A Meta-Analysis of Reference Values of Leptin Concentration in Healthy Postmenopausal Women

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Abstract

Objective: There are numerous reports about the leptin concentration (LC) in postmenopausal women (PW). Changes in LC can elicit different clinical outcomes. We systematically analyzed the LC in PW.

Methods: A search was conducted in original English-language studies published from 1994 to October 2012 in the following databases: Medline (78), Cochrane Center (123) Embase (505), Biological abstracts (108), Cochrane (53) and Science Finder Scholar (0). A meta-analysis was undertaken on the correction coefficient (r) between the serum LC and body mass index (BMI) for healthy PW across studies containing a dataset and sample size. Pre-analytical and analytical variations were examined. Pre-analytical variables included fasting status (FS) and sampling timing. Analytical variation comprised assay methodology, LC in those undertaking hormone replacement therapy (HRT) and those not having HRT as well as LC change according to age.

Results: Twenty-seven studies met the inclusion criteria. Eighteen studies detected LC in the morning in a FS, 15 studies denoted the r between leptin and the BMI. A combined r was counted for the 15 studies ($r = 0.51$ [95% confidence interval (CI), 0.46–0.54], $P = 0.025$), and if sampling collection was in the FSat morning, a combined r was form 10 studies ($r = 0.54$ [95% CI, 0.45–0.54], $P = 0.299$) and heterogeneity was diminished. LC did not change between HRT users and non-users in 7 studies. Five studies analyzed changes in LC according to age.

Conclusion: Based on all studies that investigated both LC and BMI, LC was positively correlated with the BMI. No studies established reference ranges according to the Clinical and Laboratory Standards Institute (CLSI) in healthy PW, and there was a wide variation in LC values. These differences suggest that caution should be used in the interpretation and comparison between studies.

Introduction

The prevalence of obesity is the result of multiple factors. Obesity can lead to severe health problems and is a social and economic burden. Some genetic loci for obesity have been identified, including several energy homeostasis-related peptide hormones, such as leptin, cocaine-amphetamine-regulated transcript (CART) and ghrelin [1]. These hormones target special areas in the brain and regulate body metabolism; mutations in their loci or receptors can result in obesity [1–3]. Recently, disorders of the central nervous system were recognized as having a potential role in obesity [4]. Leptin, one of obesity relating factors, is an adipocyte-derived hormone important for fat metabolism, and leptin levels correlate with insulin resistance [5]. Leptin is also associated with reproductive functions [6], and immune responses [7,8]. The central targets and mechanisms of leptin action have led to a detailed understanding after more than 10 years of research. Leptin crosses the blood–brain barrier (BBB) *via* saturable transport. Leptin has a role as a sensor of fat as part of a negative feedback loop that maintains a set point for fat stores within the body [9,10]. The hormones act on specific centers in the brain that regulate the sensations of satiety, and these effects are more obvious than peripheral administration of leptin. Furthermore, leptin can improve depression-like behavior in animals by modulating synaptic plasticityin the hippocampus [11]. Based on the central action of leptin, it has been suggested that administration of leptin in the brain is more specific if it was used to treat obesity [4].

