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Examining the causal role of leptin in bone mineral density: A Mendelian randomization study

Xiang-He Meng[a,b], Li-Jun Tan[a], Hong-Mei Xiao[c], Bei-Sha Tang[c], Hong-Wen Deng[a,b,c,*]

[a] Laboratory of Molecular and Statistical Genetics, College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China
[b] Center of Bioinformatics and Genomics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, USA
[c] School of Basic Medical Science, National Clinical Research Center for Geriatric Diseases, Xiangya Hospital, Central South University, Changsha, Hunan 410013, China

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ABSTRACT

Leptin, a small polypeptide hormone secreted by the adipocytes, controls body weight and gonadal function by binding to a special receptor located in the hypothalamus. Observational studies have demonstrated a controversial association between leptin and bone mineral density (BMD), and functional studies of the relationship between leptin and BMD still largely vary by different studies. Using SNPs strongly associated with leptin levels in 52,140 individuals, we conducted a two-sample Mendelian randomization study to identify whether genetically lowered leptin levels were associated with BMD by using an inverse-variance weighted method, a weighted median method, MR-Egger and Robust Adjusted Profile Score. We found that circulating leptin levels may causally decrease lumbar spine BMD (effect size = −0.45, 95% CI: −0.82, −0.083; p value = 0.016). The association estimates of circulating leptin levels on femoral neck, forearm and total body BMD were not significant. Our study suggests that genetically predicted higher circulating leptin was associated with lower LS-BMD.

1. Introduction

Osteoporosis is a common disease characterized by low bone mineral density (BMD) and an increased susceptibility to fractures [1]. With an increase in the elderly population, osteoporosis has become a widespread disease causing physical pain, mental anguish and massive financial costs to individuals and societies [2]. However, there are still no osteoporosis treatments that are both highly effective and free of adverse effects [3]. Previous studies have suggested that pharmacological manipulation of the leptin pathway might be a potential therapeutic approach to prevent or treat osteoporosis [4].

Leptin is a small polypeptide hormone secreted primarily by the adipocytes. It controls body weight and gonadal function by binding to a special receptor located in the hypothalamus [5]. Observational studies suggest that circulating leptin level is positively correlated with BMD, especially in women [6–12]. Most of these studies were performed in small samples, or the positive associations were attenuated [11] or not significant [10] after adjusting for body mass index (BMI). In a large sample including 5815 adults from the United States, femoral BMD was positively associated with leptin concentration in men, premenopausal women and postmenopausal women [13]. However, after adjusting for BMI and other bone density-related factors, an inversed association in men emerged [13]. In addition, the associations in both premenopausal and postmenopausal women were not significant after the BMI adjustment [13].

Functional studies have shown that leptin may act on bone density via the central pathway or the peripheral pathway [14]. In the central pathway, leptin, released by adipocytes, binds to its receptor on the surface of neurons in the ventromedial hypothalamus. Then, it induces an increase in sympathetic activity that signals to osteoblasts via the b2 adrenergic receptors (Adrb2) [15]. Finally, leptin leads to bone loss by suppressing osteoblast proliferation [15] or increasing the expression of the receptor of NF-κB ligand (RANKL) activator which promotes the resorption of osteoclasts [16]. In the peripheral pathway, leptin increases bone mass by interacting with bone marrow mesenchymal stem cells (BMSCs), osteoblasts, osteoclasts and chondrocytes [14]. For example, leptin can enhance the proliferation and differentiation of BMSCs into osteoblastic lineage [17]. Leptin can also promote proliferation, collagen synthesis and mineralization of osteoblasts in vitro [18]. It inhibits osteoclast generation in vitro by decreasing the receptor activator of RANKL and increasing the expression of osteoprotegerin in stromal cells [18]. This suggests that leptin may have a positive or

* Corresponding author at: Center of Bioinformatics and Genomics, Department of Global Biostatistics and Data Science, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1610, New Orleans, LA 70112, USA.
E-mail address: hdeng2@tulane.edu (H.-W. Deng).

