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Effect of cinacalcet-mediated parathyroid hormone reduction on vitamin D metabolism in high-fat diet-induced obese mice

Tae Yeon Kim [1], **Chan Yoon Park** [2], **and Sung Nim Han** [1,3]

[1]Department of Food and Nutrition, College of Human Ecology, Seoul National University, Seoul 08826, Korea
[2]Department of Food and Nutrition, College of Health Science, The University of Suwon, Hwaseong 18323, Korea
[3]Research Institute of Human Ecology, Seoul National University, Seoul 08826, Korea

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Correspondence to
Sung Nim Han
Department of Food and Nutrition, College of Human Ecology, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea.
Tel: +82-2-880-6836,
Email: snhan@snu.ac.kr

ORCID iDs
Tae Yeon Kim
https://orcid.org/0000-0003-0733-695X
Chan Yoon Park
https://orcid.org/0000-0002-8597-7210
Sung Nim Han
https://orcid.org/0000-0003-0647-2992

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There are no financial or other issues that might lead to conflict of interest.

ABSTRACT

Purpose: Obesity is associated with alterations in vitamin D metabolism and elevation of parathyroid hormone (PTH). Increased PTH level in obesity is likely one of the factors contributing to the dysregulation of vitamin D metabolism. We investigated the effects of lowering the PTH level in high-fat diet-induced obese mice on vitamin D metabolism.
Methods: Five-week-old male C57BL/6N mice were fed either with control (10% energy as fat) or high-fat (60% energy as fat) diets *ad libitum* for 12 weeks, and vehicle or cinacalcet HCl (30 μg/g body weight) was gavaged daily during the final week of the experiment. The following groups were studied: CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl). PTH, 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), 25-hydroxyvitamin D (25[OH]D), calcium, and phosphate levels in circulation, and the expression of genes related to vitamin D metabolism in the liver and kidneys were determined.
Results: Renal 1α-hydroxylase expression in the HFD group was higher than that in the CON group despite the lack of a difference in the PTH levels between the 2 groups. The plasma PTH level in the HFD-CIN group was 60% lower than that in the HFD group ($p < 0.05$). In parallel, the HFD-CIN group had lower adipose tissue amount (9% lower), renal 1α-hydroxylase expression (48% lower), and plasma $1,25(OH)_2D$ concentration (38% lower) than the HFD group.
Conclusion: Lowering the PTH levels in high-fat diet-induced obese mice recovered the expression of renal 1α-hydroxylase and might be associated with lower amounts of white adipose tissue.

Keywords: parathyroid hormone; 1,25-dihydroxyvitamin D; 25-Hydroxyvitamin D3 1-alpha-Hydroxylase; Vitamin D3 24-Hydroxylase; obesity

INTRODUCTION

Obesity has been associated with alteration in the vitamin D endocrine system. Several studies have reported that obese subjects have lower serum 25-hydroxyvitamin D (25[OH]D) levels but that serum 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) levels are either elevated or decreased [1-3]. Moreover, the expression of 1- or 25- hydroxylases involved in vitamin

D activation has been shown to be altered with obesity [4]. Suggested underlying causes of altered vitamin D metabolism in obesity include deposition of vitamin D in body fat compartments and reduced activation and/or increased catabolism of vitamin D metabolites in association with secondary hyperparathyroidism [3-6]. However, the precise mechanisms remain unclear.

Classically, vitamin D plays a critical role in the regulation of calcium and phosphate homeostasis and in bone health [7,8]. Since vitamin D receptor (VDR) that binds to $1,25(OH)_2D$ has been identified in various tissues, vitamin D's role has been expanded to immune system and proliferation and differentiation of cells [9,10]. Vitamin D is converted to 25(OH)D, the major circulating form of vitamin D, in the liver and then hydroxylated to the active form of vitamin D, $1,25(OH)_2D$, by 1α-hydroxylase mainly in the kidney. 25(OH)D or $1,25(OH)_2D$ can also be converted to the catabolic products, $24,25(OH)_2D$ or $1,24,25(OH)_3D$, by 24-hydroxylase for inactivation and excretion [11]. The synthesis of $1,25(OH)_2D$ is tightly controlled by the levels of parathyroid hormone (PTH), serum calcium, serum phosphate, and $1,25(OH)_2D$ itself [12]. In response to low serum calcium concentration, PTH is secreted and it stimulates renal 1α-hydroxylase expression and suppresses 24-hydroxylase expression [13].