Numerous reports have focused on leptin expression in postmenopausal women (PW). Leptin expression has been associated with breast cancer [12,13], hepatocellular carcinoma [14–16] and ovarian cancer [17,18]. Hence, compressive analyses of leptin concentration (LC) is a basis to recognize these diseases in PW, specifically in breast cancer [12,13] and ovarian cancer [17,18] because its morbidity increases during menopause compared with before menopause [19].
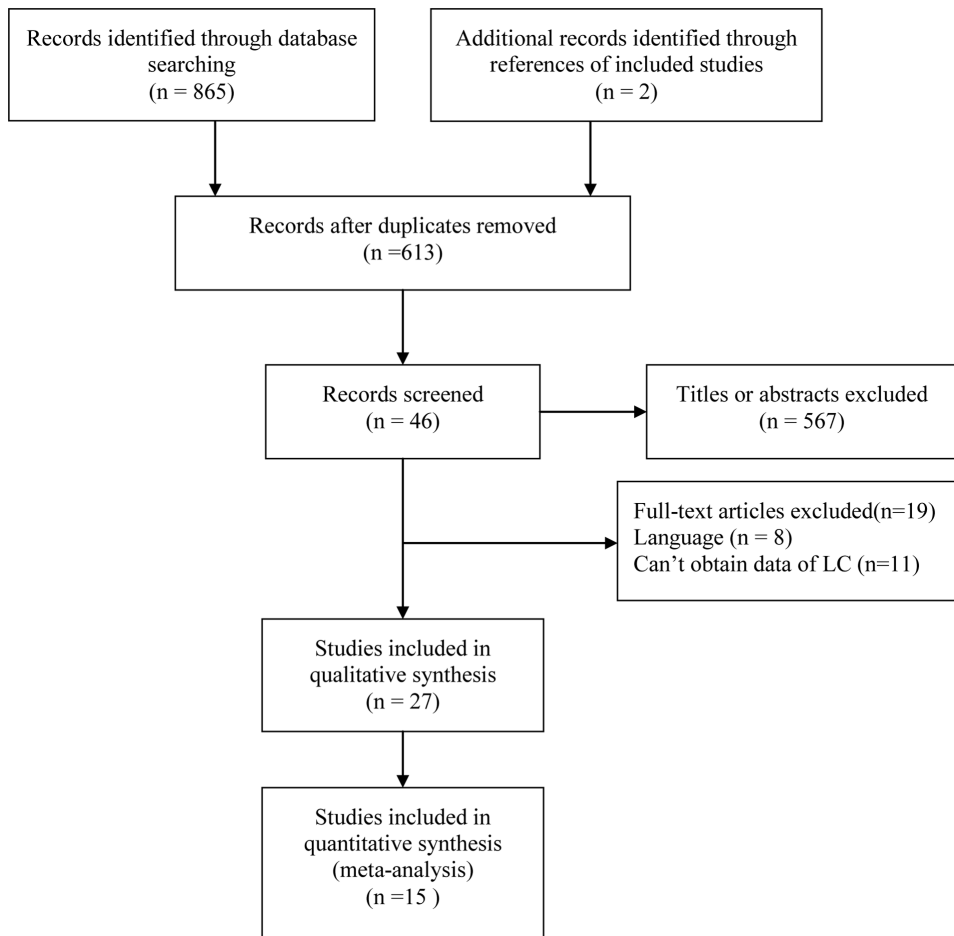
Figure 1. Flow chart from the identification of studies. LC, leptin concentration.
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Methods

Study design

This meta-analysis was conducted on literature published from January 1994 to October 2012. The databases searched were EMBASE, PubMed, Science Finder Scholar, Biological Abstracts and Cochrane. All studies were retrieved based on a search strategy in our meta-analysis using the following criteria: (i) study design – clinical cohort, cross-sectional and case–control studies were considered eligible; (ii) target population – healthy PW; (iii) specific definition of the methods used for the measurement of LC (plasma or serum), biochemical assay used [radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA)] and calculation of LC.

Data extraction

Data were extracted from the articles using a specific data form. This form included information about search yield (key words: "normal" or "healthy", "postmenopausal women" or "post menopause", "serum" or "plasma", "leptin concentration" or "leptin level" or "leptin value"). In total, 27 studies met the criteria for English language and healthy PW. None of studies gave the same sample size or LC range.

Analyses

Intercooled Stata 12 for Windows was used for all data analyses. Meta-analyses of correlations were conducted using the method described by Hedges and colleagues [20,21]. Briefly, using a Fisher's $r \rightarrow Z$ transformation to normalized $zr = \frac{1}{2} \ln \frac{1+r}{1-r}$, a combined correlation coefficient (r) was calculated for studies reporting multiple correlations between the subgroups studied. Then, transfer back was carried out using the transformation $r = \frac{e^{2Zr} - 1}{e^{2Zr} + 1}$, a variance of $Vz_r = \frac{1}{n-3}$ for the fixed-effect and $V_{Zr} = \frac{1}{n-3} + \tau^2$ for a random effect. A Q-statistic (Cochran's Q-test) was used to assess heterogeneity across studies, with significant heterogeneity noted as $P < 0.05$. All of included studies were analyzed together and, if applicable, they were also analyzed by: (i) a combined r between leptin and the body mass index (BMI); (ii) participant with or without hormone replacement therapy (HRT) (Inclusion criteria for HRT were last menstrual period from 6 months to 1 year before the date of first visit (follicle stimulating hormone >35 IU/L); absence of any significant pathology; BMI<30 kg/m^2; absence of contraindications to estrogen plus progestin therapy (EPT); no use of hormonal drugs in the past 6 months. Exclusion criteria for HRT were the presence of menstrual cycles or spotting; contraindications to EPT; hypersensitivity to progestogens and/or adhesive matrixes; obesity

