Journal of Advanced Research 62 (2024) 245–255

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Osteoprotegerin mediates adipogenesis in obesity

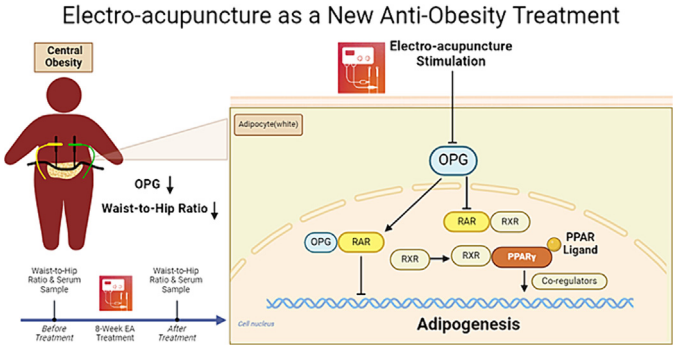
Zipan Lyu [a,1], Yau-Tuen Chan [b,1], Yuanjun Lu [b], Tsz Fung Lam [c], Xingyao Wu [c], Junyu Wu [b], Lin Xu [b], Wei Yang [c], Cheng Zhang [b], Linda Lidan Zhong [a,c,*], Ning Wang [b,**]

[a] School of Biological Sciences, Nanyang Technological University, Singapore, Singapore
[b] School of Chinese Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[c] School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China

HIGHLIGHTS

- Osteoprotegerin (OPG) significantly upregulates during the development of obesity.
- Hyperlipidaemia-induced Cbfa1 is responsible for OPG transactivation and overexpression.
- Up-regulation of OPG is responsible for the adipocyte differentiation and lipid storage.
- Targeting OPG by electroacupuncture improved obesity in mice.
- Single blinded, randomized, sham-controlled clinical trials revealed that electroacupuncture improved obesity and suppressed OPG in patients.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 February 2024
Revised 3 June 2024
Accepted 18 June 2024
Available online 19 June 2024

Keywords:
Osteoprotegerin
Obesity
Adipogenesis
PPARγ
Electroacupuncture

ABSTRACT

Introduction: Adipogenesis, the process of white adipose tissue expansion, plays a critical role in the development of obesity. Osteoprotegerin (OPG), known for its role in bone metabolism regulation, emerges as a potential regulator in mediating adipogenesis during obesity onset.
Objectives: This study aims to elucidate the involvement of OPG in adipogenesis during the early phases of diet-induced obesity and explore its therapeutic potential in obesity management.
Methods: Using a diet-induced obesity model, we investigated OPG expression patterns in adipocytes and explored the mechanisms underlying its involvement in adipogenesis. We also assessed the effects of targeted silencing of OPG and recombinant OPG administration on obesity progression and insulin resistance. Additionally, the impact of electroacupuncture treatment on OPG levels and obesity management was evaluated in both animal models and human participants.
Results: OPG expression was prominently activated in adipocytes of white adipose tissues during the early phase of diet-induced obesity. Hyperlipidemia induced Cbfa1-dependent OPG transcription, initiating and promoting adipogenesis, leading to cell-size expansion and lipid storage. Intracellular OPG physically bound to RAR and released the PPARγ/RXR complex, activating adipogenesis-associated gene expression. Targeted silencing of OPG suppressed obesity development, while recombinant OPG administration promoted disease progression and insulin resistance in obese mice. Electroacupuncture treatment suppressed obesity development in an OPG-dependent manner and improved obesity parameters in obese human participants.

* Corresponding author at: SBS-05n-09, Nanyang Technology University, Singapore.
** Corresponding author at: 6/F, 3 Sassoon Road, Pokfulam, Hong Kong, China
E-mail addresses: linda.zhong@ntu.edu.sg (L. Lidan Zhong), ckwang@hku.hk (N. Wang).
[1] Authors contributed equally.

https://doi.org/10.1016/j.jare.2024.06.018

Conclusion: OPG emerges as a key regulator in mediating adipogenesis during obesity development. Targeting OPG holds promise for the prevention and treatment of obesity, as evidenced by the efficacy of electroacupuncture treatment in modulating OPG levels and managing obesity-related outcomes.

Introduction

Obesity is closely linked to the process of the formation of fat cells through adipogenesis, which involves the differentiation of preadipocytes into mature adipocytes that store lipids[1]. Factors such as genetics, diet, sedentary lifestyle, and hormonal regulation contribute to the expansion of adipose tissue in obesity[2,3]. Adipose tissue not only serves as an energy reservoir, but also influences metabolism through the release of adipokines and the regulation of insulin sensitivity[4,5]. Disruptions in adipogenesis and altered adipokine production in obesity contribute to systemic metabolic disorders such as insulin resistance, type 2 diabetes, and cardiovascular diseases[6]. Understanding the mechanisms of obesity and adipogenesis is crucial for developing effective strategies to prevent and manage obesity-related health complications.

Osteoprotegerin (OPG) acts as a decoy receptor, binding to the receptor activator of nuclear factor-kappa B ligand (RANKL) and preventing its interaction with the receptor activator of nuclear factor-kappa B (RANK)[7,8]. Through this process, OPG inhibits osteoclast formation and bone resorption. Beyond its well-established involvement in bone metabolism, recent research has unveiled OPG's intriguing connection to metabolic diseases, particularly obesity. It is a protein that plays a crucial role in regulating adipogenesis and functions by modulating the balance between bone resorption and formation[7,9]. The pathophysiology of obesity is characterized by a state of low-grade systemic inflammation, orchestrated by various pro-inflammatory cytokines and adipokines released from dysfunctional adipose tissue into circulation [9–11]. In this milieu, the OPG-RANKL-RANK axis emerges as a significant player, influencing bone remodeling and adipose tissue dynamics. Obesity-induced alterations in adipogenesis, particularly in bone marrow, contribute to a shift from bone formation to resorption, mediated by the dysregulation of the OPG-RANKL-RANK system [12].

Notably, adipocytes themselves express and produce RANKL and OPG, suggesting a bidirectional interplay between adipose tissue inflammation and the OPG-RANKL-RANK axis [13]. Experimental studies in murine models have demonstrated elevated OPG expression in various tissues, including adipose tissue, under conditions of obesity, accompanied by inflammatory changes and metabolic disturbances [14]. Conversely, studies on OPG-deficient mice fed a high-fat diet revealed attenuated adipose tissue inflammation, highlighting a potential pro-inflammatory role of OPG in obesity [15]. Furthermore, clinical investigations have shed light on the association between obesity and the OPG-RANKL-RANK axis, although findings regarding circulating OPG levels in obese individuals remain heterogeneous [16–22]. Intriguingly, specific genetic variants in the OPG and RANK genes have been implicated in obesity risk, suggesting a genetic basis for the involvement of the OPG-RANKL-RANK axis in obesity pathogenesis [23,24]. However, the precise mechanisms by which OPG influences adipogenesis and its implications in obesity-related adipose tissue expansion are still not fully understood. Further research is needed to unravel its complex role in adipogenesis during the process of obesity to gain valuable insights for the development of targeted therapeutic interventions.

In this study, we analysed the expression of OPG in adipose tissue and its link to the development and progression of obesity. The effects were observed in obese participants and a mouse diet-induced obesity (DIO) model, which showed a particular increase in adipocytes. The results suggest a correlation between adipose tissue expansion and OPG expression during obesity. Electroacupuncture is also demonstrated as a potential therapeutic strategy that effectively suppresses OPG levels and ameliorates obesity in both rodent models and obese human participants. Collectively, these findings underscore OPG's pivotal role in adipose tissue dynamics and highlight its potential as a target for managing obesity-related complications.

Materials and Methods

Ethics statement

All biological samples were obtained from the study participants after obtaining written informed consent. Approval was obtained from the Institutional Review Board of Hong Kong Baptist University (Approval Reference: HASC/HASC/17–18/C03). This study was performed in accordance with the Declaration of Helsinki. The protocols for animal studies were approved by the Committee for the Use of Laboratory Animals for Teaching and Research of the University of Hong Kong (22–122).

Human participants and clinical treatment

The study was performed with 80 participants who met the following criteria: (i) age of 18 to 65 years, (ii) a diagnosis of central obesity (waist circumference $\geq$ 90 cm for men and $\geq$ 80 cm for women) [10], (iii) not receiving weight-loss treatment by Chinese medicine, conventional medicine, or a nutritionist in the past three months, and (iv) a body mass index (BMI) $\geq$ 25 kg/m^2 [11]. A total of 5 ml of blood was collected from each participant. Fasting blood glucose, serum insulin levels, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels, waist circumference, and waist-to-hip circumference ratio were measured at the beginning and end of the treatment. Additionally, body weight was measured every two weeks during the treatment period and the end of follow-up.

Animal models

A murine model of DIO was established by giving 5-week-old C57BL/6J male mice a high-fat diet (HFD) (60 % kcal from fat; Research Diets, USA) for 2, 4, 6 or 8 weeks. The duration of HFD feeding was indicated in the individual tests. Mice receiving a normal chow diet were used as normal controls. Each experimental group contained six mice except when noted.

Statistical analysis

Statistical significance was determined by the Student' s *t* test (two-tailed) and one-way analysis of variance (ANOVA) using GraphPad Prism 8 (GraphPad Software, La Jolla, CA). $P < 0.05$ was considered significant.

Other experimental details can be found in the supplementary materials.