Increased serum PTH concentrations have been observed with obesity [1,14-16]. Not only serum PTH showed positive correlation with body mass index (BMI) in healthy subjects [15], but it also positively predicted the 12-month change in body weight and body fat [16]. Association between increased body fat and elevated serum PTH level can be explained by several mechanisms [17-19]. PTH elevation by low calcium diet can increase $1,25(OH)_2D$, which stimulates adipocyte calcium influx and consequently increases adiposity [18]. Furthermore, elevated PTH itself has been reported to promote lipogenesis in the myocardium of rat by impairing fatty acid oxidation [17]. Since PTH increases $1,25(OH)_2D$ synthesis by enhancing 1α-hydroxylase expression, an obesity-related increase in PTH might be responsible for the alteration of vitamin D metabolism. High levels of PTH and $1,25(OH)_2D$ have been reported with obesity, but a causal relationship has not been established. Therefore, we investigated the specific effect of PTH reduction on vitamin D metabolism in obesity.

Cinacalcet HCl is the first calcimimetic, a pharmaceutical drug that mimics the action of calcium on tissues, approved by the US Food and Drug Administration (FDA) for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease and hypercalcemia in patients with parathyroid carcinoma [20]. Cinacalcet HCl functions as an allosteric modulator of the calcium-sensing receptor on parathyroid cells and enhances the sensitivity of the receptor activated by extracellular calcium, which leads to the suppression of PTH release from parathyroid cells [21].

This study was undertaken to identify whether lowering PTH can alter the vitamin D metabolism in high-fat diet-induced obese mice. Cinacalcet HCl administration was used for PTH reduction. The blood levels of vitamin D metabolites, calcium, and phosphate were measured, and the expression of genes involved in vitamin D metabolism was determined.

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METHODS

Animals and diets

Five-week-old male C57BL/6 mice were purchased from Central Laboratory Animal Inc. (Seoul, Korea) and housed in a semi-specific pathogen-free room under controlled temperature (23 ± 3°C), humidity (55 ± 10%), and light/dark cycle (12-hour dark/12-hour light 6:00 AM–6:00 PM). After 3 days of acclimation on the control diet, mice were randomly divided into one of three experimental groups. The CON group (n = 9) was fed a control diet containing 10% kcal as fat (#D12450B; Research Diets, New Brunswick, NJ, USA), and the HFD group (n = 10) and HFD-CIN group (n = 11) were fed a high-fat diet containing 60% kcal as fat (#D12492, Research Diets, USA).

Diets were fed *ad libitum* for 12 weeks. Food intake was measured 4 times per week, and body weight was measured once a week. At the end of the experimental period, mice were euthanized by CO_2 asphyxiation after 12 hours of fasting. Blood was collected by cardiac puncture and serum and plasma were separated, and liver, kidney, and white adipose tissues (WAT: epididymal, subcutaneous, perirenal-retroperitoneal, and visceral fat depots) were collected and weighed, and stored at −80°C until analysis. All experimental procedures were conducted following the protocols approved by the Institutional Animal Care and Use Committee of Seoul National University (approval No. SNU-170404-10-2).

Cinacalcet HCl preparation and *in vivo* treatment

During the final week of the experiment, all unanesthetized mice were gavaged with either cinacalcet HCl (30 μg/g body weight) or vehicle (20% sulfobutylether-β-cyclodextrin in distilled water) every day. Cinacalcet HCl or vehicle (MedChemExpress, Monmouth Junction, NJ, USA) was administered at 24-hour intervals, and blood samples were collected 2 hours after the final administration.

Determination of plasma PTH and vitamin D metabolites, and serum calcium and phosphate concentrations

Plasma PTH concentrations were measured by a commercial mouse intact PTH 1-84 ELISA kit (Immutopics Inc., San Diego, CA, USA). Plasma 25(OH)D and $1,25(OH)_2D$ concentrations were measured using an EIA kit (ImmunoDiagnostic Systems Ltd., Boldon Colliery, UK). Serum calcium concentrations were determined using a commercial calcium colorimetric assay kit (Abcam, Cambridge, UK). Serum phosphate concentrations were determined using a commercial phosphate assay kit (Sigma Aldrich, St. Louis, MO, USA).