Table 1. Basic characteristics of all included studies and the *r* with the BMI.

Study	Number	Sampling status	Sampling timing	r	Method
Larsson et al. [22]	130	Fasting	Morning		ELISA
Haffner et al. [23]	28	Fasting	–	0.793	RIA
Goulding et al. [24]	54	Fasting	Morning		RIA
Kristensen et al. [42]	295	Fasting	Morning	0.475	RIA
Di Carlo et al. [47]	164	Fasting	Morning	0.53	RIA
Hadji et al. [25]	352	Fasting	Morning		RIA
Hadji et al. [26]	216	Fasting	Morning	0.47	RIA
Blain et al. [27]	167	Fasting	Morning	0.41	RIA
Cagnacci et al. [43]	148	Fasting	Morning	0.5745	RIA
Douchi et al. [28]	75	–	–		RIA
Hadji et al. [29]	94	Fasting	Morning		RIA
Lambrinoudaki et al. [30]	22	–	Morning	0.726	RIA
Munoz et al. [31]	80	Fasting	Morning		RIA
Sahin et al. [32]	100	–	–	0.356	ELISA
Shaarawy et al. [33]	90	Fasting	Morning		RIA
Ushiroyama et al. [34]	115	Fasting	Morning	0.514	RIA
Carlo et al. [44]	122	Fasting	Morning	0.562	RIA
Cordero-MacIntyre et al. [35]	39	–	–	0.4	RIA
Ayub et al. [36]	34	Fasting	–	0.2	RIA
Bednarek-Tupikowska et al. [48]	154	Fasting	Morning	0.52	RIA
Jaleel et al. [37]	80	Fasting	Morning		RIA
Rolland et al. [38]	76	Fasting	Morning		RIA
Carlo et al. [45]	120	Fasting	Morning	0.564	RIA
Meyers et al. [39]	114	Fasting	–		RIA
Khokhar et al. [40]	9	Fasting	–		ELISA
Wu et al. [41]	25	Fasting	Morning	0.765	RIA
Soni et al. [46]	200	Fasting	–		RIA

(BMI>30 kg/m2) or pathologic leanness (BMI <19 kg/m2); hypertension (borderline hypertension excluded); diabetes, glucose intolerance); (iii) LC change in accordance with PW age; (iv) RIA or ELISA method; (5) LC in pre-PW and PW. To determine reference ranges, the lowest concentration and highest concentration in the included studies were obtained. The weighted mean reference range was calculated for studies that used the same assay methodology. $P < 0.05$ was considered significant.

Results

Summary of the included studies

A detailed flowchart of the selection process is shown in Figure 1. The 27 studies that met the inclusion criteria are summarized in Table 1. The study design involved 20 cross-section studies [22–41], five fixed cohort studies [42–46] and two dynamic cohort studies [47,48]. A total of 3,093 PW were in the included studies. Studies were characterized by total sample size, methods of LC detection, sampling conditions, and *r* with the BMI. In these studies, RIA ($n = 24$) was the more commonly used assay

Table 2. Range of LCs of PW in the included studies.

