=0.462, P=0.023; Table S4). However, serum leptin concentration was not significantly correlated with thermo-TRPs in the hypothalamus or BAT (Tables S2 and S3).

Chronic leptin administration did not affect either α or β diversity of gut microbiota, but did alter the composition of the gut microbiota (Fig. 6A,B). LEfSe methods identified the different microbial communities in the four groups (Fig. 6I). At the phyla level, cold significantly increased *Verrucomicrobia* ($F_{1,20}$=13.328, P=0.002; Fig. 6F) and decreased *Actinobacteria* ($F_{1,20}$=17.543, P<0.001; Fig. 6G), and the interaction of cold and leptin affected the level of *Proteobacteria* ($F_{1,20}$=11.054, P=0.003; Fig. 6H). At the genus level, leptin significantly increased the proportions of *Peptococcus* ($F_{1,20}$=17.543, P=0.023; Fig. 7A) and decreased the proportions of *Akkermansia* ($F_{1,20}$=17.543, P=0.018; Fig. 7B). Further, the proportions of *Roseburia* (cold: $F_{1,20}$=7.827, P=0.011, leptin: $F_{1,20}$=5.46, P=0.03; Fig. 7C) and *RuminococcaceaeUCG_014* (cold: $F_{1,20}$=10.289, P=0.004, leptin: $F_{1,20}$=11.211, P=0.003; Fig. 7D) were decreased by cold and leptin. In addition, cold temperature and

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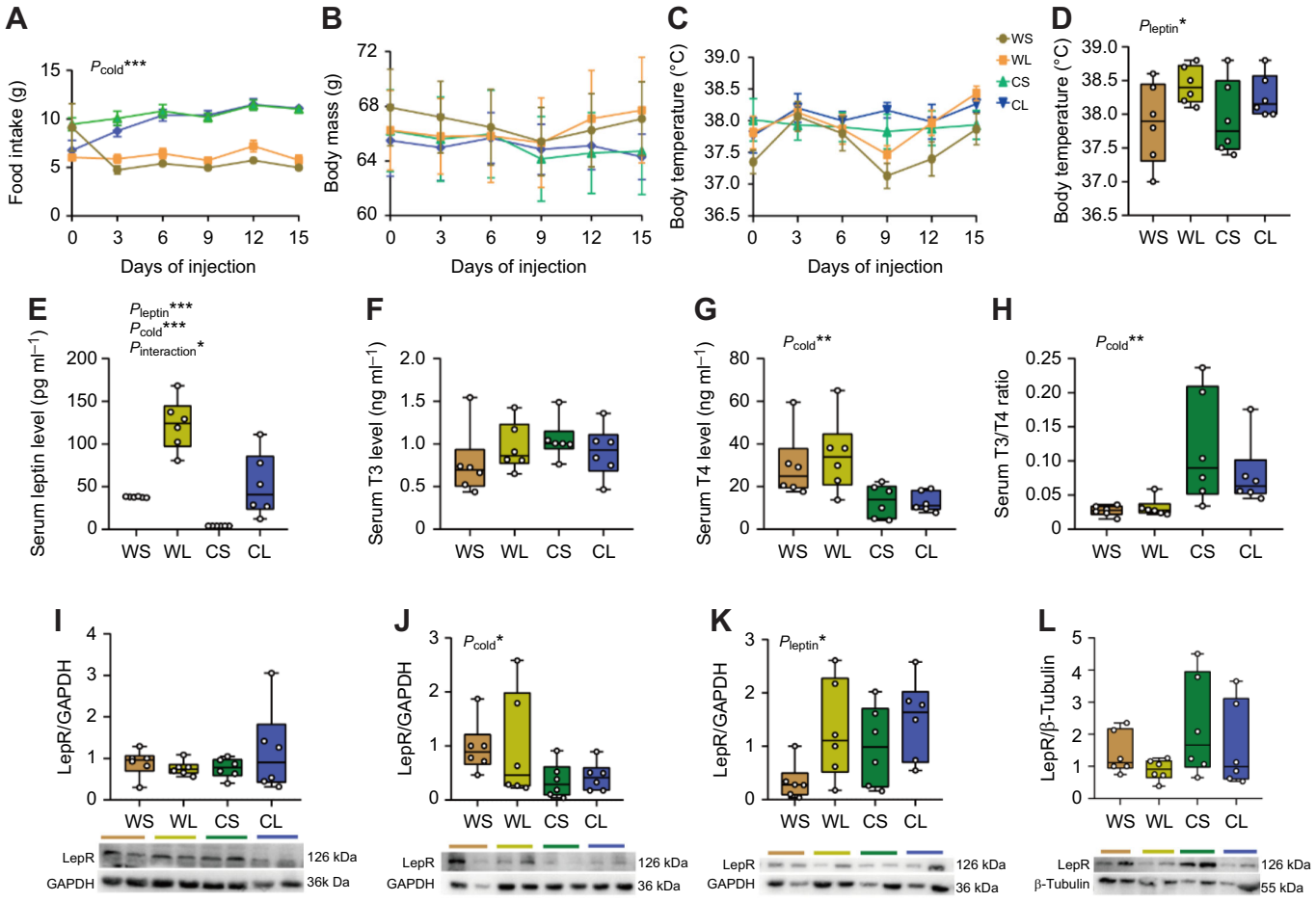

Fig. 3. The effects of chronic leptin treatment on food intake, body mass, body temperature and serum hormones in Mongolian gerbils. (A–C) Food intake, body mass and body temperature during 14 consecutive injections. (D) Body temperature after the last injection. (E) The content of leptin hormones in the serum. (F–H) The content of thyroid hormones in the serum. (I) The expression of LepR protein in the hypothalamus. (J) The expression of LepR protein in BAT. (K) The expression of LepR protein in the small intestine. (L) The expression of LepR protein in skeletal muscle. WS, gerbils kept at 23°C with saline; WL, gerbils kept at 23°C with leptin; CS, gerbils kept at 4°C with saline; CL, gerbils kept at 4°C with leptin. P_{cold}, significant effect of cold; P_{leptin}, significant effect of leptin; $P_{interaction}$, significant effect of the interaction of leptin and cold (*P<0.05, **P<0.01, ***P<0.001; n=6 for each group).

the interaction between cold temperature and leptin significantly affected the proportion of *Paracaedibacteraceae.unclassified* (cold: $F_{1,20}$=15.937, P<0.001; interaction: $F_{1,20}$=4.901, P=0.039; Fig. 7E) and *Angelakisella* (cold: $F_{1,20}$=5.592, P=0.028; interaction: $F_{1,20}$=4.977, P=0.037; Fig. 7F).

DISCUSSION

In the present study, we found that 1 mg kg^{-1} acute leptin treatment significantly increased the body temperature and the expression of anorexic neuropeptide gene in gerbils. The 5 mg kg^{-1} acute leptin group also significantly increased body temperature, and the expression of POMC, UCP1 and PPARγ was higher than that of the control group. However, in the chronic leptin treatment experiment, we found that the accumulated leptin treatment eventually led to the increase of body temperature, but it did not stimulate the NST of BAT, WAT and skeletal muscle, and gut microbiota and TRPV2 in the small intestine might be involved.

