Parathyroid hormone-related protein prevents high-fat-diet-induced obesity, hepatic steatosis and insulin resistance in mice

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Abstract. Obesity, closely related to systematic metabolic disorders, has become a major public health problem in recent decades. Here, we aimed to study the function of Parathyroid hormone-related protein (PTHrP) on high fat diet (HFD) induced murine obesity. Male C57BL/6J mice were transduced with adeno-associated virus vector encoding PTHrP (AAV-PTHrP) or adeno-associated virus control vector (AAV-Vehicle), following with HFD for 8 weeks. In addition, mice without transduction were fed on normal diet or HFD, respectively. Histological, metabolic and biochemical changes were detected. At the endpoint of experiment, body weight of mice treated with AAV-PTHrP did not increase as much as mice with AAV-Vehicle, but similar as mice with normal diet. Food efficiency ratio and weight of interscapular brown adipose tissue and epididymal white adipose tissue in mice overexpressed PTHrP were also lower than mice transduced with AAV-Vehicle. Besides, administration of AAV-PTHrP inhibited HFD-induced adipocyte hypertrophy. Protein level of PKA signaling pathway and thermogenic gene in adipose tissue exhibited a significant raise in HFD + AAV-PTHrP group, whereas transcription of inflammatory gene were decreased. Additionally, PTHrP overexpression ameliorated HFD-induced dyslipidemia, hepatic steatosis and insulin sensitivity. In HFD-induced murine obesity model, PTHrP is crucial to maintain metabolic homeostasis. PTHrP drives white adipose tissue browning and inhibits whitening of brown adipose tissue. Most importantly, PTHrP prevented HFD-induced obesity, hepatic steatosis and insulin resistance.

Key words: Parathyroid hormone-related protein (PTHrP), Adipose tissue, Obesity, Hepatic steatosis, Insulin resistance
obesity [6]. Several studies on human subjects have put forward the role of BAT in protecting against obesity, as the negative correlation between BAT prevalence and body mass index [7, 8]. Study on mice challenged with high fat diet (HFD) also showed that the transplantation of BAT dramatically reduced body weight, and improved insulin sensitivity and glucose metabolism [9]. Therefore new alternatives focusing on adipose tissue function therapeutically has gained a lot of attention in the field of obesity.

Parathyroid Hormone-related Protein (PTHrP), part of a small gene family associative with parathyroid hormone, was originally discovered as a cause of elevated calcium levels in patients with cancer [10]. Previous study has shown that PTHrP has salutary effects on the development of bones, cartilage, teeth, and the mammary gland [11]. Whereas, recent study revealed that PTHrP promoted progress of cachexia through inducing WAT browning [12]. Moreover, while driving WAT browning, PTHrP hold the potential to enhance thermogenesis and fatty acid β-oxidation [12, 13]. Combined with role of BAT on protecting against obesity mentioned above, proper PTHrP application in people with obesity might hold promise for fighting obesity and its complications.

Here, we employed a HFD induced murine obesity model to study the role of PTHrP on systemic metabolic changes. We found that PTHrP not only promoted WAT browning, but also inhibited BAT whitening in mice subjected to HFD. More importantly, PTHrP prevented HFD induced obesity, hepatic steatosis and insulin resistance. Collectively, our data demonstrated that PTHrP was crucial to maintain metabolic homeostasis when challenged with HFD.

Materials and Methods

Mice
C57BL/6J wild-type mice were purchased from Guangdong Medical Laboratory Animal Center and housed in the Southern Medical University Animal Experiment Center (Guangzhou, China). All procedures followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal study was approved by the Laboratory Animal Care and Use Committee of Southern Medical University.