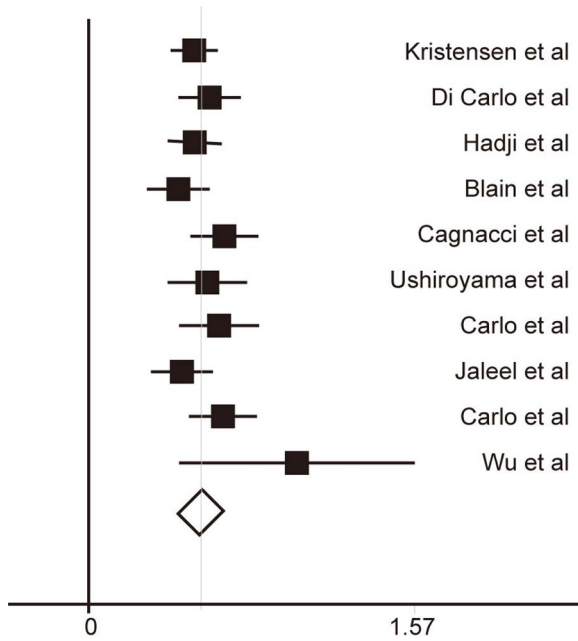| Study	Lower concentration (ng/mL)	Upper concentration (ng/mL)
Goulding et al. [24]	1.0	80.8
Blain et al. [27]	2	66
Douchi et al. [28]	1.8	48.3
Munoz et al. [31]	5.2	63.6
Lambrinoudaki et al. [38]	2	54

Figure 2. Forest plot indicating the _r_ between LC and body mass index (BMI) of postmenopausal women (PW). Horizontal lines denote 95% confidence interval (CIs); the diamond indicates the combined r with the corresponding 95% CI.
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Table 4. _r_ between LC and age in PW in the included studies.

Study	Age [SD]	r^*
Douchi et al. [28]	61.7 [7.7]	N
Sahin et al. [32]	55.1 [6.3]	N
Ushiroyama et al. [34]	53.3 [1.5]	0.25
Rolland et al. [38]	54.14 [4.24]	0.23
Khokhar er al. [40]	52.78 [4.92]	−0.081

*N: no correction.
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methodology relative to ELISA ($n = 3$), there was no clear difference in LC between the two methodologies. Of the 27 studies, 18 studies detected LC in the morning in a fasting state (FS) (Table 1). Five studies gave the complete mean [SD] and range of LCs (1.01–80.8 ng/mL) (Table 2). Fifteen studies analyzed the _r_ between leptin and the BMI of PW. Seven studies compared the LC between PW using HRT and those not using HRT. One study detected LC continually during a period of slimming in PW. None of the studies clearly explained the detection of free LC.

Six studies specified the sampling population (2 studies in Caucasians [22,43], 2 studies in Japanese subjects [28,34], 1 study in Mexican–Americans and non-Hispanic whites [23], and 1 study in Caucasian and Afro-Americans [31]. One of the included studies noted different LCs in different races [31]. One report stated that racial differences were the reason for heterogeneity [49], a factor that must be borne in mind if combining LC values across studies.

The BMI and LC of PW

Leptin is produced by adipose tissue. It is hypothesized that its level is higher in the obese than in the normal-weight population. In the 15 studies that gave the _r_ between the BMI and LC, the _r_ was from –0.075 to 0.793, and the combined _r_ was 0.51 ([95% confidence interval (CI), 0.46–0.54], Q = 26.08, _P_ = 0.025) in a fixed model and ([95% CI, 0.46–0.56], Q = 26.08, _P_ = 0.025) in a random model. If we ignore the timing of sample collection in the 11 studies, then the combined _r_ was 0.52 ([95% confidence interval (CI), 0.46–0.53], Q = 25.14, _P_ = 0.027) in a fixed model and ([95% CI, 0.46–0.55], Q = 25.23, _P_ = 0.026) in a random model, and after ignoring the sampling status of the 11 studies, the the combined _r_ was 0.52 (95% CI, 0.46–0.52, Q = 25.34, _P_ = 0.024) in a fixed model and (95% CI, 0.46–0.53, Q = 25.31, _P_ = 0.026) in a random model. However, a combined _r_ was taken from the 10 studies in which samples were collected in the FS in the morning [26,27,34,41–45,47,48]; the combined _r_ was 0.54 ([95% CI, 0.45–0.54], Q = 10.67, _P_ = 0.299) in a fixed model and ([95% CI, 0.46–0.54], Q = 10.67, _P_ = 0.299) in a random model, but heterogeneity diminished (Figure 2). LC explained 21% of the variation in the BMI.