Results

Upregulation of OPG in adipose tissue during the development of obesity

To understand the role of OPG in the expansion of adipose tissue during obesity, we first retrieved data from transcriptomic analysis on the vesical adipose tissue of lean and obese individuals from the Gene Enrichment Onimus (GEO) database. The expression of OPG was significantly elevated in the needle biopsy samples collected from 9 obese participants compared with those from lean participants (Fig. S1a)[12]. A similar observation was found in another study cohort, where OPG expression was significantly higher in obese individuals compared with lean individuals, regardless of whether the obese individuals were developing metabolic syndrome or not (Fig. S1b)[13]. In contrast, in the GSE165932 study cohort, the expression of OPG was more elevated in obese individuals with insulin resistance, suggesting that OPG expression may be involved in not only the early expansion of fat tissue, but also the progression of obesity-related diseases such as type 2 diabetes (Fig. S1c)[14].

We then established a diet-induced obesity (DIO) model to comprehensively measure the expression and distribution of OPG in the adipose tissues during the progression of obesity. Compared to mice fed with normal chow, the HFD-induced obese mice presented a gradual increase in the expression of OPG in the circulation and adipose tissue over the study period (Fig. 1a and 1b). As OPG is commonly known as a blocker of RANKL, we examined the activity of RANKL-induced molecules in adipose tissue. The expression levels of TRAF6 and c-Src, which are induced in response to RANKL activation, were strongly inhibited during the expansion of adipose tissues (Fig. 1c), confirming the upregulation of OPG. Furthermore, the expression of OPG was particularly upregulated in the intra-abdominal white adipose tissue (eWAT), but not brown adipose tissue (BAT) or subcutaneous fat pad (sWAT) (Fig. 1d).

We then isolated the adipocytes from stromal vascular fractions (SVFs) and the CD45$^+$ immune population to study the tissue distribution of OPG expression (Fig. 1e). The results showed that OPG was particularly increased in adipocytes, but not cells in SVFs or immune cells in HFD-induced obese mice (Fig. 1f). This suggested that OPG upregulation in adipocytes provides a major contribution to OPG overexpression in the adipose tissue during obesity. A correlation between adipose tissue expansion and the OPG expression in this tissue during the progression of obesity was observed.

Promotion of adipocyte differentiation by OPG expression

To explore the regulation of OPG expression in adipocytes, we established adipogenesis models *in vitro* using two adipocyte progenitor lines: C3H10T1/2 and 3T3 cells. Both progenitor cell lines were cultured with an induction cocktail and exhibited a gradual accumulation of intracellular lipid droplets at days 0, 3, 6, and 9, which was evidenced by increased positive staining by Oil red O (Fig. S2a). Increases in OPG expression were observed at the mRNA and protein levels in the early days of adipocyte differentiation, and the intracellular OPG expression reached its peak level and was maintained on later days (Fig. 2a and 2b). No significant change in osteoporosis-related gene expression was observed (Fig. S2b).

The expression of OPG was particularly increased in the adipocyte progenitors that were differentiated into white adipose tissue-like cells but not BAT-like cells (Fig. 2c). This suggests that it has a potential role in adipogenesis, but not thermogenesis. To further examine its roles in adipocytes, we supplemented recombinant OPG protein into the culture medium of C3H10T1/2 and 3T3 during adipocyte differentiation. OPG supplementation significantly accelerated the accumulation of lipid droplets (Fig. 2d), suppressed the proliferation of adipocyte progenitors (Fig. 2e), and induced the expression of adipogenesis-related genes, but not lipolysis-related genes (Fig. 2f). Interestingly, UCP-1 and IRS-1 expressions were significantly decreased in adipocytes treated with recombinant OPG

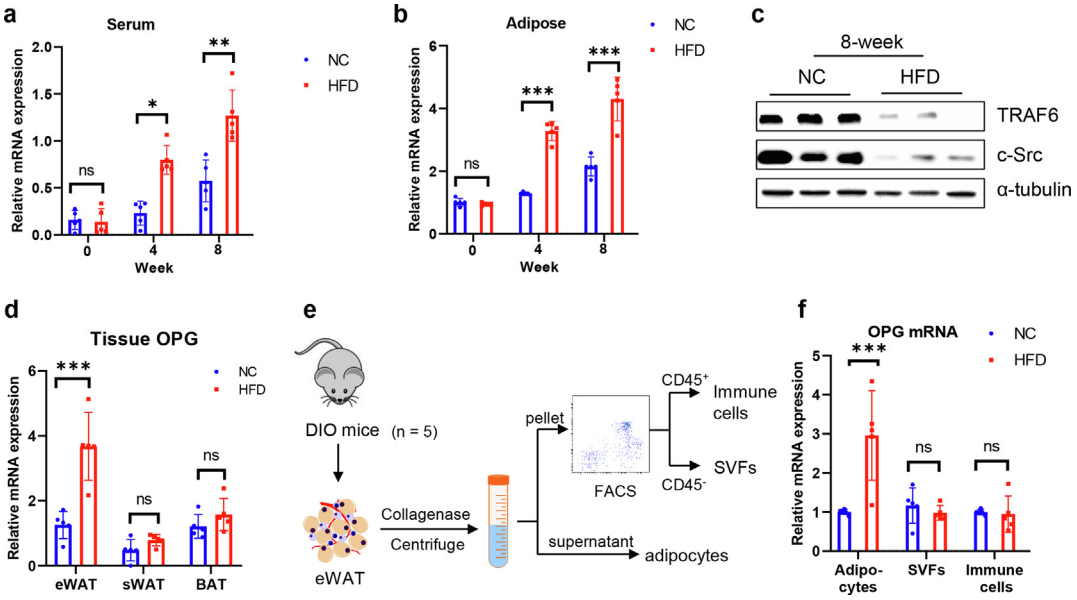


Fig. 1. Overexpression of OPG in adipocytes during obesity development. a. C57BL/6J mice were fed with high-fat diet (HFD) or normal chow (NC) for 4 and 8 weeks. Circulating OPG was increased in HFD-fed mice but not NC-fed mice (n = 5). **b.** Epididymal adipose tissue expressing OPG was induced in time-dependent manner in mice with diet-induced obesity (DIO) (n = 5). **c.** Protein levels of TRAF6 and c-Src in eWAT from lean and diet-induced obese mice (after 8 weeks of NC or HFD, respectively). **d.** HFD-induced expression of OPG was exclusively observed in eWAT but not BAT or sWAT of DIO mice (n = 5). **e.** Flow chart for isolation of different fractions and types of cells from eWAT of mice fed with NC or HFD for 8 weeks (n = 5). **f.** OPG was strongly upregulated in adipocytes but not in other stroma and immune populations of eWAT in the mice after 8 weeks of NC or HFD (n = 5). Data represent mean values ± s.e.m. Differences between NC and HFD were determined by ANOVA with Bonferroni's post hoc test; *P < 0.05, **P < 0.01, ***P < 0.001 (from post hoc test).