Experimental design
Male C57BL/6J mice (6 to 8 weeks) were given a single injection of adeno-associated virus control vector (AAV9-Vehicle, Ohio Technology, China) or adeno-associated virus vector encoding PTHrP (AAV9-PTHrP, Gene ID: NM_008970, Ohio Technology, China) via caudal vein at a dose of 10^12 V.G. in 200 uL. One week after injection, mice were subjected to HFD (D12492; Research Diets, New Brunswick, NJ) for 8 weeks. In addition, mice without transduction were fed on normal diet or HFD, respectively. In briefly, based on the treatment mice received, they were randomly divided into four groups including normal control (NC) group, HFD group, HFD plus AAV9-Vehicle (HFD + AAV-Vehicle) group, and HFD plus AAV9-PTHrP (HFD + AAV-PTHrP) group. Body weight and food intake were measured weekly. Mice were fasted for 16h before sacrificed and tissues were harvested for further analysis.

Histological analysis
Liver and pancreatic tissue were fixed in 10% formalin, while adipose tissue were fixed in adipose tissue fixative solution (Leagene Biotechnology, China), and then embedded in paraffin respectively. Histological analysis was evaluated with H&E and oil red O staining. Six viewing fields from each slide were captured under fluorescent microscope. Pancreatic islets and adipocytes area were measured with Image J. For immunohistochemical staining, WAT and BAT sections were deparaffinized in xylene, rehydrated in graded alcohols, blocked with 10% normal goat serum and incubated overnight at 4°C with anti-UCP1 primary antibody (Proteintech, Chicago, IL, USA). To estimate the intracellular mitochondria of BAT, electron microscopy was performed using Hitachi H-7500 TEM, located at Central Laboratory, Southern Medical University.

Analysis of metabolic and biochemical changes
Mice were fasted for 16 h before the tests. For the glucose tolerance tests (GTT), 2 g/kg glucose was injected intraperitoneally and plasma glucose levels were measured before the injection and at 15, 30, 60, 90, 120 and 150 min after the injection. For insulin tolerance tests (ITT), 0.75 U/kg insulin was given intraperitoneally and glucose levels in plasma were also determined before the injection and at 15, 30, 60, 90, 120 and 150 min after the injection. Fasting insulin levels was detected by an ELISA kit (EZRMI-13K, Millipore, Billerica, MA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin concentration (mU/L) × fasting glucose concentration (mmol/L)/22.5. Hepatic and plasma triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and alanine aminotransferase (ALT) were measured by enzymatic assay kits (A110-2, A111-2, A113-2, C009-2 Nanjing Jiancheng Bioengineering Institute, China). Serum levels of calcium and phosphate was determined by spectrophotometry (BC1655, BC0720, Beijing Solarbio Science & Technology Co. Ltd, China).
Quantitative real-time PCR

RNA was extracted from different tissue using RNA isoplus reagent (Takara, Dalian, China) and reversely transcribed to cDNA with PrimeScript™ RT Master Mix (Takara). qPCR was performed using SYBR Green mix (Takara). The mRNA levels of various genes were calculated with the 2-ΔΔCt method after normalizing with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Primers and their sequences are as follows:

ACSL1: 5'-GATCTGGTTGAAACGAGCAA-3', 5'-CTTGGGCTTCTGGAGGCTTG-3'
FASN: 5'-AGTGTTGATAGCCGGTATGT-3', 5'-TGGGTAATCCATAGGCTTG-3'
PPARγ: 5'-TGCTGTTATGGGTGAAACTCTG-3', 5'-TGTTGTCAACCATGGTAATTTCTT-3'
PGRα: 5'-TATTCCGGCTGAAGCTGTGAT-3', 5'-TGTCGGCTTCTGGAGGCTTG-3'
ACOX1: 5'-GCCCAACTGTGACTTCCATTAA-3', 5'-GTAGCACTCCCCTCGAGTGAT-3'
CPT1α: 5'-AGGACCCTGAGGCATCTATT-3', 5'-AGGACCTGAGGCATCTATT-3'
ATGL: 5'-CAGCACATTTATCCCGGTGTAC-3', 5'-AAAATGCCGCCATCCACATAG-3'
FGF21: 5'-GGGGATTCAACACAGGAGAA-3', 5'-ATGGGTCAGGTTCAGACTGG-3'
UCP1: 5'-CACTCAGGATTGGCCTCTACGAC-3', 5'-GCTCTGGGCTTGCATTCTGAC-3'
UCP2: 5'-ACTCTGCCTTGGGCCAGTAT-3', 5'-GCCTAAGGTCTGGAGGCTTG-3'
PCC1α: 5'-AAACTTGCTAGCGGTCCTCA-3', 5'-TGCGCTGGTGCCAGTAAGAG-3'
HSL: 5'-GCTGGGCTGTCAAGCACTGT-3', 5'-GTAACTGGGTAGGCTGCCAT-3'
IL-1β: 5'-TCCAGGATGAGGACATGAGCAA-3', 5'-AACGTCACACCCGACACACC-3'
TNFa: 5'-GCCACCAACCGCCTTCTGTCT-3', 5'-GGTCTGGGACATGAGGCTTG-3'