HRT and LC

HRT is recommended for use in PW. It can reduce some syndromes in PW [50–52]. Findings from the Women's Health Initiative (WHI) and other studies suggest that individual formulation for PW is more appropriate [53–55]. In 7 studies of the included studies (Table 3), there were no difference in LC in 5 studies and LC decreased in 2 studies after comparison of those using HRT and those not using HRT. However, in the study by DiCarlo et al. [47], the authors did not consider the effect of obesity on LC, so their conclusion was not robust. In the study by Carlo et al. [45], LC did not change from the initiation of the study

Table 3. LC in healthy PW and HRT users in the included studies.

Study	Duration of HRT	Comparison between PW using HRT and those not using HRT*
Haffner et al. [23]	7 years	N
Kristensen et al. [42]	5 years	N
Di Carlo et al. [47]	21.5–51 months	Lower in HRT group (p = 0.004)
Hadji et al. [25]	–	N
Lambrinoudaki et al. [30]	–	N
Munoz et al. [31]	1–72 months	N
Carlo et al. [45]	12 months	Lower in HRT group (p<0.05)

*N: no difference.
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to that after 12 months of HRT. The authors also mentioned that the duration of HRT did not affect LC [31,47], and concluded that HRT did not affect LC.

LC and age

The age range of subjects in the included studies was ≈46–90.5 years. Five studies measured the *r* between age and LC; the age was ≈49–68 years (Table 4). Two studies found a positive correlation and two studies did not find a correlation between them. One study showed a negative correlation. Even if a meta-analysis cannot be done, the factor of age very weakly affects the LC.

Nine studies analyzed the LC in pre-PW and PW [23,26,28,29,36,40,41,47,48]. Three studies found no difference between them [23,28,48], 4 studies found that LC was higher in PW [26,41,43,47] and the other 2 studies found LC was lower in PW [36,40]. One of the 9 studies compared LC in pre-PW, peri-PW and PW [41]. The authors found that LC increased after the menopause, and that it did not differ between peri-PW and PW. A meta-analysis of subgroups could not be carried out because of appreciable heterogeneity. However, combination of the data about LC changing according to age, one could conclude that age did not affect LC very much.

Publication bias

For excluding heterogeneity, we testedonly the ten studies that detected LC in FS in the morning.The funnel plot and Egger's linear regression testdetected no publication bias among these studies ($P = 0.053$) (Figure 3).

Discussion

The BMI allows health professionals to discuss overweight and underweight problems objectively with their patients [56]. The BMI has limitations but is used widely [57].

Most of the studies included in this meta-analysis involved blood collection in the early-to-late morning (06:00 h to 9:00 h). However, 4 studies did not specify the state (e.g., FS) at collection [22,28,30,32] and 8 studies did not specify the time of blood collection [23,28,32,35,36,39,40,46] and both factors were not specified in 3 studies [28,32,35]. These variations in the timing or status of specimen collection could significantly affect the obtained LC value in the studies.Licinio *et al.* found that serum LC

Figure 3. No publication bias was detected using a filled funnel plot with pseudo-95% (CIs) of results of 10 studies. Zr, transformed correlation coefficient (r); s.e., standard error.
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exhibited a pattern of pulsatile release, with 32.0 ± 1.5 pulses every 24 h and a pulse duration of 32.8 ± 1.6 min in healthy men [58]. Adult men and women shared with a similar pulse frequency of leptin within 24 h even though the concentration was higher in women than in men [59]. Hence, the LC is dynamic, and timing can appreciably affect the measurement of leptin in the blood [10]. Leptin has a short half-time (about 5–7.5 min) [60,61], Price *et al.* modified the leptin structure and expanded its half-time to 32.3 min [61]. Reports have clearly shown the leptin level to be lowest in the morning after an overnight fast [59,62–64] and that LC increased after feeding [64,65]. Our meta-analysis focused on the pre-analytical sources (BMI, age, HRT status) and analytical sources (analytical method, sampling time) affecting PW. The heterogeneity diminished if the analysis was conducted in a FS in the morning, suggesting that the LC is dynamic during the day. However, Hancox *et al.* reported no difference in the leptin concentration between semi-fasting and overnight fasting [66]. It could be concluded that plasma levels of leptin reflect primarily the total adipose mass rather than short FS, meal consumption or the dietary energy source [64,66]. All of these sources of variation make it difficult to accurately determine a reference range of LC in healthy PW.