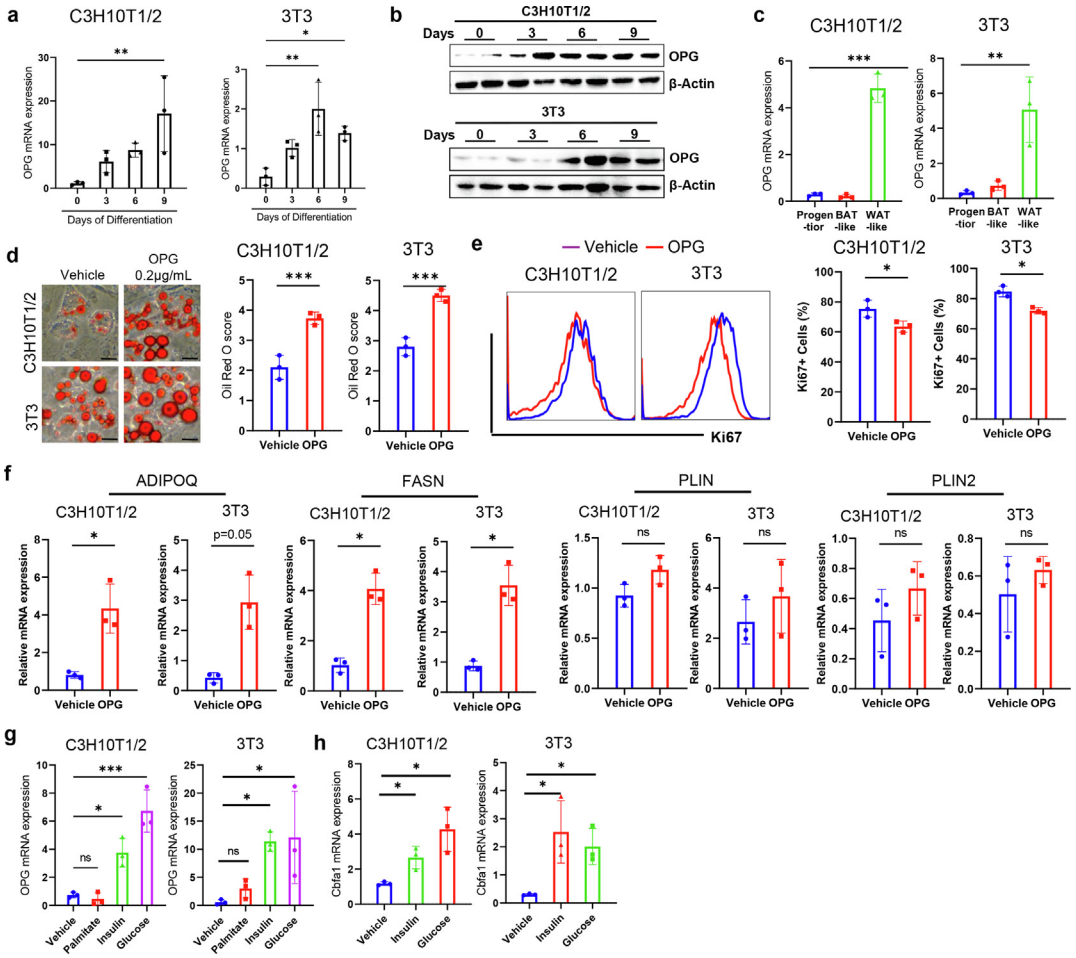


Fig. 2. Promotion of adipogenesis by OPG. Adipocyte progenitor cells C3H10T1/2 and 3T3 were treated with induction cocktails, **a.** mRNA and **b.** protein expression of OPG was significantly induced during adipocyte differentiation. **c.** Adipocyte progenitor cells were differentiated into either white adipose tissue-like or BAT-like cells, and induction of OPG expression was only found in white adipose tissue-like adipocytes. C3H10T1/2 and 3T3 cells were treated with recombinant OPG protein, which **d.** increased lipid droplet accumulation (scale bars 100 μm), **e.** reduced cell proliferation, and **f.** increased expression of adipogenesis-related (ADIPOQ & FASN) but not lipolysis-related genes (PLIN & PLIN2). **g.** C3H10T1/2 and 3T3 cells were exposed to hyperglycaemia (glucose, 10 mM), hyperinsulinemia (insulin, 100 nM), and hyperlipidaemia (BSA-conjugated palmitate, 500 μM) conditions, and high glucose and insulin levels facilitated the transcription of OPG expression. **h.** High glucose and insulin treatment induced expression of Cbfa1. n ≥ 3 biologically independent replicates. Data are presented as mean values ± SEM. Significant differences were analysed by two-tailed, unpaired *t*-test (d-f) or one-way ANOVA (a, c, g) with Bonferroni post hoc tests. *P < 0.05, **P < 0.01, ***P < 0.001 (from post hoc test).

protein (Fig. S2c), which reflects the role of OPG overexpression in mediating metabolic dysfunction in obese individuals during disease progression.

To identify whether metabolic stress could initiate the upregulation of OPG in adipocytes during the early development of obesity, we treated adipocyte progenitors with various metabolic factors, such as 10 mM glucose[15], 100 nM insulin[16], and 500 μM BSA-conjugated palmitate[17]. This was done to mimic the conditions of hyperglycaemia, hyperinsulinemia, and hyperlipidaemia, respectively[18]. Glucose and insulin treatment, but not palmitate, significantly facilitated the transcription activation of OPG in adipocyte progenitors (Fig. 2g). Consistent with previous studies, the expression of osteoblast-specific transcription factor Cbfa1 was induced by high glucose and insulin treatment in adipocyte progenitors (Fig. 2h)[19]. These observations suggested that OPG transcription was activated by metabolic stress during adipocyte differentiation and promoted adipogenesis.

Regulation of PPARϒ signalling during adipogenesis by OPG

We then explored the possible mechanisms involved in the regulation of OPG in adipogenesis-related signalling during adipocyte differentiation. C3H10T1/2 cells were treated with an induction cocktail in the presence or absence of recombinant OPG treatment for 6 days and subjected to transcriptomics analysis. Differential changes in gene expression were observed (Fig. 3a). We then shortlisted the upregulated and downregulated genes upon OPG treatment (Fig. 3b). Gene Ontology enrichment analysis was then performed to investigate the involvement of possible biological processes (Fig. 3c). OPG-induced gene expression was mostly enriched in PPARϒ-associated signalling, and the transcriptional expression of PPARϒ-downstream genes was activated upon OPG exposure (Fig. 3d). Gene set enrichment analysis (GSEA) confirmed that OPG exposure favoured the activation of PPARϒ-associated signalling in adipocytes compared to cells with vehicle treatment (Fig. 3e). The activation of PPARϒ and its downstream gene expression upon OPG treatment were validated in both C3H10T1/2 and 3T3 cells (Fig. S3a and 3b).

To confirm the role of PPARϒ in mediating OPG-induced adipogenesis, we treated differentiated C3H10T1/2 and 3T3 cells with OPG in the presence or absence of 10 μM PPARϒ antagonist GW9662. GW9662 significantly suppressed the OPG-induced expressions of lipogenesis-associated genes (Fig. 3f), while the lipolysis-associated genes remained unchanged (Fig. 3g).

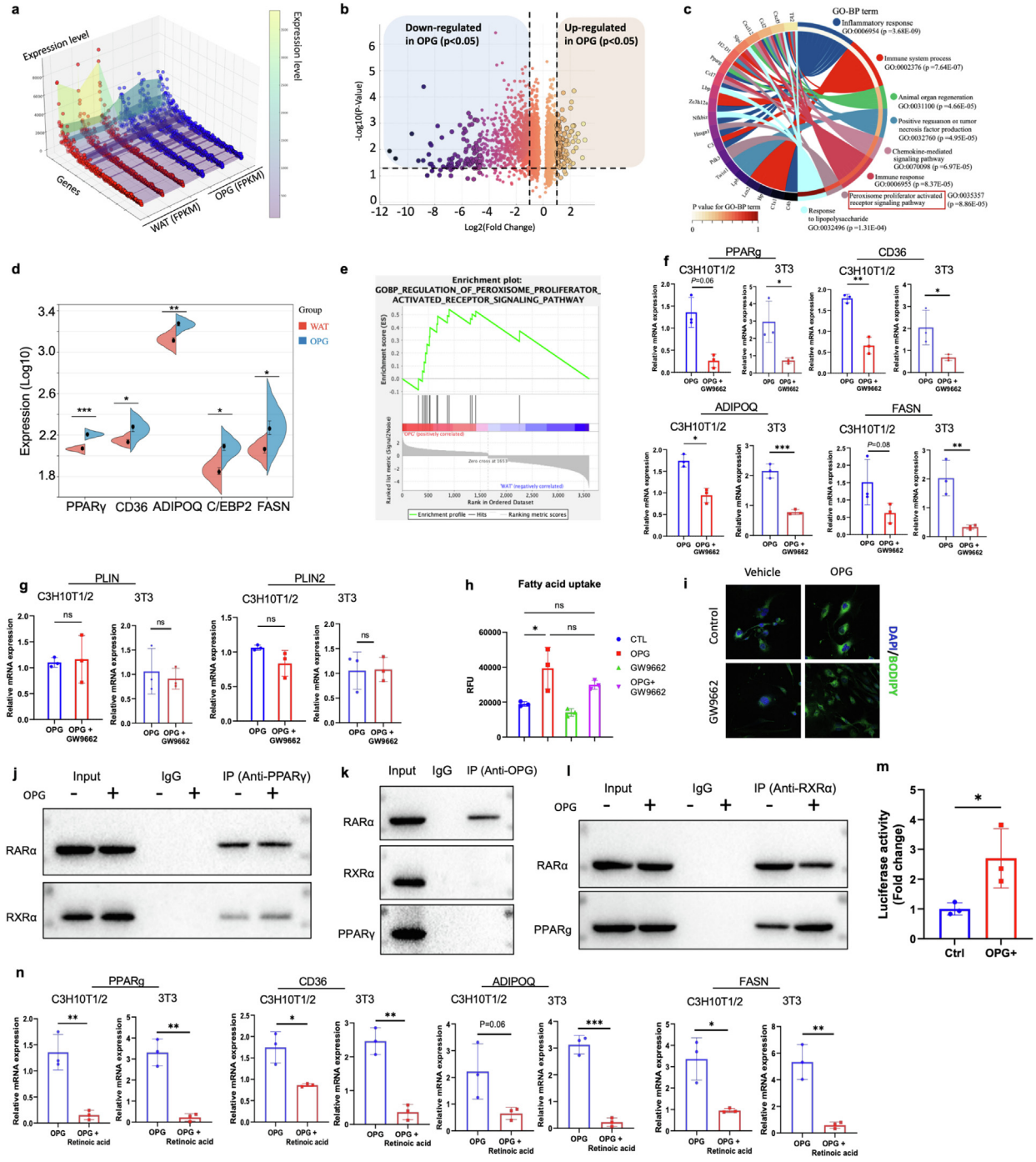


Fig. 3. Regulation of adipogenesis by OPG through PPARγ. a. Transcriptomic analysis of adipocytes treated C3H10T1/2 cells with or without recombinant OPG was conducted by RNA sequencing. **b.** Volcanic plot of differential gene expression. **c.** Gene Ontology analysis of biological process that was enriched by differentially expressed genes upon OPG treatment. **d.** Treatment with recombinant OPG induced expression of PPARγ-downstream genes. **e.** GSEA analysis showed enrichment of OPG-induced gene expression in PPARγ-related pathway. The adipocytes were then co-treated with OPG, the PPARγ inhibitor GW9662, or both of these two. GW9662 reduced **f.** OPG-induced expression of adipogenesis-related genes (PPARγ, CD36, ADIPOQ, and FASN), **g.** without changing the expression of lipolysis-related genes (PLIN and PLIN2), as well as suppressed OPG-induced **h.** fatty acid update and **i.** fatty acid deposition in adipocytes (scale bars 10 μm). **j.** Treatment with recombinant OPG induced association of PPARγ with RXR. **k.** Pull-down OPG showed that OPG was bound to endogenous RAR but not PPARγ or RXR. **l.** OPG treatment reduced the association between RXR and RAR. **m.** Treatment with retinoic acid in OPG-treated adipocytes significantly blocked the activation of adipogenesis-related gene (PPARγ, CD36, ADIPOQ, and FASN) expression. n ≥ 3 biologically independent replicates. *p < 0.05, **p < 0.01, ***p < 0.001.