Western blot assays

Tissue lysates were extracted using RIPA buffer (Beyotime, China) containing protease cocktail inhibitor (Sigma, USA), separated on 10%–15% SDS-PAGE, transferred to PVDF membranes (Millipore, USA) and probed with anti-HSL (Cell Signal Technology, USA), anti-p-CREB (Cell Signal Technology, USA), anti-adiponectin (Cell Signal Technology, USA), anti-p-AKT primary antibodies (Cell Signal Technology, USA), anti-VEGFA (Santa Cruz Biotechnology, USA) followed by anti-rabbit IgG secondary antibodies (Proteintech, USA). Images were developed by a chemiluminescence ECL detection kits (Millipore, USA).

Statistical analysis

All experiments were performed at least for 3 times. Representative and reproducible results were shown. Statistical analysis was performed with GraphPad Prism software (GraphPad Prism Software 6.0, La Jolla, CA). Data were expressed as means ± SEM. The significance of the differences was assessed by student’s t-test (for two groups) or one-way ANOVA (for multiple groups), p < 0.05 was considered statistically significant.

Results

PTHrP protects mice against HFD induced obesity

The energy intake of mice per week did not differ among HFD, HFD + AAV-Vehicle and HFD + AAV-PTHrP groups, and were higher than that of NC group (Fig. 1C). However, there is no much difference between HFD + AAV-PTHrP group and NC group on body weight change, which did not increase as much as that of HFD group and HFD + AAV-Vehicle group (Fig. 1D). At the endpoint of experiment, mice of HFD and HFD + AAV-Vehicle group gained about 60% body weight, whereas HFD + AAV-PTHrP group gained about 20%, similar as NC group (Fig. 1E). Consistent with body weight change, mice size of HFD + AAV-Vehicle group resembled HFD group, and were larger than mice from HFD + AAV-PTHrP group and NC group (Fig. 1A). As a consequence, food efficiency ratio (FER, Body weight gain/Energy intake) of HFD and HFD + AAV-Vehicle groups exceeded NC and HFD + AAV-PTHrP groups (Fig. 1B), indicative of lower metabolic efficiency and/or higher energy expenditure in PTHrP treated mice. In addition, we found that weight of both eWAT and iBAT in HFD + AAV-PTHrP group decreased significantly compared to that of HFD + AAV-Vehicle group (Fig. 1F and 1G). However, serum levels of calcium and phosphate have not been influenced by AAV-PTHrP (Fig. S1). Taken together, these results demonstrated that PTHrP protected mice from obesity when subjected to a HFD challenge.