The major function of leptin is the modulation of food intake (Zhang and Chua, 2018), along with energy homeostasis (Cowley et al., 2001). Previous studies have shown that the increase in energy expenditure by leptin is dependent on sympathetic innervation of BAT, thereby increasing thermogenesis of the BAT, including increasing the expression of UCP (Scarpace and Matheny, 1998; Scarpace et al., 1997). Some studies support that leptin stimulates a hypothalamus–adrenal medulla–BAT axis, which is necessary and sufficient to induce lipolysis and, as a result, increase body temperature (de Git et al., 2019; Perry et al., 2020). Other studies have found that the thermogenic effect of leptin is affected by ambient temperature (Yang et al., 2011; Fisher et al., 2016; Kaiyala et al., 2016). ob/ob mice injected with leptin below the thermoneutral temperature (22°C or 14°C) significantly increased the body temperature. However, ob/ob mice injected with leptin at thermoneutral temperature did not change their core body temperature, but UCP1 mRNA expression increased significantly (Kaiyala et al., 2016). Another study has suggested that leptin stimulates thermogenesis when interacting with other hormones, such as insulin (Davis et al., 2010). However, the thermogenic effect of leptin has been controversial (Fischer et al., 2020). Some studies suggest that leptin does not modify body temperature (Enriori et al., 2011; Tang et al., 2015). Furthermore, some research has indicated that the decrease in core temperature of leptin-deficient mice is caused by increased heat loss (i.e. increased thermal conductivity) in these animals, and not NST defects (Fishman and Dark, 1987; Kaiyala et al., 2016), suggesting that leptin may have a pyrexic effect rather than a thermic effect. Therefore, leptin may affect thermoregulation through vasomotor or heat conduction mechanisms, rather than by directly affecting BAT thermogenesis. In addition, some studies

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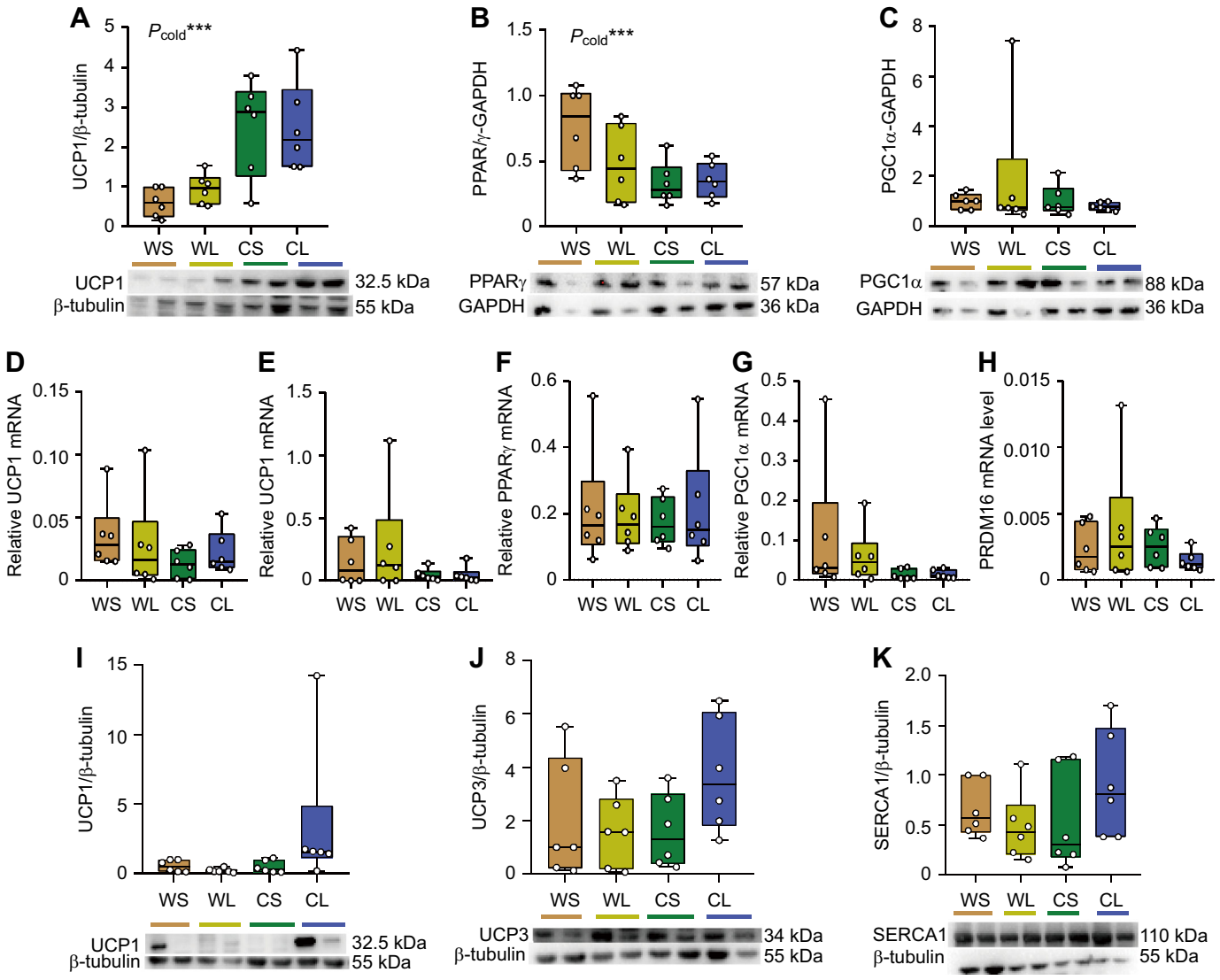

Fig. 4. The effects of chronic leptin treatment on the expression of molecular thermogenic markers on different tissue of Mongolian gerbils. (A–C) The protein expression of UCP1, PPARγ and PGC1α in BAT. (D–H) The mRNA expression of UCP1, PPARα, PPARγ, PGC1α and PRDM16 in WAT. (I-K) The protein expression of UCP1, UCP3 and SERCA1 in skeletal muscle. WS, gerbils kept at 23°C with saline; WL, gerbils kept at 23°C with leptin; CS, gerbils kept at 4°C with saline; CL, gerbils kept at 4°C with leptin. P_{cold}, significant effect of cold (*P<0.05, ***P<0.001; n=6 for each group).

suggest that the effect of exogenous leptin therapy does not accurately reflect endogenous physiology. For example, animals fed a high-fat diet (HFD) are resistant to exogenous leptin, but remain fully sensitive to endogenous leptin (Ottaway et al., 2015). That is, the development of leptin resistance could raise the set point for leptin in specific neurons, causing neurons to require a higher concentration of leptin to activate downstream signaling (Caron et al., 2018).

Over the past few years, studies have found that UCP1 was expressed not exclusively in BAT, but also in WAT, and subsequently confirmed the thermogenic potential of WAT browning process (Commins et al., 1999; Wu et al., 2012). Previous studies demonstrated that leptin treatment induced UCP1 expression and enhanced the browning of WAT in mice (Commins et al., 1999, 2000). However, our results demonstrated that leptin did not promote the browning of WAT in gerbils. Consistent with our results, Dodd et al. (2015) demonstrated that leptin ICV infusion alone had little effect on browning, whereas the co-infusion of insulin and leptin strikingly enhanced browning, suggesting that the effect of leptin on WAT browning may need be realized through the interaction with other factors.

Skeletal muscle is an important part of whole-body energy expenditure, accounting for 20–30% of the total resting state oxygen uptake (Mainieri et al., 2006). Furthermore, there is evidence of developmental overlap and cross-talk between BAT and skeletal muscle (Pani et al., 2022), and skeletal muscle is also considered to be one of the most important tissues in regulating NST (Liu et al., 2022b). Studies have confirmed that leptin can stimulate glucose metabolism and fatty acid metabolism in skeletal muscle (Minokoshi et al., 2012), and the thermogenic function of leptin in skeletal muscle requires LepR and phosphatidylinositol 3-kinase signaling (Dulloo et al., 2002). However, the effect of leptin on skeletal muscle NST remains unclear. Based on the fact that LepR is expressed in skeletal muscle (Tartaglia et al., 1995), we investigated whether leptin can directly stimulate NST in skeletal muscle of Mongolian gerbils, but our results found that leptin supplementation did not affect the expression of NST-related proteins in skeletal muscle of Mongolian gerbils. However, studies in HFD mice have shown that leptin induces NST in oxidative muscle through the leptin–AMPK axis (Kus et al., 2008).