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negative effect on bone [14]. Thus it remains unknown whether (and by what mechanism) the two pathways interact with each other in the total collective net effect of leptin on BMD of different skeletal sites.

Given that previous studies were either based on small samples or only studied the correlation between circulating leptin concentrate and BMD, there is also a need to investigate the causal relationship in large-scale samples. Furthermore, observational epidemiological studies are susceptible to confounding and reverse causation [19]. In the era of genome-wide association studies (GWAS) and high-throughput genomic technologies, genetic data are routinely available from large studies. Mendelian randomization (MR) is an effective, powerful and efficient method for establishing causal relationships between two correlated phenotypes [20]. MR, which can use the summary statistics from GWAS, is a widely used causal inference method that uses genetic variants as instrumental variables (IVs) to detect whether the exposure phenotype has a causal effect on the outcome phenotype [21,22]. In this study, we used single nucleotide polymorphisms (SNPs) strongly associated with circulating leptin concentration as instrumental variables. Because there is no need to have both the exposure and the outcome data from one sample with a great sample size, two-sample MR which uses the effect of IVs on the exposure phenotype and the outcome phenotype from two independent studies has shown that the power of detecting causality can be greatly increased compared with using the data from a single sample study [23]. To investigate the potential role of leptin in BMD, we conducted a two-sample Mendelian randomization study.

2. Material and methods

2.1. BMD GWAS summary statistics

Femoral neck, lumbar spine and forearm are three common skeletal sites where osteoporotic fractures are most prevalent [24]. The GEnetic Factors for OSteoporosis (GEFOS) consortium performed a large meta-analysis to identify genetic variants associated with femoral neck BMD (FN-BMD), lumbar spine BMD (LS-BMD) or forearm BMD (FA-BMD) in 53,236 individuals of European ancestry [24]. Each SNP was tested for association with BMD (bone mass per unit of area scanned), adjusting for sex, age, age^2 and weight. BMD was standardized to have a mean of zero and a standard deviation of one in GWAS analyses.

Total body BMD (TB-BMD) GWAS summary data were used to estimate the general effect of leptin on whole body BMD. A previous meta-analysis of 30 GWASs of TB-BMD including 66,628 individuals was performed to investigate the genetic determinants of TB-BMD variation [25]. The additive effect of each SNP on the normalized BMD residuals was estimated via linear regression [25]. The meta-analyzed effect size estimates were used in this study. The summary association statistics were all downloaded from the GEFOS website (http://www.gefos.org/).

2.2. Leptin GWAS summary statistics

A large GWAS in 52,140 individuals of European ancestry identified 5 loci putatively associated ($p < 1 \times 10^{-6}$) with circulating leptin levels (log-transformed ng/mL) after adjusting for sex, age, age^2 and BMI [26].

2.3. SNP validation

In a Mendelian randomization study, SNPs were ideally expected to not be in linkage disequilibrium (LD), since using SNPs in strong LD may cause biased results. In this study, we measured LD between selected SNPs using HapMap 2 CEU as a reference panel. For all pairs of SNPs with $r^2 > 0.01$, the SNP with a larger association p value would be removed.

MR analysis assumes that the selected IVs should act on the outcome only through the exposure variable (no pleiotropic effects exist). Potential pleiotropic effects of selected SNPs on BMD were verified via the Ensembl project (http://www.ensembl.org). We searched each SNP one by one. When a SNP was associated with another phenotype other than leptin, we checked whether the associated phenotype was associated with BMD by conducting a literature search. Pleiotropy was defined here as a SNP influencing bone mass through a phenotype other than leptin.