PTHrP induces WAT browning in mice fed on HFD

As shown in Fig. 2C, size distribution of lipid droplets in mice from HFD group were similar to HFD + AAV-Vehicle group, so were they to mice from NC group and HFD + AAV-PTHrP group. Whereas compared among HFD feeding mice, size distribution of lipid droplets from mice with PTHrP overexpression was left shifted, and average size was also smaller (Fig. 2C and 2D), indicating that PTHrP overexpression suppresses enlargement of lipid droplets induced by HFD. Accordingly,
multilobular adipocytes, a typical characteristic of beige adipocytes, brought out in HFD + AA V-PTHrP group (Fig. 2A). Consistent with the change of lipid droplets, we also found that uncoupling protein 1 (UCP1), a hallmark of BAT located in mitochondrial inner membrane [14], was dramatically induced in mice transduced with AA V-PTHrP than that with AA V-Vehicle, as reflected with higher mRNA expression of UCP1 and stronger immunochemical staining intensity of UCP1 in HFD + AA V-PTHrP treated mice (Fig. 2B and 2F), suggesting that overexpression of PTHrP contributed to activation of beige adipocytes in WAT. In addition, PTHrP overexpression also triggered transcriptional levels of thermogenic gene in WAT, as reflected with higher mRNA expression of uncoupling protein 2 (UCP2), and peroxisome proliferator-activated receptor-C coactivator (PGC1α) in HFD + AA V-PTHrP treated mice than mice treated with HFD + AA V-Vehicle (Fig. 2F).

Compared with mice in NC group, PKA signaling transduction in WAT was inhibited with HFD treated mice (HFD, HFD + AA V-Vehicle), while PTHrP overexpression reverted the PKA signal transduction, as shown with higher expression of PKA RI-α/β and PKA phosphorylated substrates, including p-HSL and p-CREB (Fig. 2E). Adiponectin expression in mice treated with HFD + AA V-PTHrP was also elevated (Fig. 2E). Taken
together, PTHrP stimulated WAT browning in mice challenged with HFD treatment.

**PTHrP inhibits whitening of BAT in mice subjected to HFD**

We first confirmed that HFD feeding mice showed an increased total BAT weight (Fig. 1E) in association with the accumulation of enlarged lipid droplets in BAT cells (Fig. 3A), giving the appearance of BAT whitening. However, with HFD + AAV-PTHrP treatment, accumulation of enlarged BAT lipid droplets were suppressed (Fig. 3A). Whitened BAT in HFD mice expressed lower UCP1, while treated with AAV-PTHrP inhibited reduction of UCP1 (Fig. 3B). In addition, number of mitochondria

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**Fig. 2** PTHrP induced WAT browning in mice fed on HFD.

A. H&E stained sections of iWAT from NC, HFD, HFD + AAV-Vehicle, HFD + AAV-PTHrP treated mice. Scale bar: 50 μm (n = 5). B. UCP1 immunohistochemical staining of iWAT from mice in different groups. Scale bar: 50 μm (n = 5). C. Frequency distribution of WAT adipocyte area of mice from different groups (n = 5). D. Average cell size of white adipocytes (n = 5, student’s t-test). E. Western blotting analysis of essential molecules of PKA signaling transduction and adiponectin of WAT from mice in different groups (n = 3, student’s t-test). F. Relative mRNA levels of thermogenic gene including UCP1, UCP2 and PGC1α in WAT from mice administrated with HFD + AAV-Vehicle or HFD + AAV-PTHrP (n = 3, student’s t-test). *p < 0.05, **p < 0.001. Comparison was made between NC, HFD, HFD + AAV-Vehicle and HFD + AAV-PTHrP group.
in BAT declined sharply in HFD feeding mice, while PTHrP prevented it (Fig. 3C).

Consistent with what we found about WAT tissue, PTHrP overexpressed in BAT from mice transduced with AAV-PTHrP (Fig. S2). We further observed that HFD treatment (HFD, HFD + AAV-Vehicle) suppressed the PKA signaling transduction, while PTHrP overexpression (HFD + AAV-PTHrP) released it, thus increasing the transcription of UCP1, UCP2 and PGC1α in BAT (Fig. 3D and 3E). Besides, PTHrP elevated vascular endothelial growth factor A (VEGFA) expression (Fig. 3D). HFD-induced obesity causes BAT dysfunction, also