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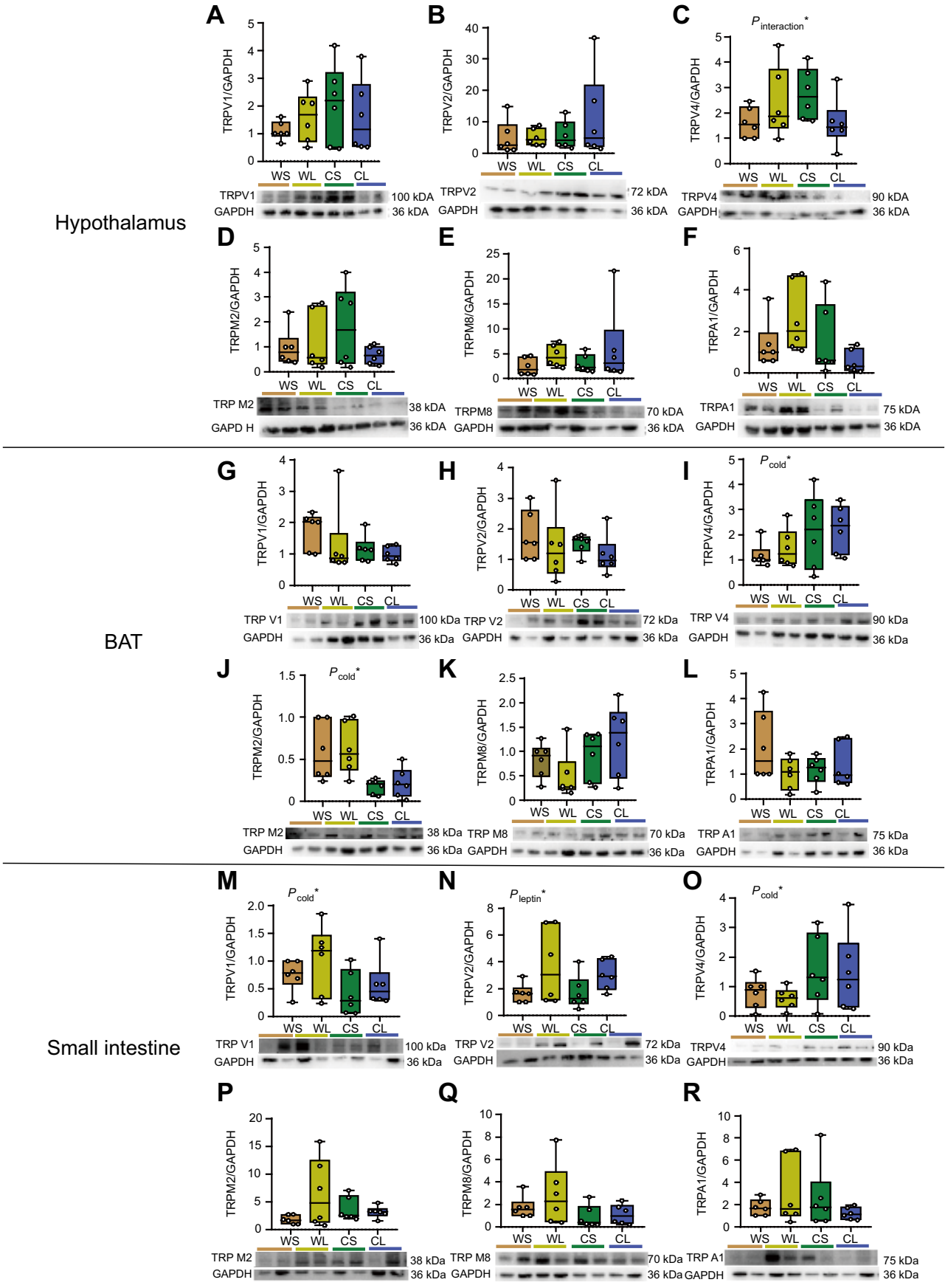

Fig. 5. The effects of chronic leptin treatment on expression of thermo-TRPs in the hypothalamus, BAT and small intestine in Mongolian gerbils. (A–F) The expression of thermo-TRP proteins in hypothalamus. (G–L) The expression of thermo-TRP proteins in BAT. (M–R) The expression of thermo-TRPs proteins in small intestine. WS, gerbils kept at 23°C with saline; WL, gerbils kept at 23°C with leptin; CS, gerbils kept at 4°C with saline; CL, gerbils kept at 4°C with leptin. P_{cold}, significant effect of cold; P_{leptin}, significant effect of leptin; $P_{interaction}$, significant effect of the interaction of leptin and cold (*P<0.05; n=6 for each group).

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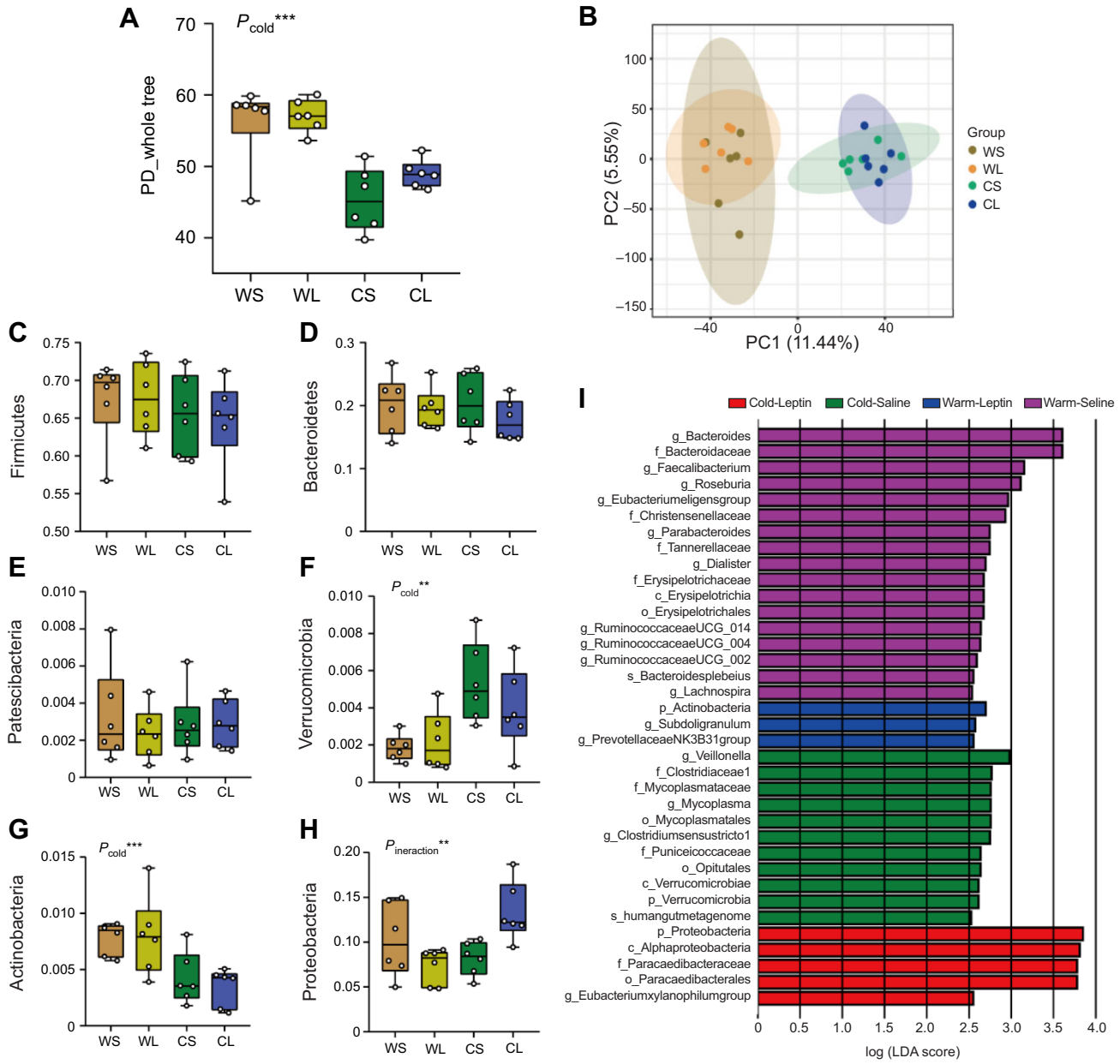

Fig. 6. The effects of chronic leptin treatment on bacterial diversity and composition of cecum microbiota at the phylum level in Mongolian gerbils. (A) Alpha diversity (PD_whole tree) of bacterial communities across groups. (B) NMDS plot based on unweighted UniFrac distance metrics representing the differences in fecal microbial community structure in four groups (ANOSIM). (C–H) Relative abundance at the phylum of chronic leptin administration. (I) Differential bacterial taxonomy selected by LEfSe analysis with LDA score >2.5 in the cecal microbiota community of the four groups with different injection concentration. WS, gerbils kept at 23°C injected by saline; WL, gerbils kept at 23°C injected by leptin; CS, gerbils kept at 4°C injected by saline; CL, gerbils kept at 4°C injected by leptin. P_{cold}, significant effect of cold; $P_{interaction}$, significant effect of the interaction of leptin and cold (**P<0.01, ***P<0.001; n=6 for each group).