2.4. Mendelian randomization estimates

The theories and methods for MR analysis have been well established [27]. MR analysis uses genetic variants as instrumental variables to estimate the causative effect of exposure variables on an outcome. We performed a meta-analysis using a fixed-effects model to obtain the combined effect of leptin SNPs on BMD at the three skeletal sites. The effects of leptin SNPs on BMDs were estimated from independent studies [24,25]. We harmonized the exposure and outcome data to ensure that the effect of a SNP on the exposure, and the effect of that same SNP on the outcome, correspond to the same allele. Here, we assumed that the effect size of the ith (i = 1, 2, …, I) SNP identified from GWAS on the exposure X (i.e., leptin level) and the outcome Y (i.e., BMD) were β_{Xi} and β_{Yi}, respectively. The standard error for each was σ_{Xi} and σ_{Yi}, respectively. The MR estimate of the causal effect of the exposure on the outcome using the ith SNP was $\beta_{XYi} = \beta_{Yi}/\beta_{Xi}$, and the variance of the estimate is σ_{Yi}/β_{Xi} [21]. The pooled effects of the exposure on the outcome using the fixed-effect inverse-variance weighted (IVW) method can be expressed as $\beta_{XY} = \frac{\sum_i \beta_{Xi}\beta_{Yi}\sigma_{Yi}^{-2}}{\sum_i \beta_{Xi}^2\sigma_{Yi}^{-2}}$, and the standard error of this estimate can be given by $se(\beta_{XY}) = \sqrt{\frac{1}{\sum_i \beta_{Xi}^2\sigma_{Yi}^{-2}}}$ [21]. A heterogeneity test was performed using Cochran's Q test to identify whether there was a higher heterogeneity between causal effects estimated using the variants individually than would be expected by chance [21].

To assess whether there are horizontal pleiotropic effects where instrumental variables affect BMD via other biological pathways, we performed MR-Egger regression [28]. The intercept deviated from the origin may provide evidence for potential pleiotropic effects across the instrumental variables. While the estimate for MR-Egger regression slope provides the pleiotropy-corrected causal effect, previous studies have confirmed that the weighted median approach affords some distinct superiorities over MR-Egger, like its improved power of causal effect detection and lower type I error [29]. In this study we also used the weighted median method to complement the MR-Egger regression to provide more robust MR estimates. The weighted median method may generate correct estimates even if up to 50% of SNPs are invalid instrumental variables [29].

Since we included many weak instrumental variables in the analyses, to make our results more reliable, we carried out a recently proposed method called Robust Adjusted Profile Score (MR.RAPS) [30]. This method can give a robust inference for Mendelian randomization with many weak instruments [30]. The analyses were performed by the Two Sample MR package in R software environment.

2.5. Sensitivity analyses

Since heterogeneity and unknown pleiotropy effects may exist, MR estimates were reperformed after excluding SNPs potentially influenced by pleiotropy.

All the analyses were conducted using R version 3.4.2. And the R code can be found in [21].

3. Results

Five SNPs (rs10487505, rs780093, rs900400, rs6071166, and rs6738627) were reported to be associated with log-transformed

circulating leptin levels after adjusting for BMI and other covariates [26]. None were excluded due to linkage disequilibrium (Table 1). Although the intronic rs6738627 variant in COBLL1 did not reach genome-wide significance, knockdown of Cobll1 significantly decreased leptin protein secretion by 16% [26]. This suggests that COBLL1 may be a causal gene regulating leptin levels. The association of rs900400, which is located 67 kb upstream from CCNL1, could be mediated by its association with birth weight, due to a mechanism shared between birth weight and leptin levels [26,31]. Variants in LD with rs780093 ($r^2 > 0.9$) were reported to be associated with > 25 metabolic traits, including fasting glucose, fasting insulin, high-density lipoprotein cholesterol, lower total cholesterol, low-density lipoprotein cholesterol, triglycerides, C-reactive protein and circulating uric acid levels [26]. A previous study suggested that circulating uric acid may play a protective role in preventing bone loss [32]. Observational studies have reported associations of C-reactive protein with osteoporosis [33,34]. Rs780093 was found to be associated with TB-BMD (p value = 2.33×10^{-7}). These studies suggested that rs780093 and those variants in strong LD with rs780093 may influence bone mass by means other than leptin. Therefore, we excluded this SNP in further analyses due to its potential pleiotropic effects. No potential pleiotropic effects of other SNPs relevant to BMD were identified. The estimated effect sizes of the remaining four SNPs on both the exposure (leptin) and BMD outcomes are displayed in scatter plots (Fig. S1).