Fig. 3  PTHrP inhibited whitening of BAT in mice subjected to HFD.
A. H&E stained sections of BAT from NC, HFD, HFD + AAV-Vehicle, HFD + AAV-PTHrP treated mice. Scale bar: 50 μm (n = 3).
B. UCP1 immunohistochemical staining of BAT from mice in different groups. Scale bar: 50 μm (n = 3).
C. Mitochondrial electron micrographs of BAT from mice in different groups. Scale bar: 2 μm (n = 3).
D. Western blotting analysis of essential markers of PKA signaling, adiponectin and VEGFA in BAT from mice in different groups (n = 3, student’s t-test).
E/F. Relative mRNA levels of UCP1, UCP2, PGC1α, HSL, IL-1β and TNFα of BAT from mice subjected to HFD + AAV-Vehicle or HFD + AAV-PTHrP treatment (n = 3, student’s t-test). * p < 0.05, ** p < 0.01. Comparison was made between NC, HFD, HFD + AAV-Vehicle and HFD + AAV-PTHrP group.
manifested with inflammatory responses. Our data displayed that PTHrP overexpression improved the HFD-induced inflammatory responses, as evidenced by lower expression of IL-1β and TNFα (Fig. 3F). Overall, our data demonstrated that PTHrP prevented the HFD-induced BAT whitening.

**PTHrP ameliorates HFD-induced hepatic steatosis and liver dysfunction**

We next examined the role of PTHrP on HFD-induced hepatic steatosis. As shown in Fig. 4A, oil red O staining clearly showed that HFD feeding induced lipid accumulation in liver, especially in hepatocytes. Noteworthy, PTHrP overexpression dramatically alleviated HFD-induced hepatic steatosis as reflected in significantly reduced lipid accumulation in liver (Fig. 4A). In addition, overexpression of PTHrP also improved hepatic or systemic lipid metabolism as shown with reduced TG and LDL-C concentration in serum of mice administered to AA V-PTHrP (Fig. 4G–I). On the other hand, mRNA expression of fatty acid synthesis genes, such as ACLS1, FASN, PPARγ, were dramatically decreased, while the mRNAs encoded by genes involved in lipolysis, such as ATGL, ACOX1, CPT1α and PPARα were strikingly increased in mice subjected to HFD + AA V-PTHrP, compared to HFD + AA V-Vehicle (Fig. 4J).

To further determine the effect of PTHrP on hepatic morphology, H&E staining and NAFLD Activity Score (NAS) were performed to assess the impairment. Mice challenged with HFD and HFD + AA V-Vehicle gained more than 5 points which was largely taken as diagnostic of non-alcoholic steatohepatitis (NASH) (Fig. 4B and 4C). Whereas mice administrated with normal diet or HFD + AA V-PTHrP were ordinarily considered not NASH, as the score they gained were below 2 (Fig. 4B and 4C). In agreement with liver damage, ALT level in serum significantly increased in HFD-fed mice but not in mice overexpressing PTHrP (Fig. 4D). Collectively, these data implicated that PTHrP overexpression attenuated HFD induced hepatic steatosis and injury.

**PTHrP prevents HFD mice from insulin resistance**

As shown in Fig. 5B and 5C, there was a significant induction of glucose intolerance in the HFD and HFD + AA V-Vehicle treated mice, but, not in the mice administrated with normal diet or HFD + AA V-PTHrP (Fig. 5B and 5C). Similarly, HFD and HFD + AA V-Vehicle treatment significantly blunted the hypoglycemic effect of insulin injection, while, mice from NC and HFD + AA V-PTHrP group rapidly answered it (Fig. 5E and 5F). In addition, mice treated with HFD + AA V-Vehicle were tested with much higher level of glucose and insulin. Whereas, glucose and insulin level in mice overexpressed PTHrP were significantly lower than that in mice treated with HFD + AA V-Vehicle (Fig. 5A and 5D). Consistent with above findings, homeostatic model assessment of insulin resistance (HOMA-IR) also indicated that mice from NC and HFD + AA V-PTHrP group were much more insulin sensitive than mice from HFD and HFD + AA V-Vehicle group (Fig. 5G). Besides, no enlargement of pancreatic islets was observed in mice of HFD + AA V-PTHrP group (Fig. 5I and 5J). FGF21 has been reported for its abilities to lower blood glucose and circulating lipid concentrations, and reduce body weight via ameliorating obesity [15]. Intriguingly, the mRNA expression of FGF21 in liver was significantly elevated in mice from HFD + AA V-PTHrP group (Fig. 5H). Taken together, these data suggested that PTHrP overexpression alleviated HFD treatment induced insulin resistance and glucose intolerance.