Thyroid hormone regulates a wide range of physiological processes after its activation from the prohormone thyroxine (T4) to the active form, triiodothyronine (T3) (Bianco et al., 2005; Obregon, 2014). Studies have shown that thyroid hormone plays an important role in the physiological effects of leptin (Kristensen et al., 1999; Feldt-Rasmussen, 2007; Wang et al., 2011). For example, the effect of thyroid hormone on leptin production in primary cultured rat brown and white adipocytes was studied (Medina-Gomez et al., 2004). In this experiment, T3 was added to the primary culture, and leptin hormone and mRNA levels were measured. The results showed that T3 inhibited leptin in a dose-dependent manner. In addition, some studies have shown that leptin is related to the hypothalamic–pituitary–thyroid axis. Circulating TSH and thyroid hormone levels decreased after leptin treatment in fasting rats (Ahima et al., 1996; Mantzoros et al., 2001). And leptin administration prevents the fasting-induced changes of TSH secretion and results in a slight increase of FT4 (Chan et al., 2003). However, in our results, cold temperature significantly increased the level of thyroid hormone, but leptin did not have a significant effect on thyroid hormone, which may be because the effect of cold temperature on thyroid hormone is extremely significant (Rezai-Zadeh and Münzberg, 2013), so the effect of leptin is not so prominent.

Many studies have shown that leptin of species living in cold regions has the specificity to adapt to cold. For example, Yang et al. (2011)

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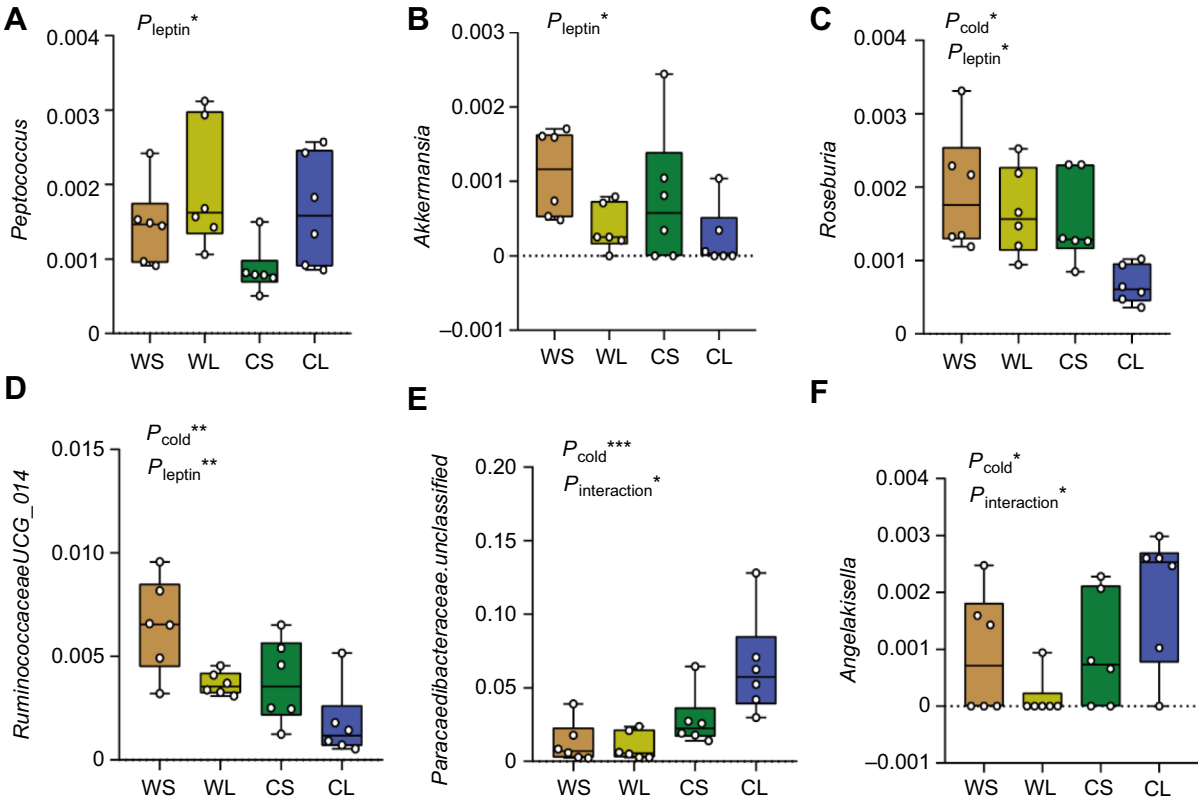

Fig. 7. The effects of chronic leptin treatment on composition of cecal microbiota at the genus level in Mongolian gerbils. Relative abundance of (A) *Peptococcus*, (B) *Akkermansia*, (C) *Roseburia*, (D) *RuminococcaceaeUCG_014*, (E) *Paracaedibacteraceae.unclassified* and (F) *Angelakisella* in the fecal microbiota community of the four groups at genus level. WS, gerbils kept at 23°C injected by saline; WL, gerbils kept at 23°C injected by leptin; CS, gerbils kept at 4°C injected by saline; CL, gerbils kept at 4°C injected by leptin. P_{cold}, significant effect of cold; P_{leptin}, significant effect of leptin; $P_{interaction}$, significant effect of the interaction of leptin and cold (*P<0.05, **P<0.01, ***P<0.001; n=6 for each group).

found that pika leptin demonstrates a superior induced capacity for adaptive thermogenesis, which is reflected in a more enhanced β-oxidation, mitochondrial biogenesis and heat production. Moreover, leptin treatment combined with cold stimulation has a significant synergistic effect on adaptive thermogenesis (Yang et al., 2011). Likewise, leptin seems to be one of the factors responsible for enhancing the survival of raccoon dogs (*Nyctereutes procyonoides*) and blue foxes (*Alopex lagopus*) in wintering (Nieminen et al., 2001), suggesting that leptin has evolved in thermogenic function to enhance the survival of wild animals in their natural environment.

Our results indicated that six thermo-TRPs were expressed in the hypothalamus, BAT and small intestine. It has been shown that thermosensitive TRPs in the hypothalamus can sense slight changes in temperature; for example, the activation temperature of TRPM2 in preoptic area (POA) neurons is slightly higher than the physiological temperature of 37°C, which allows hypothalamic TRPM2 to mediate responses to temperature increases in the POA (Kamm et al., 2021; Song et al., 2016). However, our study lacked measurements of temperature in specific brain regions, and although our results showed that leptin significantly increased body temperature in gerbils, it had little effect on thermo-TRP expression in the hypothalamus. Likewise, leptin had no impact on the thermo-TRPs in BAT. It is worth noting that our results showed that a leptin-induced rise in body temperature increased TRPV2 expression in the small intestine. TRPV2 is activated by non-physiological heat exceeding 50°C, but oxidation reduces the temperature threshold for the activation of TRPV2 to body temperature (37°C) or even lower (Fricke et al., 2019). Moreover, researchers unveiled the role of intestinal cells in thermosensation (Xiao et al., 2013). Activation of TRPV2 induces the release of NO from intestinal inhibitory motor neurons, thereby relaxing intestinal tone and promoting intestinal motility and contributing to gastrointestinal transport *in vivo* (Mihara et al., 2010).