Real-time polymerase chain reaction (PCR) analysis of genes related to vitamin D metabolism in liver and kidney

Total RNA was extracted from the liver and the kidney using RNAiso plus (Takara Bio Inc., Tokyo, Japan). Two μg of total RNA was reverse-transcribed into cDNA with a PrimeScript 1st strand cDNA synthesis kit (Takara Bio Inc.).

The mRNA levels of 25-hydroxylases (*Cyp2r1, Cyp27a1*) in the liver and those of 1α-hydroxylase (*Cyp27b1*), 24-hydroxylase (*Cyp24a1*), and PTH receptor (*Pthr*) in the kidney were measured by real-time PCR using StepOne Real-time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq (Takara Bio Inc., Japan). Calculations were performed by a comparative method ($2^{-\Delta\Delta CT}$) using the housekeeping gene *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase) as an endogenous control, and expressed as relative mRNA levels

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compared to the average levels of the control group. The following primer sequences were used: mouse *Cyp2r1*: 5'-TGG TGA GGT AAA TGA GGC TTTC-3'(forward) and 5'-TGC CAG TGC TCC AGT CTTC-3' (reverse); mouse *Cyp27a1*: 5'-CCA AGG CAA GGT GGT AGA GA-3' (forward) and 5'-CTT CAT CGC ACA AGG AGA GC-3' (reverse); mouse *Cyp27b1*: 5'-GAC GAT GTT GGC TGT CTT CC-3' (forward) and 5'- ATC TCT TCC CTT CGG CTT TG-3' (reverse); mouse *Cyp24a1*: 5'-TCC CTG AGT AAT GGG CTT TG-3' (forward) and 5'-CAC GGT AGG CTG CTG AGA TT-3' (reverse); mouse *Pthr*: 5'- CCA GCG TGA AGC CAG AGT AG-3' (forward) and 5'- GGG AAC GGG AGG TAT TTG AC-3' (reverse); mouse *Gapdh*: 5'-GGA GAA ACC TGC CAA GTA-3' (forward) and 5'-AAG AGT GGG AGT TGC TGT TG-3' (reverse).

Statistical analysis

All statistical analyses were performed using SPSS statistical software version 23.0 (IBM Corp., Armonk, NY, USA). All data were expressed as means ± standard error of means. Significant differences ($p < 0.05$) were determined using a one-way analysis of variance (ANOVA) test to evaluate overall effects of treatments, followed by Fisher's least significant difference (LSD) post-hoc test to compare differences among individual groups.

RESULTS

Body weight, weight gain, white adipose tissue weight, and food intake

After 12 weeks of feeding, body weight and weight gain were significantly higher in the HFD and HFD-CIN groups than the CON group ($p < 0.001$) (**Table 1**). The WAT weight was significantly higher in the HFD group than the CON group ($p < 0.001$). The HFD-CIN group showed significantly lower WAT weight than the HFD group ($p < 0.05$) and significantly higher WAT weight than the CON group ($p < 0.001$).

The average food intake for 12 weeks (g/day) was significantly lower in the HFD and HFD-CIN groups than the CON group ($p < 0.001$), whereas the average energy intake was significantly higher in the HFD and HFD-CIN groups. There was no significant difference in average food intake (g/day) or energy intake (kcal/day) between HFD and HFD-CIN groups.

Plasma PTH concentration

Cinacalcet treatment was effective in lowering PTH levels, as the HFD-CIN group (34.3 ± 1.3 pg/mL) had significantly lower plasma PTH concentration than the CON (90.9 ± 8.4 pg/mL)

Table 1. Body weight, weight gain, adipose tissue weight, and food intake of mice in the CON, HFD and HFD-CIN groups