GW9662 also potently suppressed OPG-induced fatty acid uptake (Fig. 3h) and the fatty acid deposition in adipocytes (Fig. 3i). To confirm if OPG particularly regulates PPARγ, the expression level of other transcriptional regulators including Wnt, CEBPβ, and CEBPα, were checked and no significant alterations were observed (Fig. S3c). This suggests that PPARγ-activated transcription of downstream lipogenesis genes was responsible for OPG-induced adipogenesis in adipocytes.

Furthermore, we immunoprecipitated PPARγ to explore the mechanisms underlying the regulation of OPG in the transcription activity of PPARγ. OPG treatment significantly improved the association of PPARγ with RXR, which is a transcription co-activator

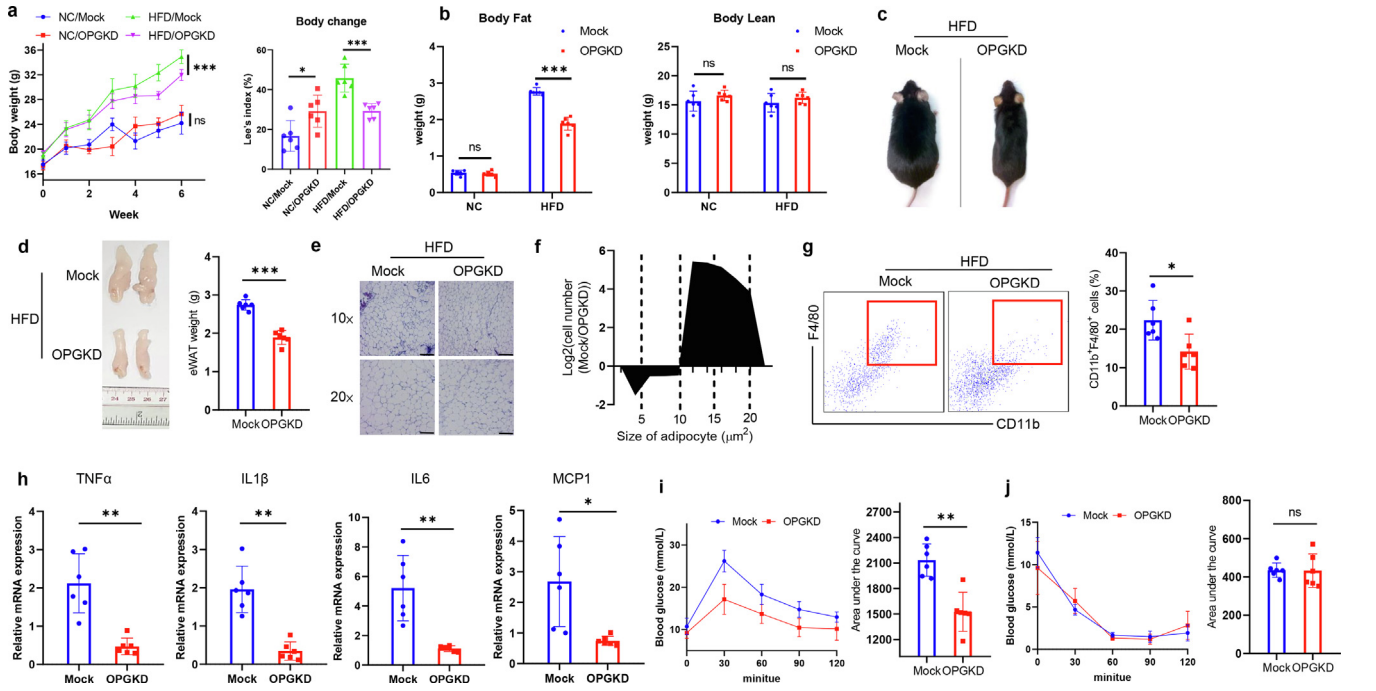


Fig. 4. Improvement of obesity in DIO mice by OPG knockdown. DIO mice were injected intravenously with AAV8-carried shRNA against OPG every 2 weeks. **a.** OPG knockdown in DIO mice suppressed body weight gain, **b.** reduced the percentage of body fat, **c.** slimmed body shape, and **d.** reduced the fat pad size. **e&f.** Histological analysis of eWAT (n = 3 images per mouse) showed a small size of adipocytes upon OPG knockdown in adipose tissues. Scale bar: 100 μm (10X), 50 μm (20X). **g.** OPG knockdown suppressed infiltration of pro-inflammatory macrophages ($F4/80^+$ and $CD11b^+$) and **h.** expression of pro-inflammatory cytokines (TNFα, IL1β, IL6, and MCP1) in adipose tissue. OPG knockdown **i.** improved IPGTT significantly **j.** but not ITT in DIO mice. Unless otherwise indicated, n = 6 for each group and measurements taken after 6 weeks on the HFD. Data are presented as mean values ± SEM. Significant differences were analysed by two-tailed, unpaired *t*-test (b, d-h) or one-way ANOVA (a, i, and g) with Bonferroni post hoc tests. *P < 0.05, **P < 0.01, ***P < 0.001 (from post hoc test).

that mediates PPARɤ-induced adipogenesis (Fig. 3j). Interestingly, immunoprecipitation of OPG did not pull down any endogenous PPARɤ or RXR, but OPG was physically bound to the RAR, which has been reported to be an endogenous inhibitor of PPARɤ/RXR-mediated transcription activation of adipogenesis-associated gene expression through its physical binding with RXR (Fig. 3k)[20].

A co-immunoprecipitation assay suggested that OPG treatment strongly reduced the association between RXR and RAR and increased the association between PPARɤ and RXR, suggesting that OPG may compete with the binding of RAR with RXR and release RXR, which can thus form a transcription activation complex with PPARɤ (Fig. 3l). Furthermore, the luciferase reporter assay result showed that, the obligate heterodimer formed by RXR and PPARɤ bound to the PPAR response elements (PPREs) on the upstream of adipogenesis genes, and the luciferase intensity was induced with the treatment of recombinant OPG (Fig. 3m). Moreover, exposure of OPG-treated adipocytes to retinoic acid, a RAR agonist, significantly blocked the activation of adipogenesis-associated gene expression, underscoring the competitive interaction between OPG and RAR for RXR binding (Fig. 3n). Our findings suggested that OPG regulates adipogenesis by activating PPARɤ/RXR-associated gene transcription.

Adipose-specific knockdown of OPG and attenuation of adipogenesis in high fat diet-induced obesity

To further understand the role of OPG in mediating adipogenesis in vivo, we used an Adeno-Associated Virus Vectors (AAV vectors)-delivered shRNA to knock down the expression of OPG in adipose tissue. 1×10^{12} units of AAV8-carried shRNA against OPG were intravenously injected into C57BL/6J mice every 2 weeks via the tail vein to suppress the HFD-induced OPG and PPARɤ expression in the eWAT but not BAT (Fig. S4a). The mice were fed with normal chow or HFD for 6 weeks, and the body weight was measured weekly. OPG knockdown significantly reduced the body weight gain induced by HFD, while knockdown of OPG did not cause significant changes in body weight in mice fed normal chow (Fig. 4a). No significant changes in food intake were observed after OPG knockdown (Fig. S4b). Since tail vein injection of AAV8 is often related to the effect on the liver, we also measured the level of OPG in the liver tissues (Fig. S4c), as well as the liver function in case of any liver damage (Fig. S4d).

The analysis of the whole body composition showed that reductions of fat were more significant than lean mass reductions when OPG was knocked down (Fig. 4b). After 6 weeks of HFD feeding, mice with OPG knockdown showed a slimmer body shape than the wild-type (WT) littermates (Fig. 4c), and the vesical fat pad was significantly smaller (Fig. 4d). Histological analysis showed that the adipocyte size was significantly smaller in diameter in mice with OPG knockdown (Fig. 4e and 4f). They also had significantly reduced expression of adipogenesis-associated genes (Fig. S4e), while lipolysis-associated genes remained unchanged (Fig. S4f). The corresponding gene expressions in the liver remain not affected by OPG knockdown (Fig. S4g).

Delayed development of obesity was also evidenced by strongly reduced inflammation of adipose tissue after OPG knockdown. OPG knockdown-associated suppression of adipogenesis resulted in reduced infiltration of $CD11b^+F4/80^+$ adipose-tissue macrophages (Fig. 4g) and expression of pro-inflammatory cytokines in the adipose tissues (Fig. 4h). As a result, mice with OPG knockdown showed significant improvement in glucose tolerance (Fig. 4i), while no improvement in insulin sensitivity was observed (Fig. 4j). These findings show the functional role of OPG in mediating adipogenesis during obesity development.

Supplementation of OPG accelerated obesity and promote insulin resistance

To further explore the role of OPG in the development of obesity-associated diseases, we supplemented DIO C57BL/6J mice with murine recombinant OPG (rOPG) three times per week via tail vein injection, which significantly increased OPG levels in the serum (Fig. S5a) and visceral fat (Fig. 5a). As expected, increased serum OPG had minimal effect on the food intake of mice with either normal chow or HFD (Fig. S5b). Furthermore, rOPG supplementation significantly induced body weight gain caused by HFD in mice, but this effect was subtle in mice fed with normal chow (Fig. 5b). rOPG supplementation significantly increased the percentage of body fat in HFD-fed mice (Fig. 5c), which resulted in bigger sizes of the whole body and visceral fat pad (Fig. 5d and 5e).