Accumulating data show that the gut microbiota is a vital endogenous factor in regulating thermoregulation (Bo et al., 2019; Li et al., 2019), including browning in WAT and NST in BAT (Xu et al., 2020). Our study found that acute treatment with a high concentration of leptin changed the composition and structure of gut microbiota and increased the expression of UCP1 protein in BAT compared with the control group. A previous study found that leptin directly acts on the intestinal epithelial cells, potentially by controlling the expression of gut antimicrobial peptides (AMPs), which, secreted by Paneth cells, represent a major mechanism by which the host influences the gut microbiome (Rajala et al., 2014). Metabolites secreted by gut microbiota act on BAT through the free fatty acid receptor-2 [FFAR2; a receptor for short chain fatty acid (SCFAs)] and Takeda-G-protein-receptor-5 (TGR5; a receptor for bile acids), then activates the cAMP–PKA–pCREB signaling pathway in BAT and finally increases the expression of UCP1 protein in BAT (Liu et al., 2022a; Zhang and Wang, 2022). However, in our CL experiment, although leptin affected T_b and the composition of gut microbiota to a certain extent, it did not result in changes in BAT and WAT thermogenic markers. One possible explanation for this is that gut microbiota can directly warm their host; in fact, Rosenberg and Zilber-Rosenberg (2016) explored in

detail the relationship between gut microbes and body temperature. Like all cells, microbes produce heat as long as they are alive, and they convert heat more efficiently than animals, for example, microbial metabolism in the gut produces 61 kcal h^{-1}, which corresponds to approximately 70% of the total heat production of an average person at rest (Rosenberg and Zilber-Rosenberg, 2016). Another explanation may be the microbial metabolites. Numerous studies have shown that microbial metabolites might play an important role in the crosstalk between gut microbiota and other metabolic organs, especially the SCFAs (Zhou et al., 2019; Ramos Meyers et al., 2022). The major SCFAs in serum and the caecum included acetate, propionate and butyrate, which have been demonstrated to play important roles in thermogenesis (Chambers et al., 2018). For example, it has been previously shown that the bacteria belonging to *Akkermansia* and *Ruminococcus* genera produce SCFAs, as acetate, to activate BAT thermogenesis and promote WAT browning in mice (Hu et al., 2016). However, SCFAs were not measured in our study, and remain a future research topic.

Our study found that AL treatment significantly decreased the *Actinobacteria* proportion at phylum level, and significantly increased the level of *Peptococcus* and decreased the level of *Sphingmonas* at genus level. In the CL treatment, gerbils administrated with leptin had lower levels of *Akkermansia*, *Roseburia* and *RuminnococcaceaeUCG_014*, and had higher levels of *Peptococcus*. The majority of microbes forming the gut microbiota can be assigned to four major phyla: *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* (Arumugam et al., 2011). *Firmicutes* and *Bacteroidetes* accounted for more than 90% of the relative abundance of the gut microbiota, with *Actinobacteria* and *Proteobacteria* accounting for the remaining 10% (Segata et al., 2012). Although they represent only a small percentage, *Actinobacteria* are pivotal in the maintenance of gut homeostasis (Binda et al., 2018). Using metabolomics and 16S rRNA gene sequencing analyses in the rat model, Wen et al. (2019) showed a correlation between *Peptococcus* and key metabolic pathways, also including tryptophan metabolism and tyrosine metabolism. *Roseburia* produces butyrate in the colon, and has been shown to prevent intestinal inflammation and maintain energy homeostasis by producing metabolites (Nie et al., 2021). Some studies have reported that *Akkermansia muciniphila* increases thermogenesis and glucagon-like peptide-1 (GLP-1) secretion in HFD-induced C57BL/6J mice by induction of UCP1 in BAT and systemic GLP-1 secretion (Depommier et al., 2019; Yoon et al., 2021), and the beneficial metabolic effects of *Akkermansia muciniphila* could partly be due to the attenuation in intestinal absorptive functions, promoting a situation of caloric restriction in the mammalian host (Moreno-Navarrete and Fernandez-Real, 2019). Further, another study showed a negative correlation of the relative abundance of *Akkermansia* and *Ruminococcus* genera with cold-induced BAT volume and activity (Ortiz-Alvarez et al., 2023). *Sphingomonas* of the *Proteobacteria* phylum plays the most important role as a bacterium, and is positively correlated with metabolites related to protein digestion and absorption (Wen et al., 2021).

In summary, our study found the particular physiological significance of Mongolian gerbils containing high serum leptin, as well as its possible underlying underlying mechanism. Hyperleptin can cause gerbils to have a higher body temperature, which is not achieved by activating BAT thermogenesis, but TRPV2 and gut microbiota were implicated. Subsequent studies should further explore the influence of leptin on intestinal metabolites, further reveal the specific mechanism of leptin's influence on body temperature, and refine the results of the hypothalamic region, focusing on POA. In addition, the relationship between TRPV2 and the intestine still warrants further study.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: L.T., X.Z., D.W.; Methodology: L.T., J.L.; Software: L.T.; Formal analysis: L.T.; Investigation: L.T.; Data curation: L.T., J.L.; Writing – original draft: L.T.; Writing – review & editing: L.T., X.Z., D.W.; Visualization: L.T.; Supervision: X.Z., C.-Z.W., D.W.; Funding acquisition: D.W.

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Data availability
All relevant data can be found within the article and its supplementary information.

ECR Spotlight
This article has an associated ECR Spotlight interview with Liqiu Tang.

References
Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E. and Flier, J. S. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250-252. doi:10.1038/382250a0

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J.-M. et al. (2011). Enterotypes of the human gut microbiome. *Nature* **473**, 174-180. doi:10.1038/nature09944

Avraham, Y., Davidi, N., Porat, M., Chernoguz, D., Magen, I., Vorobeiv, L., Berry, E. M. and Leker, R. R. (2010). Leptin reduces infarct size in association with enhanced expression of CB2, TRPV1, SIRT-1 and leptin receptor. *Curr. Neurovasc Res.* **7**, 136-143. doi:10.2174/156720210791184943

Bal, N. C., Maurya, S. K., Sopariwala, D. H., Sahoo, S. K., Gupta, S. C., Shaikh, S. A., Pant, M., Rowland, L. A., Bombardier, E., Goonasekera, S. A. et al. (2012). Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat. Med.* **18**, 1575-1579. doi:10.1038/nm.2897

Bal, N. C., Maurya, S. K., Singh, S., Wehrens, X. H. T. and Periasamy, M. (2016). Increased reliance on muscle-based thermogenesis upon acute minimization of brown adipose tissue function. *J. Biol. Chem.* **291**, 17247-17257. doi:10.1074/jbc.M116.728188

Bianco, A. C., Maia, A. L., da Silva, W. S. and Christoffolete, M. A. (2005). Adaptive activation of thyroid hormone and energy expenditure. *Biosci. Rep.* **25**, 191-208. doi:10.1007/s10540-005-2885-6

Binda, C., Lopetuso, L. R., Rizzatti, G., Gibiino, G., Cennamo, V. and Gasbarrini, A. (2018). Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig. Liver Dis.* **50**, 421-428. doi:10.1016/j.dld.2018.02.012

Bo, T.-B., Zhang, X.-Y., Wen, J., Deng, K., Qin, X.-W. and Wang, D.-H. (2019). The microbiota–gut–brain interaction in regulating host metabolic adaptation to cold in male Brandt's voles (*Lasiopodomys brandtii*). *ISME J.* **13**, 3037-3053. doi:10.1038/s41396-019-0492-y

Buonfiglio, D., Parthimos, R., Dantas, R., Cerqueira Silva, R., Gomes, G., Andrade-Silva, J., Ramos-Lobo, A., Amaral, F. G., Matos, R., Sinésio, J. et al. (2018). Melatonin absence leads to long-term leptin resistance and overweight in rats. *Front. Endocrinol. (Lausanne)* **9**, 122. doi:10.3389/fendo.2018.00122

Caron, A., Lee, S., Elmquist, J. K. and Gautron, L. (2018). Leptin and brain–adipose crosstalks. *Nat. Rev. Neurosci.* **19**, 153-165. doi:10.1038/nrn.2018.7

Ceddia, R. B., William, W. N. and Curi, R. (2001). The response of skeletal muscle to leptin. *Front. Biosci.* **6**, D90-D97. doi:10.2741/ceddia

Chambers, E. S., Preston, T., Frost, G. and Morrison, D. J. (2018). Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. *Curr. Nutr. Rep.* **7**, 198-206. doi:10.1007/s13668-018-0248-8

Chan, J. L., Heist, K., DePaoli, A. M., Veldhuis, J. D. and Mantzoros, C. S. (2003). The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J. Clin. Invest.* **111**, 1409-1421. doi:10.1172/JCI200317490

Chua, S. C., Chung, W. K., Wu-Peng, X. S., Zhang, Y., Liu, S. M., Tartaglia, L. and Leibel, R. L. (1996). Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* **271**, 994-996. doi:10.1126/science.271.5251.994

Chua, S. C., Koutras, I. K., Han, L., Liu, S. M., Kay, J., Young, S. J., Chung, W. K. and Leibel, R. L. (1997). Fine structure of the murine leptin receptor gene: splice

site suppression is required to form two alternatively spliced transcripts. *Genomics* **45**, 264-270. doi:10.1006/geno.1997.4962