Variables	CON (n = 9)	HFD (n = 10)	HFD-CIN (n = 11)	p-value[1)]
Body weight at week 0 (g)	19.8 ± 0.3	19.9 ± 0.3	19.8 ± 0.4	0.952
Body weight at week 12 (g)	29.8 ± 0.4^{b}	39.1 ± 0.6^{a}	38.3 ± 0.5^{a}	< 0.001
Weight gain[2)] (g)	10.0 ± 0.4^{b}	19.2 ± 0.5^{a}	18.5 ± 0.4^{a}	< 0.001
WAT weight[3)] (g)	2.49 ± 0.13^{c}	5.55 ± 0.21^{a}	5.08 ± 0.12^{b}	< 0.001
Average food intake[4)] (g/day)	2.67 ± 0.04^{a}	2.33 ± 0.04^{b}	2.28 ± 0.01^{b}	< 0.001
Average energy intake (kcal/day)	10.30 ± 0.15^{b}	12.22 ± 0.21^{a}	11.93 ± 0.06^{a}	< 0.001

Data are presented as means ± standard error of means. Groups were CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl).
ANOVA, analysis of variance; WAT, white adipose tissues.
[1)]One-way ANOVA was used to determine the significant differences among groups followed by Fisher's LSD test.
[2)]Average weight gain (g) for 12 weeks.
[3)]WAT includes perirenal, intraperitoneal, epididymal, and subcutaneous fat.
[4)]Average food intake (g/day) for 12 weeks.
Different letters indicate significant differences at $p < 0.05$ by Fisher's LSD multiple comparison test.

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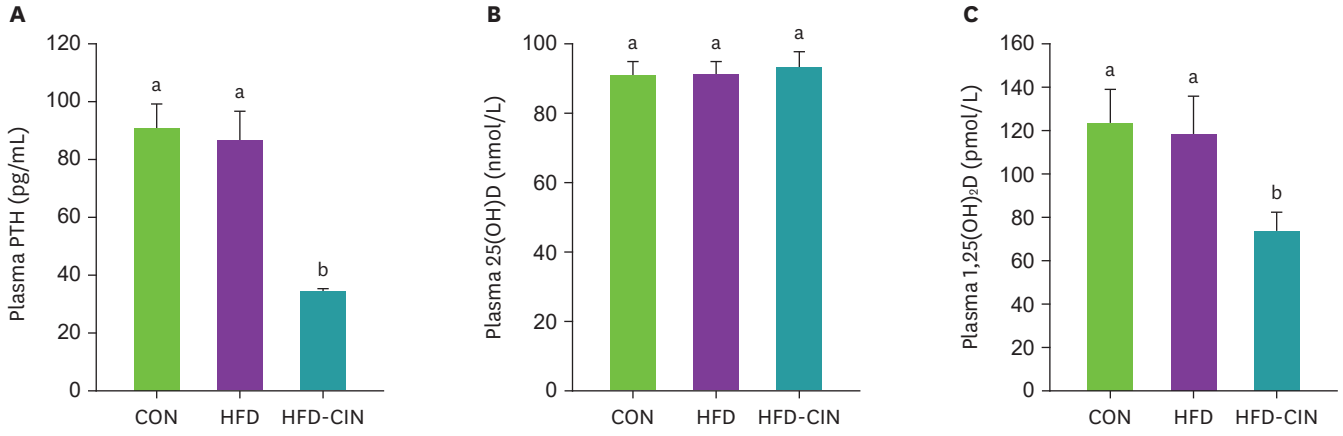

Fig. 1. Plasma levels of (A) PTH, (B) 25(OH)D, and (C) 1,25(OH)$_2$D.
Plasma levels of PTH and vitamin D metabolites were measured by enzyme-linked immunosorbent assay. Values are presented as means ± standard error of means (n = 8 to 11 per group). Groups were CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl).
PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D.
Different letters indicate significant differences at $p < 0.05$ by Fisher's LSD multiple comparison test.

and HFD groups (86.6 ± 10.2 pg/mL) ($p < 0.001$). The CON and HFD groups did not show a significant difference in PTH levels (**Fig. 1A**).

Plasma concentrations of vitamin D metabolites

Plasma 25(OH)D concentrations were not different among the three groups (**Fig. 1B**), while plasma 1,25(OH)2D levels were significantly lower in the HFD-CIN group than the CON and HFD groups (123.4 ± 15.1 pmol/L in the CON group, 118.0 ± 17.3 pmol/L in the HFD group, and 73.5 ± 8.6 pmol/L in the HFD-CIN group, $p < 0.05$) (**Fig. 1C**).