The MR-Egger regression results showed that the estimated value for the intercept term was null for leptin and all BMDs (Table 2), suggesting that horizontal pleiotropy did not heavily influence the results.

Using the IVW method with fixed effects, we calculated the causal effects of circulating leptin level on BMD of three skeletal sites and total body, respectively (Fig. 1). We observed a significant association of genetically lowered circulating leptin level with increased LS-BMD (−0.45, 95% CI: −0.82, −0.083; p value = 0.016) with no heterogeneity (I^2 = 0%, 95% CI: 0%–79.7%; p value = 0.52). This result could be explained as one standard deviation increase in log-transformed circulating leptin levels decreased LS-BMD by 0.45 standard deviation. The causal estimate of circulating leptin level on FN-BMD was marginally significant (−0.30, 95% CI: −0.62, 0.011; p value = 0.059) with relatively large yet non-significant heterogeneity (I^2 = 56.4%, 95% CI: 0%–85.5%; p value = 0.076). The causal effect of circulating leptin level on FA-BMD was estimated as −0.36 (95% CI: −1.01, 0.30; p value = 0.28) with no heterogeneity (I^2 = 0%, 95% CI: 0%–0%; p value = 0.96). The estimated effect size of leptin on TB-BMD was −0.04 (95% CI: −0.28, 0.20; p value = 0.73; I^2 = 0%, 95% CI: 0%, 80%; p value = 0.96). The results are also displayed graphically in Fig. 1.

The estimates of other methods including MR-Egger regression, weighted median and MR.RAPS were consistent with the IVW results for FA-BMD, FN-BMD and TB-BMD. The slope estimation of circulating leptin level on LS-BMD using MR-Egger was not significant (beta = −0.012; 95% CI: −0.14, 0.12; p value = 0.74). The results estimated by weighted median method (beta = −0.46; 95% CI: −0.91, −0.06; p value = 0.034) and MR.RAPS (beta = −0.46; 95% CI: −0.85, 0.067; p value = 0.022) were consistent with the IVW result that an increase in log-transformed circulating leptin level was associated with decreased LS-BMD (Table 2).

There was large heterogeneity in the effect of leptin on FN-BMD, the direction of the estimated effect of leptin on FN-BMD using rs6071166 was different from that of the estimates using other SNPs. We conducted a sensitive analysis after excluding rs6071166. After removal of this SNP, we observed a clear association between leptin and FN-BMD (beta = −0.50; 95% CI: −0.87. -0.14; p-value = 0.0067) with no heterogeneity (I^2 = 0%, 95% CI: 0%–89.0%; p value = 0.39). The results of weighted method and MR.RAPS also showed this strong association, although the MR-Egger result was not significant (Table 3).

Table 1
Characteristics of SNPs associated with circulating leptin levels.

SNP	CHR	Gene	EA/OA[a]	EAF[b]	Association with leptin		Association with FN-BMD		Association with LS-BMD		Association with FA-BMD		Association with TB-BMD	
					β (se)	p value	β (se)[b]	p value	β (se)[c]	p value	β (se)[c]	p value	β (se)[c]	p value
rs10487505	7	LEP	G/C	0.50	0.029 (0.004)	1.99×10^{-12}	−0.0074 (0.0075)	0.34	−0.013 (0.0087)	0.14	−0.0041 (0.016)	0.79	0.0055 (0.0057)	0.33
rs780093	2	GCKR	C/T	0.61	0.024 (0.004)	3.80×10^{-10}	0.015 (0.0077)	0.057	0.022 (0.0089)	0.017	0.039 (0.016)	0.016	0.0302 (0.0058)	2.33×10^{-7}
rs900400	3	CCNL1	T/C	0.60	0.021 (0.004)	1.17×10^{-7}	−0.018 (0.0078)	0.017	−0.020 (0.0090)	0.030	−0.0080 (0.0016)	0.62	−0.0044 (0.0059)	0.45
rs6071166	20	SLC32A1	C/A	0.37	0.024 (0.004)	1.74×10^{-8}	0.0075 (0.0079)	0.35	−0.0024 (0.0091)	0.80	−0.14 (0.016)	0.40	−0.0049 (0.0059)	0.41
rs6738627	2	COBLL1	A/G	0.37	0.020 (0.004)	1.92×10^{-6}	−0.012 (0.0077)	0.11	−0.0076 (0.0090)	0.41	−0.010 (0.016)	0.54	−0.0028 (0.0058)	0.63