Collins, S., Kuhn, C. M., Petro, A. E., Swick, A. G., Chrunyk, B. A. and Surwit, R. S. (1996). Role of leptin in fat regulation. *Nature* **380**, 677. doi:10.1038/380677a0

Commins, S. P., Watson, P. M., Padgett, M. A., Dudley, A., Argyropoulos, G. and Gettys, T. W. (1999). Induction of uncoupling protein expression in brown and white adipose tissue by leptin. *Endocrinology* **140**, 292-300. doi:10.1210/endo.140.1.6399

Commins, S. P., Watson, P. M., Levin, N., Beiler, R. J. and Gettys, T. W. (2000). Central leptin regulates the UCP1 and ob genes in brown and white adipose tissue via different beta-adrenoceptor subtypes. *J. Biol. Chem.* **275**, 33059-33067. doi:10.1074/jbc.M006328200

Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdán, M. G., Diano, S., Horvath, T. L., Cone, R. D. and Low, M. J. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411**, 480-484. doi:10.1038/35078085

Davis, J. F., Choi, D. L. and Benoit, S. C. (2010). Insulin, leptin and reward. *Trends Endocrinol. Metab.* **21**, 68. doi:10.1016/j.tem.2009.08.004

de Git, K. C. G., den Outer, J. A., Wolterink-Donselaar, I. G., Luijendijk, M. C. M., Schéle, E., Dickson, S. L. and Adan, R. A. H. (2019). Rats that are predisposed to excessive obesity show reduced (leptin-induced) thermoregulation even in the preobese state. *Physiol. Rep.* **7**, e14102.

Denis, R. G. P., Williams, G. and Vernon, R. G. (2003). Regulation of serum leptin and its role in the hyperphagia of lactation in the rat. *J. Endocrinol.* **176**, 193-203. doi:10.1677/joe.0.1760193

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., Falony, G., Raes, J., Maiter, D., Delzenne, N. M. et al. (2019). Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat. Med.* **25**, 1096-1103. doi:10.1038/s41591-019-0495-2

Dodd, G. T., Decherf, S., Loh, K., Simonds, S. E., Wiede, F., Balland, E., Merry, T. L., Münzberg, H., Zhang, Z.-Y., Kahn, B. B. et al. (2015). Leptin and insulin act on POMC neurons to promote the browning of white fat. *Cell* **160**, 88-104. doi:10.1016/j.cell.2014.12.022

Duggal, P., Guo, X., Haque, R., Peterson, K. M., Ricklefs, S., Mondal, D., Alam, F., Noor, Z., Verkerke, H. P., Marie, C. et al. (2011). A mutation in the leptin receptor is associated with *Entamoeba histolytica* infection in children. *J. Clin. Invest.* **121**, 1191-1198. doi:10.1172/JCI45294

Dulloo, A. G., Stock, M. J., Solinas, G., Boss, O., Montani, J. P. and Seydoux, J. (2002). Leptin directly stimulates thermogenesis in skeletal muscle. *FEBS Lett.* **515**, 109-113. doi:10.1016/s0014-5793(02)02449-3

Enriori, P. J., Sinnayah, P., Simonds, S. E., Garcia Rudaz, C. and Cowley, M. A. (2011). Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. *J. Neurosci.* **31**, 12189-12197. doi:10.1523/JNEUROSCI.2336-11.2011

Feldt-Rasmussen, U. (2007). Thyroid and leptin. *Thyroid* **17**, 413-419. doi:10.1089/thy.2007.0032

Fischer, A. W., Hoefig, C. S., Abreu-Vieira, G., de Jong, J. M. A., Petrovic, N., Mittag, J., Cannon, B. and Nedergaard, J. (2016). Leptin raises defended body temperature without activating thermogenesis. *Cell Rep* **14**, 1621-1631. doi:10.1016/j.celrep.2016.01.041

Fischer, A. W., Cannon, B. and Nedergaard, J. (2020). Leptin: is it thermogenic? *Endocr. Rev.* **41**, 232-260. doi:10.1210/endrev/bnz016

Fishman, R. B. and Dark, J. (1987). Sensory innervation of white adipose tissue. *Am. J. Physiol.* **253**, R942-R944.

Fricke, T. C., Echtermeyer, F., Zielke, J., de la Roche, J., Filipovic, M. R., Claverol, S., Herzog, C., Tominaga, M., Pumroy, R. A., Moiseenkova-Bell, V. Y. et al. (2019). Oxidation of methionine residues activates the high-threshold heat-sensitive ion channel TRPV2. *Proc. Natl. Acad. Sci. USA* **116**, 24359-24365. doi:10.1073/pnas.1904332116

Grases-Pintó, B., Abril-Gil, M., Castell, M., Rodríguez-Lagunas, M. J., Burleigh, S., Fåk Hållenius, F., Prykhodko, O., Pérez-Cano, F. J. and Franch, À. (2019). Influence of leptin and adiponectin supplementation on intraepithelial lymphocyte and microbiota composition in suckling rats. *Front. Immunol.* **10**, 2369. doi:10.3389/fimmu.2019.02369

Guo, X., Roberts, M. R., Becker, S. M., Podd, B., Zhang, Y., Chua, S. C., Myers, M. G., Duggal, P., Houpt, E. R. and Petri, W. A. (2011). Leptin signaling in intestinal epithelium mediates resistance to enteric infection by *Entamoeba histolytica*. *Mucosal Immunol.* **4**, 294-303. doi:10.1038/mi.2010.76

Heiss, C. N., Mannerås-Holm, L., Lee, Y. S., Serrano-Lobo, J., Håkansson Gladh, A., Seeley, R. J., Drucker, D. J., Bäckhed, F. and Olofsson, L. E. (2021). The gut microbiota regulates hypothalamic inflammation and leptin sensitivity in Western diet-fed mice via a GLP-1R-dependent mechanism. *Cell Reports* **35**, 109163. doi:10.1016/j.celrep.2021.109163

Hermann, G. E., Barnes, M. J. and Rogers, R. C. (2006). Leptin and thyrotropin-releasing hormone: cooperative action in the hindbrain to activate brown adipose thermogenesis. *Brain Res.* **1117**, 118-124. doi:10.1016/j.brainres.2006.08.018

Hoffstaetter, L. J., Bagriantsev, S. N. and Gracheva, E. O. (2018). TRPs et al.: a molecular toolkit for thermosensory adaptations. *Pflugers Arch.* **470**, 745-759. doi:10.1007/s00424-018-2120-5

Hu, J., Kyrou, I., Tan, B. K., Dimitriadis, G. K., Ramanjaneya, M., Tripathi, G., Patel, V., James, S., Kawan, M., Chen, J. et al. (2016). Short-chain fatty acid acetate stimulates adipogenesis and mitochondrial biogenesis via GPR43 in brown adipocytes. *Endocrinology* **157**, 1881-1894. doi:10.1210/en.2015-1944

Kaiyala, K. J., Ogimoto, K., Nelson, J. T., Muta, K. and Morton, G. J. (2016). Physiological role for leptin in the control of thermal conductance. *Mol. Metab.* **5**, 892-902. doi:10.1016/j.molmet.2016.07.005

Kamm, G. B., Boffi, J. C., Zuza, K., Nencini, S., Campos, J., Schrenk-Siemens, K., Sonntag, I., Kabaoglu, B., El Hay, M. Y. A., Schwarz, Y. et al. (2021). Darby. *Neuron* **109**, 3283-3297.e11. doi:10.1016/j.neuron.2021.10.001

Kristensen, K., Pedersen, S. B., Langdahl, B. L. and Richelsen, B. (1999). Regulation of leptin by thyroid hormone in humans: studies in vivo and in vitro. *Metabolism* **48**, 1603-1607. doi:10.1016/s0026-0495(99)90252-4

Kus, V., Prazak, T., Brauner, P., Hensler, M., Kuda, O., Flachs, P., Janovska, P., Medrikova, D., Rossmeisl, M., Jilkova, Z. et al. (2008). Induction of muscle thermogenesis by high-fat diet in mice: association with obesity-resistance. *Am. J. Physiol. Endocrinol. Metabolism* **295**, E356-E367. doi:10.1152/ajpendo.90256.2008

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I. and Friedman, J. M. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**, 632-635. doi:10.1038/379632a0

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D. and Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U.S.A* **102**, 11070-11075. doi:10.1073/pnas.0504978102

Li, X.-S. and Wang, D.-H. (2007). Photoperiod and temperature can regulate body mass, serum leptin concentration, and uncoupling protein 1 in Brandt's voles (*Lasiopodomys brandtii*) and Mongolian gerbils (*Meriones unguiculatus*). *Physiol. Biochem. Zool.* **80**, 326-334. doi:10.1086/513189

Li, X.-S., Wang, D.-H. and Yang, M. (2004). Effects of cold acclimation on body weight, serum leptin level, energy metabolism and thermogenesis in the Mongolian gerbil *Meriones unguiculatus*. *Acta Zool. Sin.* **50**, 334-340.