Serum concentrations of calcium and phosphate

Serum calcium concentrations were significantly lower in the HFD-CIN group than the CON (33% lower, $p < 0.05$) and HFD groups (45% lower, $p < 0.001$) (**Table 2**). Serum phosphate concentrations were significantly higher in the HFD-CIN group than the CON (14% higher, $p < 0.05$) and HFD groups (19% higher, $p < 0.05$) (**Table 2**). There was no significant difference between the CON and HFD groups in serum calcium or phosphate concentrations.

Hepatic mRNA levels of 25-hydroxylases

The *Cyp2r1* mRNA levels were significantly higher in the HFD and HFD-CIN groups compared with the CON group (53% higher in the HFD group, 41% higher in the HFD-CIN group, $p < 0.05$). There was no difference in *Cyp2r1* expression between the HFD and HFD-CIN groups (**Fig. 2**). Hepatic *Cyp27a1* mRNA levels did not show significant difference among the groups.

Table 2. Serum levels of calcium and phosphate

Variables	CON	HFD	HFD-CIN	p value[1)]
Serum calcium (mM)	2.9 ± 0.4^a	3.6 ± 0.3^a	2.0 ± 0.3^b	< 0.05
Serum phosphate (mM)	255.2 ± 11.2^b	245.2 ± 7.7^b	291.1 ± 12.5^a	< 0.05

Data are presented as means ± standard error of means, n = 8 to 11 per group. Groups were CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl).
ANOVA, analysis of variance; LSD, least significant difference.
[1)]One-way ANOVA was used to determine the significant differences among groups followed by Fisher's LSD test.
Different letters indicate significant differences at $p < 0.05$ by Fisher's LSD multiple comparison test.

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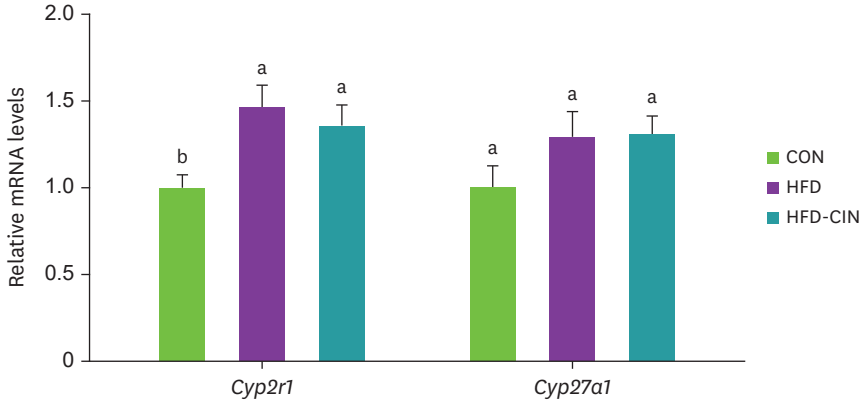

Fig. 2. mRNA levels of genes related to vitamin D metabolism in liver.
The mRNA levels of *Cyp2r1* and *Cyp27a1* in the liver were measured by quantitative real-time PCR. Values were normalized to the levels of housekeeping gene *Gapdh* and expressed as relative mRNA levels compared with the average levels of the CON group. Values are presented as means ± standard error of means (n = 8 to 11 per group). Groups were CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl).
PCR, polymerase chain reaction; LSD, least significant difference.
Different letters indicate significant differences at $p < 0.05$ by Fisher's LSD multiple comparison test.

Renal mRNA levels of 1α-hydroxylase, 24-hydroxylase, and parathyroid hormone 1 receptor

Renal 1α-hydroxylase expression was different among the groups ($p < 0.05$). Renal *Cyp27b1* mRNA levels were significantly higher in the HFD group than the CON group (257% higher, $p < 0.05$). Renal *Cyp27b1* expression was down-regulated by cinacalcet HCl treatment. Renal *Cyp27b1* mRNA levels were significantly lower in the HFD-CIN group than the HFD group (48% lower, $p < 0.05$). Renal *Cyp27b1* mRNA levels were similar between the HFD-CIN and CON groups.

Renal *Cyp24a1* mRNA levels were significantly higher in the HFD-CIN group than the HFD group (224% higher, $p < 0.05$). There was no significant difference in renal *Pth1r* expression among the groups (**Fig. 3**).