CHR: chromosome; EA: effect allele; OA: other allele; EAF: effect allele frequency.
[a] Allele associated with higher circulating leptin level.
[b] The frequency of effect allele was calculated from leptin study.
[c] Change in BMD (expressed as standard deviations of FN-BMD, LS-BMD, FA-BMD and TB-BMD) per additional exposure-increasing allele.

Table 2
Mendelian randomization estimates of circulating leptin level on BMDs.

BMDs	IVW			MR-Egger			WMM		MR.RAPS	
	Beta	p-Value	slope	p-Value	Intercept	p-Value	Beta	p-Value	Beta	p-Value
LS-BMD	−0.45 (−0.82, −0.08)	0.016	−0.012 (−0.14, 0.12)	0.74	0.027 (−5.54, 5.60)	0.99	−0.46 (−0.91, −0.06)	0.034	−0.46 (−0.85, 0.067)	0.022
FN-BMD	−0.30 (−0.62, 0.012)	0.059	−0.033 (−0.21, 0.15)	0.51	1.06 (−6.49, 8.61)	0.61	−0.33 (−0.76, 0.090)	0.12	−0.31 (−0.78, 0.16)	0.21
FA-BMD	−0.36 (−1.01, 0.30)	0.28	−0.021 (−0.25, 0.21)	0.73	0.52 (−9.11,10.16)	0.84	−0.28 (−1.05, 0.49)	0.48	−0.36 (−1.03, 0.31)	0.30
TB-BMD	−0.04 (−0.28, 0.20)	0.73	−0.025 (−0.11, 0.059)	0.33	0.82 (−2.54, 4.53)	0.35	−0.15 (−0.45, 0.15)	0.34	−0.042 (−0.29, 0.21)	0.74

4. Discussion

In this study, we used summary statistics from GWASs to identify the causal relationships between circulating leptin level and BMD at different skeletal sites. Results suggested that circulating leptin level causally decreased LS-BMD. The estimated effect sizes of leptin on FA-BMD and FN-BMD were not significant, and leptin did not increase or decrease TB-BMD. These results suggest that the effects of leptin on BMD at different skeletal sites may be differentially associated with various components of bone, since bone from different skeletal sites may differ in composition (e.g., different proportions of trabecular and cortical bones). It is established that there are differences of genetic determination among various skeletal sites [35]. Previous studies suggested that leptin decreases bone density mainly through the central nervous system [4,36]; however, the effects of leptin on bone density may vary between different skeletal regions and between cortical and trabecular moieties [4,37]. For example, the deficiency of leptin increased lumbar BMD, and decreased femoral BMD was found in leptin-deficient mice [37]. Another study found that leptin had no effect on cortical bone, but the deficiency of leptin increased trabecular bone volume [4]. Previous studies suggested that leptin had no effect on cortical bone, but the deficiency of leptin increased trabecular bone volume [4]. Lumbar spine has the highest percentage of cancellous bone in the skeletal sites studied here [38], and we found that the genetically decreased leptin caused an increase in LS-BMD. Total body has the lowest percentage of cancellous bone [38] for the BMD phenotypes studied here, and the estimated effect size of leptin on TB-BMD was almost zero.

The largest heterogeneity (I^2 = 56.4%) was observed in the causal estimate of circulating leptin level on FN-BMD, although the test of the causal estimate was nearly significant (p = 0.059). The sensitivity analysis identified a causal effect of genetically predicted leptin on FN-BMD. This result might indicate a potential effect of pleiotropy. The confidence interval of estimated effect size of circulating leptin level on FA-BMD was quite wide. Since relatively small-scale samples (n = 10,805) were included in the association test for FA-BMD compared with the three other tests (for LS-BMD, FN-BMD and TB-BMD), sample size may contribute to the relatively large standard error of effect size generated [24]. Therefore, the variance and the 95% CIs of the causal estimate of circulating leptin level on FA-BMD were relatively large. This observation may partially explain why the causal estimate of circulating leptin level on FA-BMD was not significant. The limited number of IVs used in MR may be another contributing cause underlying the lack of significant direct causal relationships between leptin levels and FN-BMD, FA-BMD and TB-BMD.