Li, B., Li, L., Li, M., Lam, S. M., Wang, G., Wu, Y., Zhang, H., Niu, C., Zhang, X., Liu, X. et al. (2019). Microbiota depletion impairs thermogenesis of brown adipose tissue and browning of white adipose tissue. *Cell Rep* **26**, 2720-2737.e5. doi:10.1016/j.celrep.2019.02.015

Liu, X.-Y., Xu, D.-L. and Wang, D.-H. (2012). High body weight associated with impaired nonshivering thermogenesis but improved glucose tolerance in Mongolian gerbils (*Meriones unguiculatus*). *J. Therm. Biol.* **37**, 524-530. doi:10.1016/j.jtherbio.2012.06.005

Liu, W., Yang, G., Liu, P., Jiang, X. and Xin, Y. (2022a). Modulation of adipose tissue metabolism by microbial-derived metabolites. *Front. Microbiol.* **13**, 1031498. doi:10.3389/fmicb.2022.1031498

Liu, M., Zhang, X.-Y., Wang, C.-Z. and Wang, D.-H. (2022b). Recruitment of muscle genes as an effect of brown adipose tissue ablation in cold-acclimated Brandt's voles (*Lasiopodomys brandtii*). *Int. J. Mol. Sci.* **24**, 342. doi:10.3390/ijms24010342

Magoč, T. and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957-2963. doi:10.1093/bioinformatics/btr507

Mainieri, D., Summermatter, S., Seydoux, J., Montani, J.-P., Rusconi, S., Russell, A. P., Boss, O., Buchala, A. J. and Dulloo, A. G. (2006). A role for skeletal muscle stearoyl-CoA desaturase 1 in control of thermogenesis. *FASEB J.* **20**, 1751-1753. doi:10.1096/fj.06-5934fje

Mantzoros, C. S., Ozata, M., Negrao, A. B., Suchard, M. A., Ziotopoulou, M., Caglayan, S., Elashoff, R. M., Cogswell, R. J., Negro, P., Liberty, V. et al. (2001). Synchronicity of frequently sampled thyrotropin (TSH) and leptin concentrations in healthy adults and leptin-deficient subjects: evidence for possible partial TSH regulation by leptin in humans. *J. Clin. Endocrinol. Metab.* **86**, 3284-3291. doi:10.1210/jcem.86.7.7644

Medina-Gomez, G., Calvo, R.-M. and Obregón, M.-J. (2004). T3 and Triac inhibit leptin secretion and expression in brown and white rat adipocytes. *Biochim. Biophys. Acta* **1682**, 38-47. doi:10.1016/j.bbalip.2004.01.007

Mihara, H., Boudaka, A., Shibasaki, K., Yamanaka, A., Sugiyama, T. and Tominaga, M. (2010). Involvement of TRPV2 activation in intestinal movement through nitric oxide production in mice. *J. Neurosci.* **30**, 16536-16544. doi:10.1523/JNEUROSCI.4426-10.2010

Minokoshi, Y., Toda, C. and Okamoto, S. (2012). Regulatory role of leptin in glucose and lipid metabolism in skeletal muscle. *Indian J. Endocrinol. Metab.* **16**, S562-S568. doi:10.4103/2230-8210.105573

Moreno-Navarrete, J. M. and Fernandez-Real, J. M. (2019). The gut microbiota modulates both browning of white adipose tissue and the activity of brown adipose tissue. *Rev. Endocr. Metab. Disord.* **20**, 387-397. doi:10.1007/s11154-019-09523-x

Myers, M. G., Cowley, M. A. and Münzberg, H. (2008). Mechanisms of leptin action and leptin resistance. *Annu. Rev. Physiol.* **70**, 537-556. doi:10.1146/annurev.physiol.70.113006.100707

Nie, K., Ma, K., Luo, W., Shen, Z., Yang, Z., Xiao, M., Tong, T., Yang, Y. and Wang, X. (2021). Roseburia intestinalis: a beneficial gut organism from the discoveries in genus and species. *Front. Cell Infect. Microbiol.* **11**, 757718. doi:10.3389/fcimb.2021.757718

Nieminen, P., Asikainen, J. and Hyvärinen, H. (2001). Effects of seasonality and fasting on the plasma leptin and thyroxin levels of the raccoon dog (*Nyctereutes procyonoides*) and the blue fox (*Alopex lagopus*). *J. Exp. Zool.* **289**, 109-118. doi:10.1002/1097-010X(20010201)289:2<109::AID-JEZ4>3.0.CO;2-I

Nikanorova, A. A., Barashkov, N. A., Nakhodkin, S. S., Pshennikova, V. G., Solovyev, A. V., Romanov, G. P., Kuzmina, S. S., Sazonov, N. N., Burtseva, T. E., Odland, J. Ø. et al. (2020). The role of leptin levels in adaptation to cold climates. *Int. J. Environ. Res. Public Health* **17**, E1854. doi:10.3390/ijerph17061854

Nilius, B. and Flockerzi, V. (2014). Mammalian transient receptor potential (TRP) cation channels. Preface. *Handb. Exp. Pharmacol.* **223**, v-vi.

Nilius, B. and Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome Biol.* **12**, 218. doi:10.1186/gb-2011-12-3-218

Obregon, M.-J. (2014). Adipose tissues and thyroid hormones. *Front. Physiol.* **5**, 479.

Ortiz-Alvarez, L., Acosta, F. M., Xu, H., Sanchez-Delgado, G., Vilchez-Vargas, R., Link, A., Plaza-Díaz, J., Llamas, J. M., Gil, A., Labayen, I. et al. (2023). Fecal microbiota composition is related to brown adipose tissue 18F-fluorodeoxyglucose uptake in young adults. *J. Endocrinol. Invest.* **46**, 567-576. doi:10.1007/s40618-022-01936-x

Ottaway, N., Mahbod, P., Rivero, B., Norman, L. A., Gertler, A., D'Alessio, D. A. and Perez-Tilve, D. (2015). Diet-induced obese mice retain endogenous leptin action. *Cell Metab.* **21**, 877-882. doi:10.1016/j.cmet.2015.04.015

Pan, Q., Li, M., Shi, Y., Liu, H., Speakman, J. R. and Wang, D. (2014). Lipidomics reveals mitochondrial membrane remodeling associated with acute thermoregulation in a rodent with a wide thermoneutral zone. *Lipids* **49**, 715-730. doi:10.1007/s11745-014-3900-0

Pani, S., Dey, S., Pati, B., Senapati, U. and Bal, N. C. (2022). Brown to white fat transition overlap with skeletal muscle during development of larger mammals: is it a coincidence? *J. Endocr. Soc.* **6**, bvac151. doi:10.1210/jendso/bvac151

Pant, M., Bal, N. C. and Periasamy, M. (2016). Sarcolipin: a key thermogenic and metabolic regulator in skeletal muscle. *Trends Endocrinol. Metab.* **27**, 881-892. doi:10.1016/j.tem.2016.08.006

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T. and Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* **269**, 540-543. doi:10.1126/science.7624776

Perry, R. J., Lyu, K., Rabin-Court, A., Dong, J., Li, X., Yang, Y., Qing, H., Wang, A., Yang, X. and Shulman, G. I. (2020). Leptin mediates postprandial increases in body temperature through hypothalamus-adrenal medulla-adipose tissue crosstalk. *J. Clin. Invest.* **130**, 2001-2016. doi:10.1172/JCI134699

Rajala, M. W., Patterson, C. M., Opp, J. S., Foltin, S. K., Young, V. B. and Myers, M. G. (2014). Leptin acts independently of food intake to modulate gut microbial composition in male mice. *Endocrinology* **155**, 748-757. doi:10.1210/en.2013-1085