DISCUSSION

In the current study, we investigated whether the reduction of PTH could alter the $1,25(OH)_2D$ metabolism in HFD-induced obesity. The oral administration of cinacalcet HCl in HFD-induced obese mice was effective in lowering PTH levels. This reduction of PTH levels led to a down-regulation of 1α-hydroxylase mRNA levels and up-regulation of 24-hydroxylase mRNA levels, resulting in lower plasma $1,25(OH)_2D$ levels.

PTH is a key regulator of the renal 1α-hydroxylase expression [13]. In our previous studies, serum PTH levels were elevated in high-fat diet-induced obese mice compared with control mice [4,22]. Obesity was associated with secondary hyperparathyroidism in humans [23]. Cinacalcet HCl has been used for the effective treatment of secondary hyperparathyroidism in animal model and in clinical studies [24,25]. Therefore, in this study, cinacalcet HCl was used to decrease plasma PTH levels in HFD-induced obese mice. Cinacalcet HCl administration effectively lowered the PTH levels in the HFD-CIN group compared with the HFD group. Lower PTH concentrations resulted in lower 1α-hydroxylase expression in the HFD-CIN

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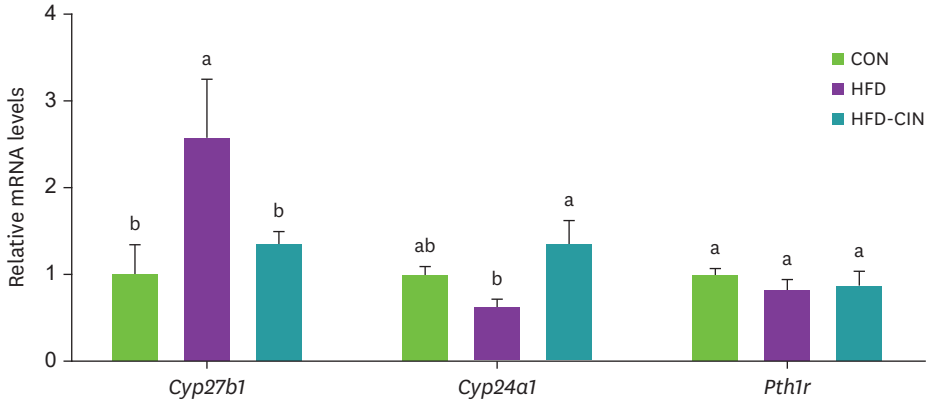

Fig. 3. mRNA levels of genes related to vitamin D metabolism in kidney.
The mRNA levels of *Cyp27b1*, *Cyp24a1* and *Pth1r* in the kidney were measured by quantitative real-time PCR. Values were normalized to the levels of housekeeping gene *Gapdh* and expressed as relative mRNA levels compared with the average levels of the CON group. Values are presented as means ± standard error of means (n = 8 to 11 per group). Groups were CON (control diet + vehicle), HFD (high-fat diet + vehicle), and HFD-CIN (high-fat diet + cinacalcet HCl).
PCR, polymerase chain reaction; LSD, least significant difference.
Different letters indicate significant differences at $p < 0.05$ by Fisher's LSD multiple comparison test.

group compared with the HFD group. As a result, plasma $1,25(OH)_2D$ concentrations were down-regulated. Expression of 24-hydroxylase, a catabolic enzyme for 25(OH)D and $1,25(OH)_2D$, is attenuated by PTH and shows a negative correlation with $1,25(OH)_2D$ [26]. In the current study, 24-hydroxylase mRNA levels were higher in the HFD-CIN group than in the HFD group, which could be due to reduced PTH levels in the HFD-CIN group. Therefore, lower $1,25(OH)_2D$ levels with the cinacalcet HCl treatment in the HFD-CIN group seemed to be the result of decreased 1α-hydroxylase and increased 24-hydroxylase expression caused by lower PTH levels.

In the current study, there was no significant difference in PTH levels between the CON and HFD groups, which is inconsistent with our previous findings [4,22] and those of others [27]. However, Camozzi *et al.* [28] reported no significant difference in PTH concentrations among normal, overweight, and obese subjects in a double-blind, randomized, placebo-controlled trial. Therefore, it is speculated that the PTH concentration is not always elevated in obesity. Also, it is controversial whether overweight is the consequence of elevated PTH or vice versa [15,29]. Excess PTH secretion induced by low calcium or vitamin D has been reported to increase intracellular calcium level in adipocytes followed by lipolysis inhibition and fatty acid synthesis that result in weight gain [29,30]. Furthermore, supplementation of calcium and vitamin D has been suggested as the alternative strategies for down-regulating PTH to lessen the risk for weight gain. This study showed that down-regulation of PTH level in obese mice can reduce white adipose tissue amount in association with reduced renal 1α-hydroxylase expression, serum 1,25(OH)2D, and serum calcium levels.