The present study may have some limitations that should be considered. First, the BMD GWAS of the three skeletal sites was adjusted for sex, age, age^2, and weight [24]. TB-BMD GWAS was corrected for age, weight, height, and genomic principal components [25]. The leptin study was adjusted for sex, age, age^2 and BMI [26]. Neither weight nor BMI distinguishes between lean and fat mass, although there were no marked differences in the effect sizes between the BMI and body fat percentage-adjusted results for the SNPs used in the meta-analysis study of circulating leptin levels [26]. Considering leptin is mainly secreted by the adipocytes, body fat percentage may be a better choice for adjustment. Second, previous studies suggested that the effect of leptin on bone metabolism was dose-dependent [39]. Administering low dose leptin was able to prevent the induced bone loss in tail-suspended rats [39]. High dose leptin was able to decrease bone formation and increase bone resorption [39]; however, we can only test the linear association between leptin and BMD using MR methods. Third, the LS-BMD association is not statistically significant at the Bonferroni corrected significance threshold (0.05/4 = 0.0125). The larger contribution of genetic variants will lead to an increased power for detecting the causal relationship by including more genetic variants as variables. When data with larger samples size are available, replication of this study is warranted.

In summary, our study found that genetically elevated circulating

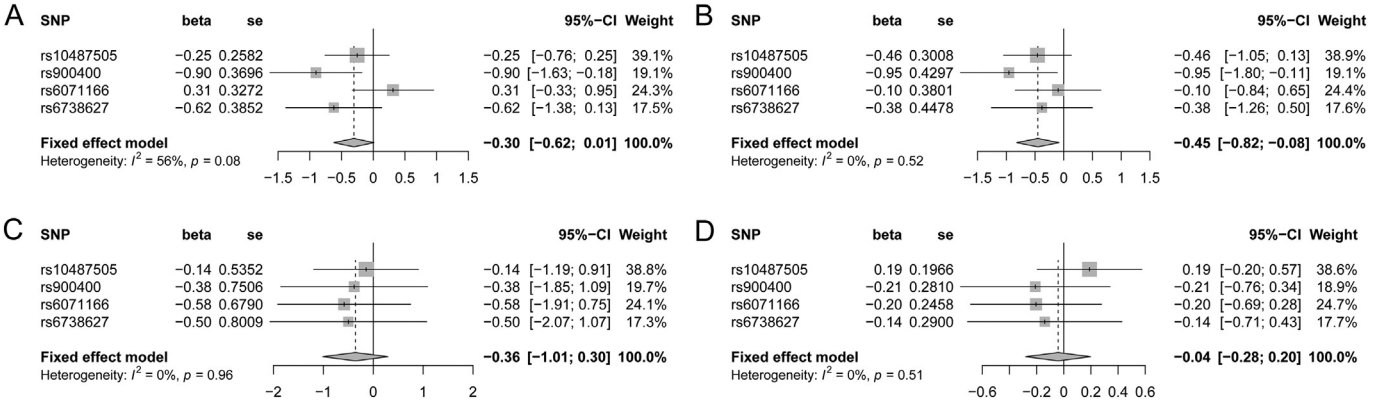

Fig. 1. Mendelian randomization estimates for the associations of leptin concentration with BMDs. Effect estimates are expressed as standard deviations of normalized FN-BMD (A), LS-BMD (B), FA-BMD (C) and TB-BMD (D) per genetically predicted one standard deviation (SD) increase in leptin concentration.

Table 3
Mendelian randomization estimates of the association of leptin and FN-BMD after excluding rs6071166 SNP.