Consistently, histological analysis revealed larger adipocyte diameters in mice supplemented with rOPG (Fig. 5f and 5 g), which also induced expression of PPARγ and its downstream lipogenesis-associated genes (Fig. S5c and Fig. S5d). Infiltration of adipose tissue macrophages and expression of pro-inflammatory cytokines in the adipose tissues were significantly increased (Fig. 5h and 5i). The fasting glucose level was increased after 6 weeks of treatment with rOPG in HFD-fed mice (Fig. 5j), and rOPG supplementation significantly increased the level of serum insulin (Fig. S5e) and the insulin resistance indicator HOMA-IR (Fig. 5k).

The intraperitoneal glucose tolerance test (IPGTT) showed that mice with rOPG supplementation were more intolerant of glucose (Fig. 5l). In addition, rOPG supplementation resulted in increased fat deposition in the liver of HFD-fed mice (Fig. S5f) and macro- and microvesicular steatosis (Fig. S5g), which are related to impaired liver functions. This was evidenced by elevated serum levels of AST and ALT (Fig. 5m). This is likely the result of the fat deposition in the liver, instead of the accumulation of OPG, as shown by the unchanged OPG levels in the liver (Fig. S5h). These observations suggest that OPG overexpression facilitates the progression of obesity and the development of associated diseases.

Suppression of obesity through electroacupuncture in an OPG-dependent manner

Currently, there is no effective systematic and localised inhibitor of OPG in vivo. To explore the potential of OPG as a targetable molecule in the management of obesity, we screened and identified possible therapeutic approaches that can inhibit OPG in obese rodents. Electroacupuncture is a traditional approach in Chinese medicine that has been shown to manage body weight in obese individuals[21]. We found that electroacupuncture can significantly reduce the serum level of OPG in DIO mice (Fig. 6a), which consistently, showed a significant suppression of the mRNA and protein expression of OPG in the visceral adipose tissues (Fig. 6b and 6c).

To explore whether electroacupuncture inhibited the development of obesity via an OPG-dependent mechanism, we supplemented the circulating adipose level of OPG via intravenous injection of rOPG in DIO mice. Electroacupuncture intervention three times per week at the particular acupoints Tianshu (ST-25)

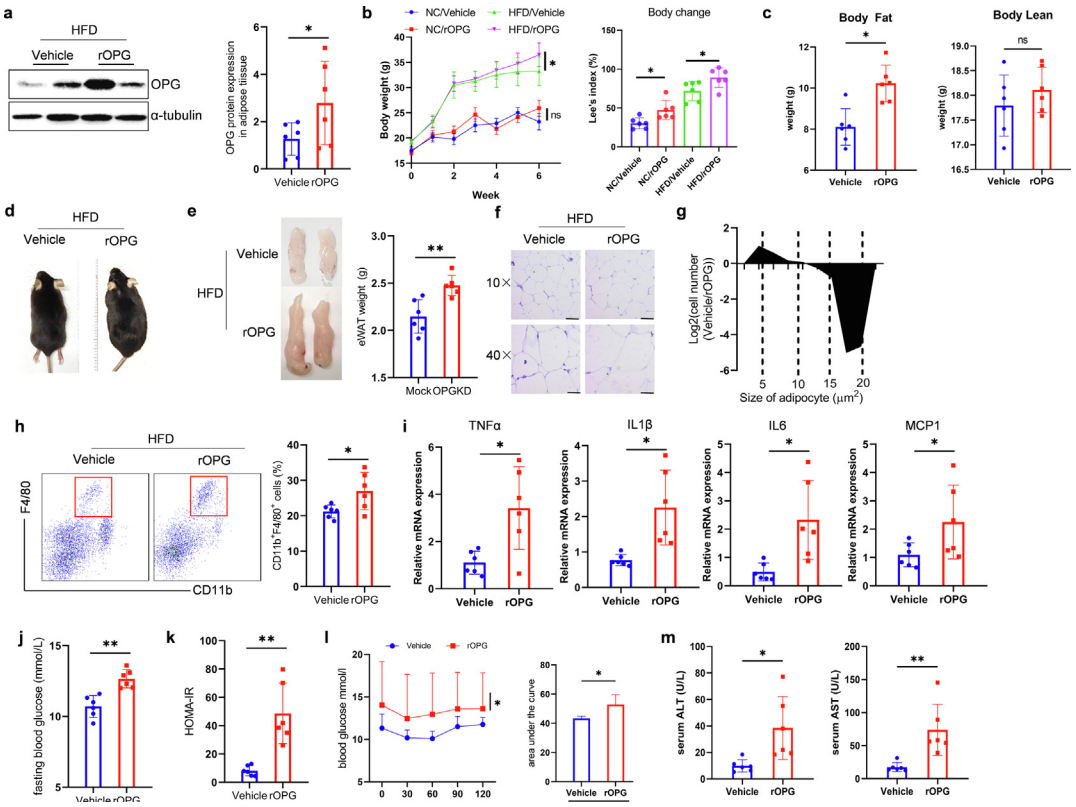


Fig. 5. Supplementation of OPG promotes obesity and metabolic syndromes in DIO mice. DIO mice were injected intravenously with 10 mg/kg recombinant OPG three times per week lasting 6 weeks. **a.** OPG supplementation in DIO mice increased OPG level in visceral fat pads, and **b.** accelerated body weight gain. Two-way ANOVA was used to compare means for different groups. **c.** rOPG increased body fat percentage of DIO mice. OPG supplementation resulted in **d.** larger body size and **e.** visceral fat pad in DIO mice. **f&g.** Histological analysis for eWAT (n = 3 images per mouse) showed a larger size of adipocytes upon OPG supplementation in the adipose tissues. Scale bar: 100 μm (10X), 25 μm (40X). OPG supplementation increased **h.**infiltration of pro-inflammatory macrophages and **i.** expression of pro-inflammatory cytokines (F4/80$^+$ and CD11b$^+$) in adipose tissue. OPG supplementation significantly worsened **j.** fasting blood glucose, **k.** insulin resistance indicator HOMA-IR, and **l.** IPGTT in DIO mice. **m.** Serum ALT and AST levels were increased after rOPG supplementation. Unless otherwise indicated, n = 6 for each group and measurements taken after 6 weeks on the HFD. Data are presented as mean values ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

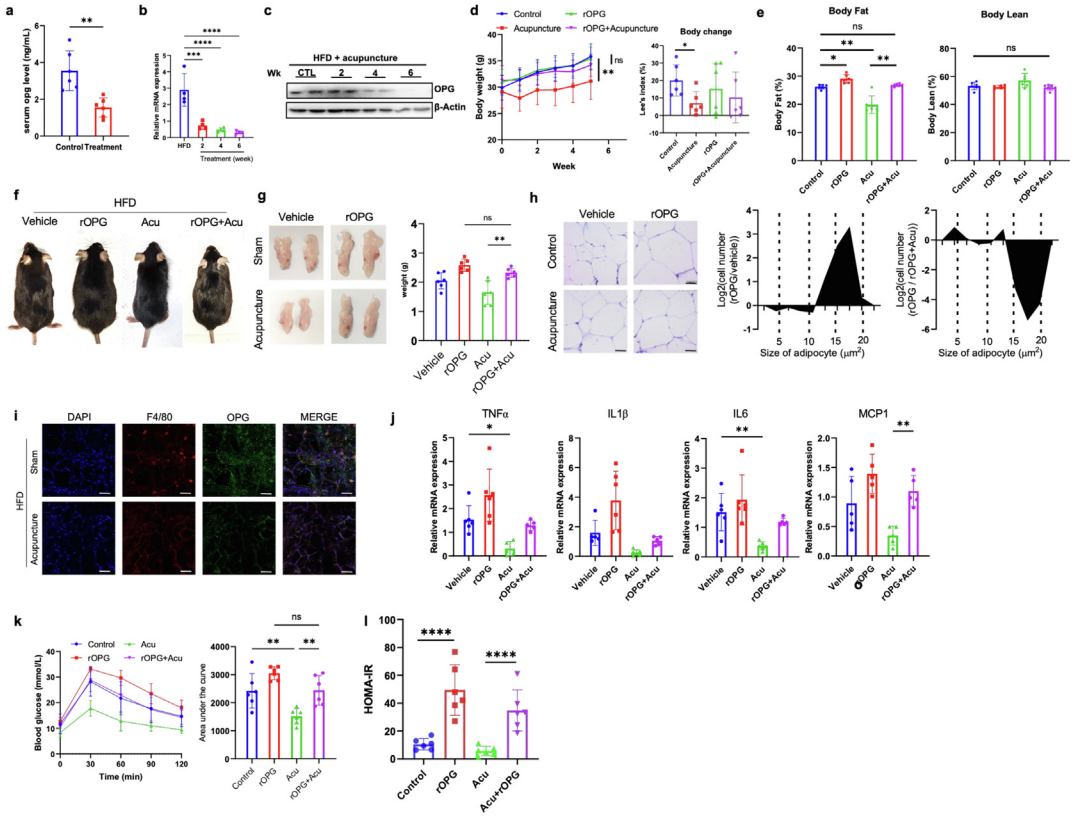


Fig. 6. Electroacupuncture and improvement of experimental obesity in an OPG-dependent manner. DIO mice received electroacupuncture three times per week at specific acupoints for 3 weeks with or without OPG (10 mg/kg, i.v. injection, three times per week). **a.** Electroacupuncture treatment significantly suppressed serum OPG levels in DIO. Electroacupuncture reduced **b.** mRNA and **c.** protein in visceral adipose tissue of DIO mice. OPG supplementation completely **d.** abolished electroacupuncture-induced body weight loss, **e.** reduction in body fat percentage, **f.** body size, **g.** visceral fat pad size, and **h.** adipocyte size reduction (n = 3 images per mouse, scale bar 25 μm). OPG supplementation ameliorated the inhibitory effect of electroacupuncture on **i.** infiltration of pro-inflammatory macrophages (n = 3 images per mouse, scale bar 10 μm) and **j.** expression of pro-inflammatory cytokines in adipose tissue and improvement of **k.** IPGTT and **l.** HOMA-IR in DIO mice. Unless otherwise indicated, n = 6 for each group and measurements taken after 5 weeks on the HFD. Data are presented as mean values ± SEM. Significant differences were analysed by two-tailed, unpaired *t*-test (a) or one-way ANOVA (b, d-h, j, k) with Bonferroni post hoc tests. *P < 0.05, **P < 0.01, ***P < 0.001 (from post hoc test).