**Discussion**

Here, our data provided a fresh perspective for the important and possible therapeutic role of PTHrP on obesity. Analogous with the prior studies, PTHrP overexpression induced WAT browning and inhibited BAT whitening even in HFD mice. We further elicited that the benefit of PTHrP in obesity and its related metabolic diseases, including hepatic steatosis and insulin resistance.

Obesity, an abnormal accumulation of fat, is a major risk factor of various metabolic diseases such as hepatic steatosis, insulin resistance and cardiovascular diseases [2]. Substantial deposition TG in liver is a major feature of NAFLD. With increasing fatty acid synthesis in hepatocyte, excessive TG deposited in liver lipid droplets, resulting in abnormal lipid accumulation [16]. Our data revealed that PTHrP reduced lipids content in liver and serum and its effect on TG is most obvious. Further experiment on lipids metabolism enzyme exhibited that PTHrP not only attenuated transcription of enzyme of fatty acid synthesis but also enhanced that of lipolysis in liver of HFD mice. Moreover, NAS below 2 eliminated the possibility of NASH in HFD + AA V-PTHrP mice. Therefore PTHrP prevented HFD induced hepatic steatosis.

NAFLD is strongly interrelated with insulin resistance [16, 17]. We further detected the effect of PTHrP on insulin resistance in mice challenged with HFD. PTHrP impeded increasing fasting blood glucose and fasting insulin levels in mice with 8 weeks of HFD. Impaired glucose tolerance and insulin sensitivity was not observed from GTT and ITT in HFD + AA V-PTHrP treated mice, similar with NC group. HOMA-IR also displayed that mice treated with AA V-PTHrP were much
more insulin sensitive. Furthermore, PTHrP elevated the adiponectin in WAT and BAT of HFD mice, a insulin sensitization hormone secreted by adipocytes that regulates energy homeostasis and glucose and lipid metabolism [18]. In fact, adiponectin decreases insulin resistance by increasing fatty-acid oxidation, which diminishes the TG content in these tissues in obese and type 2 diabetic mice [19]. Several studies have shown that there is a link between FGF21 and adiponectin. FGF21 increased the expression and secretion of
adiponectin in adipocytes and circulating concentration in mice, while FGF21 deficient mice exhibited significantly lower serum adiponectin level under both lean and HFD-induced obesity conditions [20, 21]. Intriguingly, our data revealed that PTHrP overexpression prevented HFD-induced changes in adiponectin protein level in adipose tissue. Trend in both was in agreement with the above findings, suggesting that PTHrP may enhance the effect of the FGF21-adiponectin axis. Additionally, FGF21 has also been reported for its abilities to lower blood glucose, circulating lipid concentrations and body weight via ameliorating adiposity [15, 22]. Thus, PTHrP attenuated HFD treatment induced systemic insulin resistance and glucose intolerance.