Ramos Meyers, G., Samouda, H. and Bohn, T. (2022). Short chain fatty acid metabolism in relation to gut microbiota and genetic variability. *Nutrients* **14**, 5361. doi:10.3390/nu14245361

Rezai-Zadeh, K. and Münzberg, H. (2013). Integration of sensory information via central thermoregulatory leptin targets. *Physiol. Behav.* **121**, 49-55. doi:10.1016/j.physbeh.2013.02.014

Rezai-Zadeh, K., Yu, S., Jiang, Y., Laque, A., Schwartzenburg, C., Morrison, C. D., Derbenev, A. V., Zsombok, A. and Münzberg, H. (2014). Leptin receptor neurons in the dorsomedial hypothalamus are key regulators of energy expenditure and body weight, but not food intake. *Mol. Metab.* **3**, 681-693. doi:10.1016/j.molmet.2014.07.008

Rosenberg, E. and Zilber-Rosenberg, I. (2016). Do microbiotas warm their hosts? *Gut Microbes* **7**, 283-285. doi:10.1080/19490976.2016.1182294

Scarpace, P. J. and Matheny, M. (1998). Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *Am. J. Physiol.* **275**, E259-E264.

Scarpace, P. J., Matheny, M., Pollock, B. H. and Tümer, N. (1997). Leptin increases uncoupling protein expression and energy expenditure. *Am. J. Physiol.* **273**, E226-E230.

Segata, N., Haake, S. K., Mannon, P., Lemon, K. P., Waldron, L., Gevers, D., Huttenhower, C. and Izard, J. (2012). Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol.* **13**, R42. doi:10.1186/gb-2012-13-6-r42

Song, K., Wang, H., Kamm, G. B., Pohle, J., Reis, F. d. C., Heppenstall, P., Wende, H. and Siemens, J. (2016). The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia. *Science* **353**, 1393-1398. doi:10.1126/science.aaf7537

Sun, W., Li, C., Zhang, Y., Jiang, C., Zhai, M., Zhou, Q., Xiao, L. and Deng, Q. (2017). Gene expression changes of thermo-sensitive transient receptor potential channels in obese mice. *Cell Biol. Int.* **41**, 908-913. doi:10.1002/cbin.10783

Tang, G.-B., Tang, X.-F., Li, K. and Wang, D.-H. (2015). Intracerebroventricular administration of leptin increase physical activity but has no effect on thermogenesis in cold-acclimated rats. *Sci. Rep.* **5**, 11189. doi:10.1038/srep11189

Tartaglia, L. A. (1997). The leptin receptor. *J. Biol. Chem.* **272**, 6093-6096. doi:10.1074/jbc.272.10.6093

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J. et al. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263-1271. doi:10.1016/0092-8674(95)90151-5

Trayhurn, P., Thurlby, P. L. and James, W. P. (1977). Thermogenic defect in pre-obese ob/ob mice. *Nature* **266**, 60-62. doi:10.1038/266060a0

Ukropec, J., Anunciado, R. V. P., Ravussin, Y. and Kozak, L. P. (2006). Leptin is required for uncoupling protein-1-independent thermogenesis during cold stress. *Endocrinology* **147**, 2468-2480. doi:10.1210/en.2005-1216

Wang, H. and Siemens, J. (2015). TRP ion channels in thermosensation, thermoregulation and metabolism. *Temperature (Austin)* **2**, 178-187. doi:10.1080/23328940.2015.1040604

Wang, D.-H., Wang, Y.-S. and Wang, Z.-W. (2000). Metabolism and thermoregulation in the Mongolian gerbil *Meriones unguiculatus*. *Acta Theriologica* 45, 183-192.

Wang, L., Shao, Y. Y. and Ballock, R.T. (2011). Leptin synergizes with thyroid hormone signaling in promoting growth plate chondrocyte proliferation and terminal differentiation in vitro. *Bone* **48**, 1022–1027. doi:10.1016/j.bone.2011.02.012

Wen, Z., He, M., Peng, C., Rao, Y., Li, J., Li, Z., Du, L., Li, Y., Zhou, M., Hui, O. et al. (2019). Metabolomics and 16S rRNA gene sequencing analyses of changes in the intestinal flora and biomarkers induced by gastrodia-uncaria treatment in a rat model of chronic migraine. *Front. Pharmacol.* **10**, 1425. doi:10.3389/fphar.2019.01425

Wen, M., Liu, T., Zhao, M., Dang, X., Feng, S., Ding, X., Xu, Z., Huang, X., Lin, Q., Xiang, W. et al. (2021). Correlation analysis between gut microbiota and metabolites in children with systemic lupus erythematosus. *J. Immunol. Res.* **2021**, 5579608.

Wen, J., Bo, T., Zhao, Z. and Wang, D. (2022). Role of transient receptor potential vanilloid-1 in behavioral thermoregulation of the Mongolian gerbil *Meriones unguiculatus*. *Integr. Zool.* **17**, 608-618. doi:10.1111/1749-4877.12587

Wilson, D. E. and Reeder, D. M. (2005). *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd edn. Baltimore: Hopkins University Press.

Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A. H. et al. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150**, 366-376. doi:10.1016/j.cell.2012.05.016

Xiao, R., Zhang, B., Dong, Y., Gong, J., Xu, T., Liu, J. and Xu, X. Z. S. (2013). A genetic program promotes *C. elegans* longevity at cold temperatures via a thermosensitive TRP channel. *Cell* **152**, 806-817. doi:10.1016/j.cell.2013.01.020

Xu, Y., Wang, N., Tan, H.-Y., Li, S., Zhang, C., Zhang, Z. and Feng, Y. (2020). Panax notoginseng saponins modulate the gut microbiota to promote thermogenesis and beige adipocyte reconstruction via leptin-mediated AMPKα/STAT3 signaling in diet-induced obesity. *Theranostics* **10**, 11302-11323. doi:10.7150/thno.47746

Yang, J., Bromage, T. G., Zhao, Q., Xu, B. H., Gao, W. L., Tian, H. F., Tang, H. J., Liu, D. W. and Zhao, X. Q. (2011). Functional evolution of leptin of *Ochotona curzoniae* in adaptive thermogenesis driven by cold environmental stress. *PLoS One* **6**, e19833. doi:10.1371/journal.pone.0019833

Yoon, H. S., Cho, C. H., Yun, M. S., Jang, S. J., You, H. J., Kim, J., Han, D., Cha, K. H., Moon, S. H., Lee, K. et al. (2021). *Akkermansia muciniphila* secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. *Nat. Microbiol.* **6**, 563-573. doi:10.1038/s41564-021-00880-5

Zhang, Y. and Chua, S. C., Jr (2018). Leptin function and regulation. *Compr. Physiol.* **8**, 351-369.

Zhang, Z.-Q. and Wang, D.-H. (2007). Seasonal changes in thermogenesis and body mass in wild Mongolian gerbils (*Meriones unguiculatus*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **148**, 346-353. doi:10.1016/j.cbpa.2007.05.012

Zhang, X.-Y. and Wang, D.-H. (2022). Gut microbial community and host thermoregulation in small mammals. *Front. Physiol.* **13**, 888324. doi:10.3389/fphys.2022.888324

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425-432. doi:10.1038/372425a0

Zhang, X.-Y., Chi, Q.-S., Liu, W. and Wang, D.-H. (2016). Studies of behavioral and physiological ecology in Mongolian gerbils (*Meriones unguiculatus*). *Sci. Sin. Vitae.* **46**, 120-128. doi:10.1360/N052015-00223

Zhang, X.-Y., Sukhchuluun, G., Bo, T.-B., Chi, Q.-S., Yang, J.-J., Chen, B., Zhang, L. and Wang, D.-H. (2018). Huddling remodels gut microbiota to reduce energy requirements in a small mammal species during cold exposure. *Microbiome* **6**, 103. doi:10.1186/s40168-018-0473-9

Zhou, L., Xiao, X., Zhang, Q., Zheng, J. and Deng, M. (2019). Deciphering the anti-obesity benefits of resveratrol: the "gut microbiota-adipose tissue" axis. *Front. Endocrinol.* **10**, 413. doi:10.3389/fendo.2019.00413