and Zusanli (ST-36) [22,23] significantly suppressed the body weight gain in DIO mice, while supplementation of OPG completely abolished the electroacupuncture-induced body weight loss (Fig. 6d). Similarly, the body composition analysis showed that electroacupuncture reduced the fat percentage, size of the body, and size of visceral fat pads in mice, which could be reversed by OPG supplementation (Fig. 6e-6g, and Fig. S6a). The size of adipocytes in visceral adipose tissues was also reduced by electroacupuncture treatment, which was then regained upon OPG supplementation in DIO mice (Fig. 6h).

The downstream signalling components of OPG, including PPARγ and lipogenesis-associated gene expression, were consistently inhibited by electroacupuncture intervention, which was reversed by OPG supplementation (Fig. S6b and Fig. S6c). As a result, infiltration of adipose tissue macrophages and expression of pro-inflammatory cytokines were regulated accordingly (Fig. 6i and 6j). Electroacupuncture also improved glucose tolerance and HOMA-IR in DIO mice, which was abolished by OPG supplementation (Fig. 6k and 6l). Our observations suggested that electroacupuncture works through the inhibition of OPG, which improves adipogenesis and obesity development.

Improvement of central obesity and reduced serum OPG contents in human participants through electroacupuncture

A single-blinded, randomised, controlled clinical study was conducted to assess the efficacy of electroacupuncture in the management of central obesity in 80 human participants from Hong Kong. The baseline data are shown in Supplemental Table 1. Participants received either sham or electroacupuncture at the acupoints of Tianshu (ST-25), Daheng (SP-15), Daimai (GB-26), Qihai (CV-6), Zhongwan (CV-12), Zusanli (ST-36), Fenglong (ST-40), and Sanyinjiao (SP-6) 2 times per week for 2 months (Fig. 7a) [24]. The location, function, and potential bio-mechanism for these acupoints are shown in Supplemental Table 2. Compared with the sham control, electroacupuncture significantly improved the waist-to-hip ratio and HOMA-IR value, as well as the fasting blood glucose (Fig. 7b-7d), but did not reduce body weight (Supplemental Table 3). Subgroup analysis revealed that the effect of electroacupuncture was not related to the sex or age of the participants (Fig. 7e-7f), except that HOMA-IR seemed to be higher in males than females at the baseline as well as after treatment.

To confirm the possible mechanism involving OPG inhibition, we used a cytokine array to identify the possible biomarkers of electroacupuncture treatment in 5 obese participants (Fig. 7g). Among serum biomarkers, electroacupuncture intervention significantly reduced the OPG expression and circulating pro-inflammatory cytokines (Fig. 7h-7i). Compared with the sham control, electroacupuncture strongly reduced serum levels of OPG in obese individuals (Fig. 7j). The serum and adipose OPG levels were observed to have a linear correlation, as demonstrated from our mice model sample (Fig. S7a). Although it was not possible for us to obtain the adipose OPG levels, the serum level can be a potential predictor for measuring the treatment response. On the other

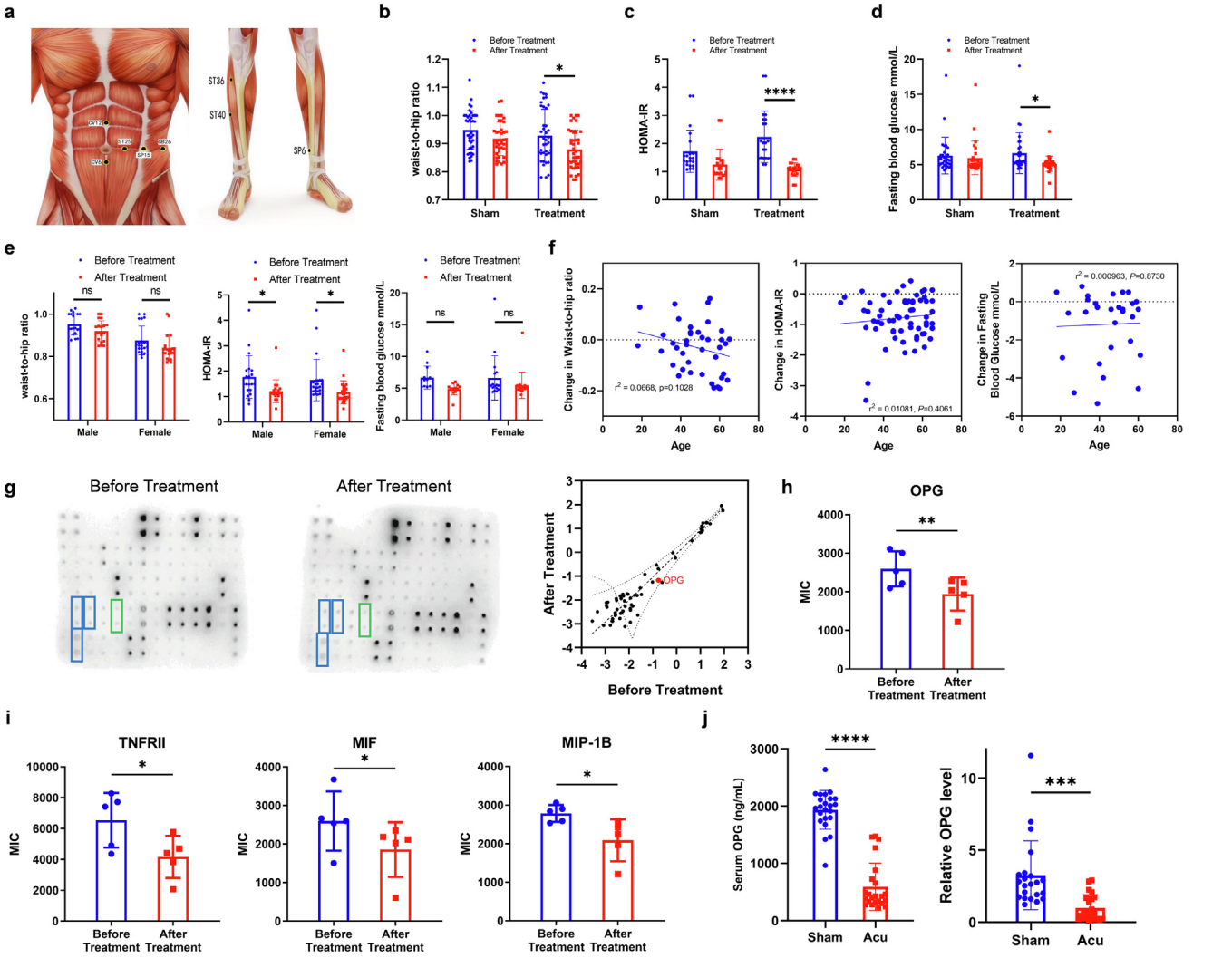


Fig. 7. Electroacupuncture suppressed OPG and improved obesity-related metabolic syndrome in obese individuals. a. Flow chart of electroacupuncture intervention for obese individuals from Hong Kong. Compared to sham control, electroacupuncture significantly **b.** improved waist-to-hip ratio (n = 40), **c.** HOMA-IR level (n = 22) and **d.** fasting blood glucose (n = 30). The effect of electroacupuncture was not dependent on **e.** sex (n = 19 in males and n = 21 in females) or **f.** age (n = 40). **g.** Serum of obese individuals was collected before and after electroacupuncture for cytokine array analysis (n = 5). **h.** Among the cytokines, OPG was one of the most down-regulated cytokines by electroacupuncture treatment (n = 5). **i.** Reduction in inflammatory cytokines (TNFRII, MIF, and MIF1β) by electroacupuncture treatment was also observed (n = 5). **j.** A significant reduction of serum OPG level and expression in obese patients was observed in patients receiving electroacupuncture compared to the sham control (n = 23). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

hand, subgroup analysis showed no significant age-related differences in serum OPG levels or relative OPG expression (Fig. S7b-c). These findings were consistent with our observation in rodents and suggested that electroacupuncture is effective for the management of central obesity in individuals with significant inhibition of serum OPG expression.