**Fig. 5** PTHrP attenuated insulin resistance in HFD mice.

A. Fasting glucose of mice from different groups (n = 5, student’s t-test). B/C. GTT and AUC (n = 5, student’s t-test). D. Fasting insulin of mice from different groups (n = 5, student’s t-test). E/F. ITT and AUC. G. HOMA-IR was calculated as fasting insulin concentration (mU/L) × fasting glucose concentration (mmol/L)/22.5 (n = 5, student’s t-test). H. The transcriptional level of FGF21 in liver from mice in different groups (n = 4, student’s t-test). I. Islets area of mice in different groups (n = 5, student’s t-test). J. Representative images of HE staining of pancreas islets from mice in different groups. Scale bar: 50 μm (n = 5–8). * p < 0.05, ** p < 0.01, *** p < 0.001. Comparison was made between NC, HFD, HFD + AAV-Vehicle and HFD + AAV-PTHrP group.
BAT is a highly vascularized organ containing abundant mitochondria and UCP1 [23]. It dissipates energy by nonshivering thermogenesis in multilocular brown adipocytes [7, 24, 25]. Norepinephrine stimulates PKA signaling pathway in BAT and then upregulates thermoregulatory gene expression and phosphorylates hormone-sensitive lipase, enhancing lipolysis [26, 27]. Quite a few studies have reported that whitening of BAT is relevant to obesity and metabolic diseases [23, 28, 29]. Recent studies reveal that PTHrP is able to induce WAT browning and enhance energy expenditure [12, 13]. UCP1, a hallmark of BAT located in mitochondrial inner membrane, uncouples oxidative phosphorylation from ATP synthesis and mediates energy expenditure by nonshivering thermogenesis [14]. Our data exhibited that PTHrP overexpression reversed the decrease of UCP1, VEGFA and mitochondria in BAT of HFD-fed mice, suggesting PTHrP restrained HFD-induced whitening of BAT. In addition, thermogenic gene UCP2 and PGC1α were also upregulated in BAT by PTHrP compared to the other two HFD groups, suggesting PTHrP strengthened the lipolysis in dietary obesity by nonshivering thermogenesis, which means the inhibition of HFD induced BAT whitening by PTHrP might prevent against obesity.

New evidence suggests that impaired metabolic pathways between liver and adipose tissue may also play a key role on ectopic lipid deposition. Laura et al. proposed an adipose-liver axis and UCP1 deficiency increased hepatic lipid content [30]. Transgenic UCP1 elevated lipolysis in a cell-autonomous way [31]. Adipose tissue releases redundant free fatty acids when subjected long-term HFD, increasing pro-inflammatory adipokines and chemokines which exacerbates ectopic lipid deposition, whereas inhibition of this inflammation suppresses HFD-induced hepatic steatosis and improves the function β cell [3, 32, 33]. PTHrP shares the same N-terminal end as parathyroid hormone and therefore it can bind to Type I PTH receptor (PTHR1). PTHR activates the PKA signaling to stimulate phosphorylation of the PKA substrates and thermogenic gene expression [13]. In accordance with these, data obtained in our research showed the higher expression of PKA RI α/β and p-HSL (Ser 563) in HFD + AAV-PTHrP mice, which is consistent with the findings in primary adipocytes [12]. As foreseen, PTHrP significantly reduced HFD induced adipocyte hypertrophy. Accordingly, without the excessive free fatty acids, the transcription of inflammatory genes is lower in BAT of mice overexpressed PTHrP, consistent with the relationship of lipid and inflammation mentioned above.

In conclusion, our current study reveals that, through transduction of AAV-PTHrP in mice following with HFD treatment, PTHrP overexpression protected mice from HFD induced obesity and obesity related metabolic disorders. Several lines of data support this novel conclusion. First, we observed that mice with PTHrP overexpression gained less weight and had smaller size when subjected to HFD challenge. Second, we found that PTHrP overexpression not only contributed to WAT browning, but also inhibited BAT Whitening. Third, we demonstrated that PTHrP overexpression also improved HFD induced hepatic steatosis and systemic insulin tolerance.

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Disclosures

The authors declare that there is no competing interest.

Author Contributions

LB, ND and BQ conceived the project, designed the studies, and proposed the experiments. BQ, ND, CQ, SH, YL and JS performed the experiments, BQ, ND, and LB wrote the manuscript.

Data and Materials Availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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