IVW			MR-Egger				WMM		MR.RAPS	
Beta	p-Value	I2 (95% CI)	Slope	p-Value	Intercept	p-Value	Beta	p-Value	Beta	p-Value
−0.50 (−0.87, −0.14)	0.0067	8.9% (0%, 90.5%)	−0.034 (−0.36, 0.29)	0.41	0.90 (−12.88, 14.69)	0.56	−0.49 (−0.93, −0.051)	0.029	−0.51 (−0.91, 0.11)	0.011

leptin levels were potentially causally associated with lower LS-BMD. Further studies are warranted to confirm the relationship between leptin and FN-BMD.

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Competing interests

The authors have no financial interests to disclose.

References

[1] S.R. Cummings, L.J. Melton, Epidemiology and outcomes of osteoporotic fractures, Lancet 359 (9319) (2002) 1761–1767.
[2] N. Harvey, E. Dennison, C. Cooper, Osteoporosis: impact on health and economics, Nat. Rev. Rheumatol. 6 (2) (2010) 99–105.
[3] T.D. Rachner, S. Khosla, L.C. Hofbauer, Osteoporosis: now and the future, Lancet 377 (2011) 1276–1287.
[4] P. Ducy, M. Amling, S. Takeda, M. Priemel, A.F. Schilling, et al., Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass, Cell 100 (2000) 197–207.
[5] J.M. Friedman, J.L. Halaas, Leptin and the regulation of body weight in mammals, Nature 395 (1998) 763–770.
[6] T. Thomas, B. Burguera, L.J. Melton 3rd, E.J. Atkinson, W.M. O'Fallon, et al., Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women, Bone 29 (2001) 114–120.
[7] M. Yamauchi, T. Sugimoto, T. Yamaguchi, D. Nakaoka, M. Kanzawa, et al., Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women, Clin. Endocrinol. 55 (2001) 341–347.
[8] M. Sato, N. Takeda, H. Sarui, R. Takami, K. Takami, et al., Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men, J. Clin. Endocrinol. Metab. 86 (2001) 5273–5276.
[9] S. Ormarsdottir, O. Ljunggren, H. Mallmin, H. Olofsson, W.F. Blum, et al., Inverse relationship between circulating levels of leptin and bone mineral density in chronic liver disease, J. Gastroenterol. Hepatol. 16 (2001) 1409–1414.
[10] N. Tanna, K. Patel, A.E. Moore, D. Dulnoan, S. Edwards, et al., The relationship between circulating adiponectin, leptin and vaspin with bone mineral density (BMD), arterial calcification and stiffness: a cross-sectional study in post-menopausal women, J. Endocrinol. Investig. 40 (2017) 1345–1353.
[11] E. Biver, C. Salliot, C. Combescure, L. Gossec, P. Hardouin, et al., Influence of adipokines and ghrelin on bone mineral density and fracture risk: a systematic review and meta-analysis, J. Clin. Endocrinol. Metab. 96 (2011) 2703–2713.
[12] K. Liu, P. Liu, R. Liu, X. Wu, M. Cai, Relationship between serum leptin levels and bone mineral density: a systematic review and meta-analysis, Clin. Chim. Acta 444 (2015) 260–263.
[13] C.E. Ruhl, J.E. Everhart, Relationship of serum leptin concentration with bone mineral density in the United States population, J. Bone Miner. Res. 17 (2002) 1896–1903.
[14] X.X. Chen, T. Yang, Roles of leptin in bone metabolism and bone diseases, J. Bone Miner. Metab. 33 (2015) 474–485.
[15] G. Karsenty, Convergence between bone and energy homeostases: leptin regulation of bone mass, Cell Metab. 4 (2006) 341–348.
[16] V. Schwetz, T. Pieber, B. Obermayer-Pietsch, The endocrine role of the skeleton: background and clinical evidence, Eur. J. Endocrinol. 166 (2012) 959–967.
[17] T. Thomas, F. Gori, S. Khosla, M.D. Jensen, B. Burguera, et al., Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes, Endocrinology 140 (1999) 1630–1638.
[18] J.O. Gordeladze, C.A. Drevon, U. Syversen, J.E. Reseland, Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: impact on differentiation markers, apoptosis, and osteoclastic signaling, J. Cell. Biochem. 85 (2002) 825–836.