Discussion

OPG is a glycoprotein that is primarily known for its role in bone metabolism, in which it regulates the balance between bone resorption and formation by inhibiting the differentiation and activation of osteoclasts[25,26]. A controversial role of OPG in obesity has been proposed, but the exact mechanisms through which it regulates obesity are not yet fully understood. Because OPG is a well-documented antagonist of NF-κB signalling[27], it may help to reduce inflammation in adipose tissue and mitigate its detrimental effects on energy metabolism[28]. OPG may also have direct effects on energy metabolism. There are reports that serum OPG level increases in obese individuals or patients with metabolic syndrome, which can promote adipose tissue proinflammatory changes [29,30].

In vitro studies have shown that OPG can increase the expression of UCP1 in BAT, which is involved in the regulation of thermogenesis and energy expenditure[31]. Additionally, OPG has been shown to promote oxidative metabolism in skeletal muscle, which could enhance insulin sensitivity and help counteract obesity[32]. Although the majority of OPG's effects are thought to be mediated through its peripheral actions, there is evidence suggesting that OPG may also play a role in the central regulation of energy balance. OPG is expressed in various regions of the brain[33], and its levels in the hypothalamus have been shown to reduce food intake and body weight in rodents, suggesting that OPG may have anorexigenic effects[34]. However, some studies have also reported that OPG levels are positively correlated with adiposity and insulin resistance[35,36], indicating that the OPG secreted by adipose tissue may act as an adipokine in regulating adipose tissue inflammation, insulin resistance, and energy metabolism[30].

Our study revealed that during the early development of obesity, metabolic challenges can stimulate the expression of OPG in

adipocytes. This activated the expression of lipogenesis-related genes, which increased lipid storage in the cells, resulting in the expansion of adipose tissue and the progression of obesity. This supports the role of localised OPG in the adipogenesis of adipose tissue. OPG is a secretable decoy receptor for RANKL, which interacts with RANKL and limits its function. OPG itself can be controlled by other molecules, including TRAIL, von Willebrand factor, and glycosaminoglycans [37]. Since the function of OPG mainly requires interactions with other signalling molecules, it is necessary to be secreted and participate in intercellular communications in an autocrine/paracrine manner. A similar relationship is observed in the RANKL/RANK/OPG axis for the osteoclast to control osteoclastogenesis [38]. These molecules are secreted by the bone and act together to maintain a balance of bone metabolism. There is also a report that OPG/RANKL secreted by adipocytes have the effects of increased osteoblast proliferation [39]. This is a demonstration of the intracellular signaling pathway. From our results and these findings, we concluded that increased OPG is secreted by adipocytes during obesity development, and the extra OPG is targeting the adipocytes itself to further increase the lipogenesis through autocrine signaling.

Moreover, we observed that OPG can regulate the transcription activity of PPARϒ. Zhang et al. reported that hepatic expression of OPG promoted liver steatosis by facilitating the binding between PPARϒ and the PPAR response element (PPRE) site of the CD36 promoter[40]. Upon ligand binding, PPARϒ forms a heterodimer with RXR and binds to PPREs in the promoter region of target genes [41]. However, it remains unclear how OPG regulates PPARϒ. Consistent with this previous study, we did not observe a significant change in the expression of the endogenous co-activator or repressor of PPARϒ in rOPG-treated adipocytes, but rOPG rearranged the interaction network between PPARϒ and RXR. Instead of promoting the association between PPARϒ and RXR directly, OPG outcompeted the interaction of RXR with its endogenous repressor RAR by directly binding it. The binding between OPG and RAR allowed for the release of RXR from the RAR/RXR heterodimer. This enhanced the formation of PPARϒ/RXR heterodimers, which can promote gene expression related to the adipogenesis of white adipose tissues[42,43]. Some previous studies have revealed that RAR/RXR heterodimers bind to specific DNA sequences called retinoic acid response elements (RAREs), which are present in the promoter regions of UCP1[44]. UCP1 is a crucial protein involved in thermogenesis and promotes the differentiation of brown and beige adipocytes[45]. Together with our study, these findings reveal that OPG may serve as a critical molecule in the determination of cell fate and differentiation of adipocyte progenitor. The presence of a high level of OPG facilitates PPARϒ/RXR heterodimer-mediated differentiation of white adipocytes, while low cellular OPG levels allow RAR/RXR heterodimer-induced cells to differentiate into brown adipocytes. Interestingly, a previous study reported that the PPARϒ/RXR heterodimer can bind to the PPRE in the promoter region of OPG, leading to the transcription inhibition of OPG[46]. This suggests a negative feedback loop in the OPG-regulating PPARϒ transcription activity during the maintenance of the ability of adipocyte progenitor to differentiate towards various cell types. We found that in the early development of obesity, adipocytes received metabolic stress from high glucose and high insulin challenge, which led to a forced expression of intracellular OPG in adipocyte progenitor. This resulted in the development of white adipose tissue in obese individuals.

Regarding the observed effects of OPG knockdown on glucose metabolism, OPG knockdown may exert its effects through the modulation of inflammatory processes and tissue-specific pathways. OPG interacts with pro-inflammatory cytokines and signaling molecules, including TNF-α and NF-κB, crucial in insulin signaling and glucose metabolism. Reduction of OPG levels could downregulate inflammatory pathways, improving insulin sensitivity and glucose tolerance. Furthermore, OPG receptors, such as RANK and RANKL, are expressed differentially in metabolic tissues. Skeletal muscle, rich in OPG receptors, plays a vital role in insulin-mediated glucose uptake, potentially enhancing glucose clearance upon OPG knockdown. Additionally, OPG receptors in adipose tissue and liver may influence lipid metabolism and gluconeogenesis, respectively, contributing to the observed differential response in glucose tolerance and insulin sensitivity tests. The basal metabolic rate and body weight to body surface area ratio may also contribute to this variation. This is an interesting observation, and it is worth investigating further in future studies.

Our findings regarding electroacupuncture demonstrated that it effectively reduces both systemic and localized OPG levels in obese mice. This reduction disrupts pathways that are implicated in obesity development while regulating key pathways related to adipogenesis and mitigating inflammation in adipose tissue, resulting in improved metabolic parameters. A similar observation was found in our clinical study, where the serum OPG levels of patients with reduced body weight after electroacupuncture were significantly reduced. The patients also improved in blood glucose level and insulin resistance. Therefore, we concluded that electroacupuncture works in an OPG-dependent manner. These outcomes are consistent with existing evidence supporting acupuncture's efficacy in obesity treatment, as highlighted by Lei Ding *et al.*[47]. Nevertheless, the establishment of electroacupuncture's efficacy across diverse populations warrants further investigation through rigorous large-scale randomized controlled trials. Our research provides important new knowledge about electroacupuncture's potential in addressing obesity and underscores the need for ongoing scientific inquiry and clinical validation to confront the global challenge of obesity comprehensively.

In conclusion, we have demonstrated that OPG could be a targetable regulatory molecule in the development of obesity. The transcription of OPG was initiated by hyperglycaemic and hyperinsulinemia conditions in adipocytes in visceral fat tissue in the early phase of obesity development, which led to the expansion of adipocytes and intracellular lipid storage. The cellular OPG could bind to RAR, which released PPARϒ/RXR for the transcription activation of adipogenesis-associated genes. Adipose-specific knockout of OPG delayed the development of obesity, while OPG supplementation accelerated the progression of obesity and insulin resistance. Clinical and experimental observations revealed that electroacupuncture could improve obesity in an OPG-dependent manner. Our study sheds light on the role of OPG as a novel target for the successful management of obesity.

Compliance with Ethics Requirements.

All biological samples were obtained from the study participants after obtaining written informed consent. Approval was obtained from the Institutional Review Board of Hong Kong Baptist University (Approval Reference: HASC/HASC/17–18/C03). This study was performed in accordance with the Declaration of Helsinki. The protocols for animal studies were approved by the Committee for the Use of Laboratory Animals for Teaching and Research of the University of Hong Kong (22–122).

Acknowledgement

This work is financially supported by the University Research Committee of The University of Hong Kong (Project Code: 104006600), Nanyang Technological University Research Committee (Project Code: #22387-00001), Research Grant Committee of Hong Kong (Project Code: 17119621), The Health and Medical Research Fund (Project Code: 19201591 and 15162961) and Innovation and Technology Fund (Project Code: PRP/028/22FX), Health and Medical Research Fund (Project Code: 15163331). The authors

would like to express their appreciation to Mr. Keith Wong, Ms. Cindy Lee, Mr. Alex Shek in the School of Chinese Medicine and Centre for PanorOmic Science (CPOS): Imaging and Flow Cytometry Core of Li Ka Shing Faculty of Medicine, The University of Hong Kong, for their technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2024.06.018.