Higher expression of renal 1α-hydroxylase has been consistently observed with high-fat diet-induced obesity despite the lack of difference in PTH concentrations between lean control and obese groups. Renal 1α-hydroxylase expression is regulated by a number of physiological factors, including PTH, insulin, and interferon gamma (INF-γ) [31,32]. Although PTH serves as a major stimulator for renal 1α-hydroxylase expression, in the current study, elevated 1α-hydroxylase expression in obese mice might be due to other factors. Levels of insulin, one of the 1α-hydroxylase regulating factors, were shown to be higher in obese mice fed a

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high-fat diet [33]. INF-γ is a strong activator of Janus kinase 3 (JAK3) via CD40 signaling [34], and JAK3 was shown to be a powerful regulator of hydroxylase expression in an animal study [35]. Since HFD-induced mice have been reported to produce more INF-γ [36], it could possibly mediate the up-regulation of 1α-hydroxylase in obese mice. In the current study, the possibility of insulin or INF-γ contributing to higher 1α-hydroxylase expression in obese mice in the absence of PTH elevation could not be determined because quantity of blood samples was not enough to measure them. Hence, further studies are needed to identify the influence of these factors.

Dissociation of 1α-hydroxylase expression and $1,25(OH)_2D$ concentration was observed in the CON and HFD groups in this study. The levels of 1α-hydroxylase mRNA levels were higher in the HFD group than the CON and HFD-CIN groups. However, it did not result in a difference in plasma $1,25(OH)_2D$ concentrations between the CON and HFD groups. Plasma $1,25(OH)_2D$ levels were lower in adult (12-month-old) rats even though there was no difference in renal *Cyp27b1* mRNA levels between adult and young (2-month-old) rats and the decreased activity of *Cyp27b1* protein caused by oxidative damage in the adult rats was suggested as one of the possible explanations [37]. Oxidative damage can arise through oxidative stress [38]. Eo et al. [39] showed that high-fat diet-induced obese mice had significantly higher protein carbonyl groups, the oxidative stress end products. Furthermore, increased BMI was associated with oxidative damage in obese subjects [40]. Therefore, the disconnection between 1α-hydroxylase expression and plasma $1,25(OH)_2D$ levels could be related to the reduction of *Cyp27b1* activity by oxidative damage due to increased oxidative stress in obese mice.

In the current study, plasma 25(OH)D concentrations and hepatic *Cyp2r1* and *Cyp27a1* mRNA levels between the HFD and HFD-CIN groups were not different. PTH seems to have no direct effect on the production of serum 25(OH)D [12]. Our results also support this as lowering of PTH did not result in changes in gene expression of 25-hydroxylases. The CON group had lower expression of *Cyp2r1* but not *Cyp24a1* compared with the HFD and HFD-CIN groups. CYP2R1 and CYP27A1 are well known as hepatic 25-hydroxylases in murine and human cells among a number of cytochrome P450 enzymes with 25-hydroxylase activity [41]. A null mutant mouse lacking *Cyp2r1* was still capable of 25(OH)D synthesis, suggesting the influence of other 25-hydroxylases on plasma 25(OH)D production [42]. Therefore, no difference in plasma 25(OH)D levels among groups in our study could be explained by no difference in other 25-hydroxylases, especially *Cyp27a1*.

SUMMARY

Altered 1α-hydroxylase mRNA levels in HFD-induced obesity can be reversed by lowering PTH levels. Although hyperparathyroidism was not observed in HFD-induced obesity, cinacalcet HCl-treatment in obese mice significantly reversed renal 1α-hydroxylase expression and reduced plasma PTH levels, $1,25(OH)_2D$ levels, and white adipose tissue amount, suggesting that the reduction of plasma PTH levels could be considered as a way to correct the vitamin D dysregulation observed with obesity.

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