[19] J. Zheng, D. Baird, M.C. Borges, J. Bowden, G. Hemani, et al., Recent developments in Mendelian randomization studies, Curr Epidemiol Rep 4 (2017) 330–345.
[20] S. Greenland, Randomization, statistics, and causal inference, Epidemiology 1 (1990) 421–429.
[21] S. Burgess, F. Dudbridge, S.G. Thompson, Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods, Stat. Med. 35 (2016) 1880–1906.
[22] N. Dalbeth, R. Topless, T. Flynn, M. Cadzow, M.J. Bolland, et al., Mendelian randomization analysis to examine for a causal effect of urate on bone mineral density, J. Bone Miner. Res. 30 (2015) 985–991.
[23] B.L. Pierce, S. Burgess, Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators, Am. J. Epidemiol. 178 (2013) 1177–1184.
[24] H.F. Zheng, V. Forgetta, Y.H. Hsu, K. Estrada, A. Rosello-Diez, et al., Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture, Nature 526 (2015) 112–117.
[25] C. Medina-Gomez, J.P. Kemp, K. Trajanoska, J. Luan, A. Chesi, et al., Life-course genome-wide association study meta-analysis of Total body BMD and assessment of age-specific effects, Am. J. Hum. Genet. 102 (2018) 88–102.
[26] T.O. Kilpelainen, J.F. Carli, A.A. Skowronski, Q. Sun, J. Kriebel, et al., Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels, Nat. Commun. 7 (2016) 10494.
[27] G. Davey Smith, G. Hemani, Mendelian randomization: genetic anchors for causal inference in epidemiological studies, Hum. Mol. Genet. 23 (2014) R89–R98.
[28] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression, Int. J. Epidemiol. 44 (2015) 512–525.
[29] J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess, Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator, Genet. Epidemiol. 40 (2016) 304–314.
[30] Zhao Q, Jingshu Wang, Jack Bowden, and Dylan S. Small. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. arXiv:1801.09652 2018.
[31] P. Atanassova, L. Popova, Leptin expression during the differentiation of subcutaneous adipose cells of human embryos in situ, Cells Tissues Organs 166 (2000) 15–19.
[32] J. Makovey, M. Macara, J.S. Chen, C.S. Hayward, L. March, et al., Serum uric acid plays a protective role for bone loss in peri- and postmenopausal women: a longitudinal study, Bone 52 (2013) 400–406.
[33] S. Berglundh, L. Malmgren, H. Luthman, F. McGuigan, K. Akesson, C-reactive protein, bone loss, fracture, and mortality in elderly women: a longitudinal study in the OPRA cohort, Osteoporos. Int. 26 (2015) 727–735.
[34] T.R. Sponholtz, X. Zhang, J.D. Fontes, J.B. Meigs, L.A. Cupples, et al., Association between inflammatory biomarkers and bone mineral density in a community-based cohort of men and women, Arthritis Care Res (Hoboken) 66 (2014) 1233–1240.
[35] Yang TL, Zhao LJ, Liu YJ, Liu JF, Recker RR, Deng HW, et al. Genetic and environmental correlations of bone mineral density at different skeletal sites in females and males.
[36] F. Elefteriou, J.D. Ahn, S. Takeda, M. Starbuck, X. Yang, et al., Leptin regulation of bone resorption by the sympathetic nervous system and CART, Nature 434 (2005) 514–520.
[37] M.W. Hamrick, C. Pennington, D. Newton, D. Xie, C. Isales, Leptin deficiency produces contrasting phenotypes in bones of the limb and spine, Bone 34 (2004) 376–383.
[38] B. Clarke, Normal bone anatomy and physiology, Clinical journal of the American Society of Nephrology : CJASN 3 (Suppl. 3) (2008) S131–S139.
[39] A. Martin, V. David, L. Malaval, M.-H. Lafage-Proust, L. Vico, et al., Opposite effects of leptin on bone metabolism: a dose-dependent balance related to energy intake and insulin-like growth factor-I pathway, Endocrinology 148 (2007) 3419–3425.