References

[1] Sárvári AK, Van Hauwaert EL, Markussen LK, Gammelmark E, Marcher AB, Ebbesen MF, et al. Plasticity of epididymal adipose tissue in response to diet-induced obesity at single-nucleus resolution. Cell Metab 2021;33:23.
[2] Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. Lancet Diabetes Endocrinol 2020;8:616–27.
[3] Shen S, Liao Q, Gu L, Zhu Y, Liu Y, Zhang X, et al. G protein-coupled receptor-biased signaling: potential drug discovery to facilitate treatment of metabolic diseases, Acta Materia. Medica 2024;3:31–45.
[4] Klein S, Gastaldelli A, Yki-Järvinen H, Scherer PE. Why does obesity cause diabetes? Cell Metab 2022;34:11–20.
[5] Hocking S, Samocha-Bonet D, Milner KL, Greenfield JR, Chisholm DJ. Adiposity and Insulin Resistance in Humans: The Role of the Different Tissue and Cellular Lipid Depots. Endocr Rev 2013;34:463–500.
[6] Ghaben AL, Scherer PE. Adipogenesis and metabolic health. Nat Rev Mol Cell Biol 2019;20:242–58.
[7] Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
[8] Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, et al. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997;390:175–9.
[9] Baud'huin M, Duplomb L, Teletchea S, Lamoureux F, Ruiz-Velasco C, Maillasson M, et al. Osteoprotegerin: Multiple partners for multiple functions. Cytokine Growth Factor Rev 2013;24:401–9.
[10] Consultation WE. Waist circumference and waist-hip ratio, Report of a WHO Expert Consultation. Geneva: World Health Organization; 2008, 2008,. p. 8–11.
[11] W.H. Organization W.H. organization, Regional office for the Western Pacific, The Asia-Pacific perspective: redefining obesity and its treatment 2000 (2000). Health Communications Australia Sydney
[12] Mutch DM, Tordjman J, Pelloux V, Hanczar B, Henegar C, Poitou C, et al. Needle and surgical biopsy techniques differentially affect adipose tissue gene expression profiles. Am J Clin Nutr 2009;89:51–7.
[13] A. Fuchs, D. Samovski, G.I. Smith, V. Cifarelli, S.S. Farabi, J. Yoshino, T. Pietka, S. W. Chang, S. Ghosh, T.M. Myckatyn, S. Klein, Associations Among Adipose Tissue Immunology, Inflammation, Exosomes and Insulin Sensitivity in People With Obesity and Nonalcoholic Fatty Liver Disease, Gastroenterology, 161 (2021) 968-+.
[14] Isidor MS, Dong WT, Servin-Uribe R, Villarroel J, Altintas A, Ayala-Sumuano JT, et al. Insulin resistance rewires the metabolic gene program and glucose utilization in human white adipocytes. Int J Obes 2022;46:535–43.
[15] Wang N, Tan H-Y, Li S, Wang D, Xu Y, Zhang C, et al. SBP2 deficiency in adipose tissue macrophages drives insulin resistance in obesity. Sci Adv 2019;5: eaav0198.
[16] Friesen M, Khalil AS, Barrasa MI, Jeppesen JF, Mooney DJ, Jaenisch R. Development of a physiological insulin resistance model in human stem cell-derived adipocytes. Sci Adv 2022;8:eabn7298.
[17] L.L. Listenberger, X. Han, S.E. Lewis, S. Cases, R.V. Farese, D.S. Ory, J.E. Schaffer, Triglyceride accumulation protects against fatty acid-induced lipotoxicity, Proceedings of the National Academy of Sciences, 100 (2003) 3077-3082.
[18] Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E, et al. Metabolic Dysfunction Drives a Mechanistically Distinct Proinflammatory Phenotype in Adipose Tissue Macrophages. Cell Metab 2014;20:614–25.
[19] Chava S, Chennakesavulu S, Gayatri MB, Reddy ABM. A novel phosphorylation by AMP-activated kinase regulates RUNX2 from ubiquitination in osteogenesis over adipogenesis. Cell Death Dis 2018;9:16.
[20] Ziouzenkova O, Orasanu G, Sharlach M, Akiyama TE, Berger JP, Viereck J, et al. Retinaldehyde represses adipogenesis and diet-induced obesity. Nat Med 2007;13:695–702.
[21] Lacey JM, Tershakovec AM, Foster GD. Acupuncture for the treatment of obesity: a review of the evidence. Int J Obes 2003;27:419–27.
[22] Liu SB, Wang ZF, Su YS, Qi L, Yang W, Fu MZ, et al. A neuroanatomical basis for electroacupuncture to drive the vagal-adrenal axis. Nature 2021;598:641-+.
[23] Gong MR, Wang XJ, Mao Z, Shao QH, Xiang XR, Xu B. Effect of Electroacupuncture on Leptin Resistance in Rats with Diet-Induced Obesity. Am J Chin Med 2012;40:511–20.
[24] Sui Y, Zhao HL, Wong VC, Brown N, Li XL, Kwan AK, et al. A systematic review on use of Chinese medicine and acupuncture for treatment of obesity. Obes Rev 2012;13:409–30.
[25] Ardeshirpour L, Dumitru C, Dann P, Sterpka J, VanHouten J, Kim W, et al. OPG Treatment Prevents Bone Loss During Lactation But Does Not Affect Milk Production or Maternal Calcium Metabolism. Endocrinology 2015;156:2762–73.
[26] Yasuda H. Discovery of the RANKL/RANK/OPG system. J Bone Miner Metab 2021;39:2–11.
[27] Lacey DL, Boyle WJ, Simonet WS, Kostenuik PJ, Dougall WC, Sullivan JK, et al. Bench to bedside: elucidation of the OPG-RANK-RANKL pathway and the development of denosumab. Nat Rev Drug Discov 2012;11:401-+.
[28] Vachliotis ID, Polyzos SA. Osteoprotegerin/Receptor Activator of Nuclear Factor-Kappa B Ligand/Receptor Activator of Nuclear Factor-Kappa B Axis in Obesity, Type 2 Diabetes Mellitus, and Nonalcoholic Fatty Liver Disease. Curr Obes Rep 2023;12:147–62.
[29] Zaky D, Ali A, Abd-Elraheem SE, Abdel-Moniem SH. Circulating osteoprotegerin level in relation to obesity in middle aged females. Int J Prev Treat 2019;8:41–5.
[30] Bernardi S, Fabris B, Thomas M, Toffoli B, Tikellis C, Candido R, et al. Osteoprotegerin increases in metabolic syndrome and promotes adipose tissue proinflammatory changes. Mol Cell Endocrinol 2014;394:13–20.
[31] Du JK, He ZH, Xu MM, Qu XH, Cui JQ, Zhang SY, et al. Brown Adipose Tissue Rescues Bone Loss Induced by Cold Exposure. Front Endocrinol 2022;12:10.
[32] Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL inhibition improves muscle strength and insulin sensitivity and restores bone mass. J Clin Invest 2019;129:3214–23.
[33] Hanada R. The role of the RANKL/RANK/OPG system in the central nervous systems (CNS). J Bone Miner Metab 2021;39:64–70.
[34] Cawley NX, Yanik T, Woronowicz A, Chang WZ, Marini JC, Loh YP. Obese carboxypeptidase E knockout mice exhibit multiple defects in peptide hormone processing contributing to low bone mineral density. Am J Physiol-Endocrinol Metab 2010;299:E189–97.
[35] Rashad NM, El-Shal AS, Shalaby SM, Abdel-Nour HM, Sarhan WM. Osteoprotegerin expression and serum values in obese women with type 2 diabetes mellitus. Mol Biol Rep 2021;48:7095–104.
[36] Ayina CNA, Sobngwi E, Essouma M, Noubiap JJN, Boudou P, Ngoa LSE, et al. Osteoprotegerin in relation to insulin resistance and blood lipids in sub-Saharan African women with and without abdominal obesity. Diabetol Metab Syndr 2015;7:4.
[37] Renema N, Navet B, Heymann M-F, Lezot F, Heymann D. RANK–RANKL signalling in cancer. Biosci Rep 2016;36.
[38] B. Xing J. Yu H. Zhang Y. Li RANKL inhibition: a new target of treating diabetes mellitus? Ther Adv Endocrinol Metab 14 2023 20420188231170754
[39] Kühn MC, Willenberg HS, Schott M, Papewalis C, Stumpf U, Flohé S, et al. Adipocyte-secreted factors increase osteoblast proliferation and the OPG/RANKL ratio to influence osteoclast formation. Mol Cell Endocrinol 2012;349:180–8.
[40] Zhang C, Luo XH, Chen JR, Zhou BY, Yang ML, Liu R, et al. Osteoprotegerin Promotes Liver Steatosis by Targeting the ERK-PPAR-γ-CD36 Pathway. Diabetes 2019;68:1902–14.
[41] Nakashima K, Yamaguchi E, Noritake C, Mitsugi Y, Goto M, Hirai T, et al. Discovery and SAR of Natural-Product-Inspired RXR Agonists with Heterodimer Selectivity to PPARδ-RXR. ACS Chem Biol 2020;15:1526–34.
[42] Noshiro M, Kawamoto T, Nakashima A, Ozaki N, Saeki M, Honda K, et al. DEC1 regulates the rhythmic expression of PPARγ target genes involved in lipid metabolism in white adipose tissue. Genes Cells 2020;25:232–41.
[43] Li Z, Luo LL, Yu WX, Li P, Ou DF, Liu J, et al. PPARγ phase separates with RXRα at PPREs to regulate target gene expression. Cell Discov 2022;8:15.
[44] Teruel T, Hernandez R, Benito M, Lorenzo M. Rosiglitazone and retinoic acid induce uncoupling protein-1 (UCP-1) in a p38 mitogen-activated protein kinase-dependent manner in fetal primary brown adipocytes. J Biol Chem 2003;278:263–9.
[45] Liu JX, Wang YT, Lin LG. Small molecules for fat combustion: targeting obesity. Acta Pharm Sin B 2019;9:220–36.
[46] Fu MG, Zhang JF, Lin YM, Zhu XJ, Willson TM, Chen YQE. Activation of peroxisome proliferator-activated receptor γ inhibits osteoprotegerin gene expression in human aortic smooth muscle cefls. Biochem Biophys Res Commun 2002;294:597–601.
[47] Ding L, Teng RF, Zhu YF, Liu FM, Wu LL, Qin LL, et al. Electroacupuncture treatment ameliorates metabolic disorders in obese ZDF rats by regulating liver energy metabolism and gut microbiota. Front Endocrinol 2023;14:15.