Interestingly, our analyses from all 15 studies that investigated both leptin concentration and BMI, showed clearly a positive relationship between LC and BMI ($P = 0.025$). At the same time we also should note that if sampling collection was restricted to the morning, the relationship between fasting LC and BMI just showed a trend ($P = 0.299$). We consider that for this no-significant relationship of FS LC and BMI, at least a possible reason is due to the reduced sample size ($n = 10$) for FS in the morning. Hence our results showed a moderate correlation between LC and BMI, serum LC increased significantly with the increase in BMI.

Our results showed that: (i) even though studies determined LC, the results were diverse and identifying a generic LC for PW according to criteria set by the Clinical and Laboratory Standards Institute is difficult; (ii) ELISA and RIA are used for the measurement of LC, but RIA is a more popular methodology that is recommended used to ascertain LC; (iii) LC has a wide concentration range in PW; (iv) obese PW had a higher mean value of LC; (v) undergoing HRT did not affect LC in PW; (vi) age affected LC only mildly. LC is higher in obese individuals than in non-obese subjects, andcan be found in other populations [1,4].

A range of 12–14 h fasting time was stated in most of the included studies. FS and food intake are two other important pre-analytical variables that should be acknowledged. During FS, glycogen stores become the primary energy source for the body through glycogenolysis [10]. Fasting longer than 12–15 h results in the depletion of glycogen stores and a consequent increase in luconeogenesis [10]. However, different LC values in the FS could not be ascertained.

Leptin administration for the obese populationhas not shown encouraging results because of its short half-life in the circulation, low potency, and poor solubility [60].It was found that the metabolic effects of leptin act predominantly *via* the brain after leptin crosses the BBB by a saturable pattern, and that even peripheral leptin receptors exist [4,67]. Leptin interacts with hypothalamic-pituitary-growth hormone as well as the hypothalamic–pituitary–adrenalandhypothalamic–pituitary–thyroid axes, and is involvedin glucose metabolism, reproduction, pubertal development, hematopoiesis and the immune system [4,68,69]. Leptin also has peripheral effects on skeletal muscle, the liver, pancreas and several other tissues [4,68]. Systemic injection of leptin in mice or rats subjected to hyperinsulinemic clamp studies improved the effects of insulin and further decreased hepatic
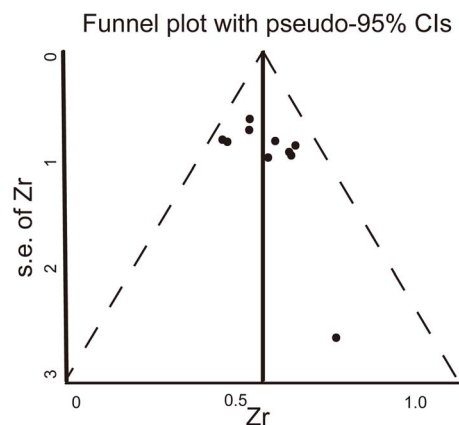
glucose production. After leptin was administered *via* the intracerebroventricular (ICV) route into the third ventricle at much lower doses in lean male rats, its metabolic effects could be almost replicated, also suggesting that leptin action in the brain is largely responsible for these effects. Furthermore, Fliedner *et al.* found that radiolabeled leptin preferentially reaches the hypothalamus and that hyperleptinemia could not block leptin transport to the brain. These findings suggested that the intranasal (IN) route of leptin administration could be a potential therapeutic method for obesity [70], but further works are needed to evaluate its effects. Clinical application of leptin was revised intensively by Yildiz *et al.* [67]and Scheler *et al.* [4].

The levels of some hormones keep changing during the postmenopausal process, especially in the early period (e.g., estrogen) [71,72]. The morbidity due to hypertension [73,74], cancer [75] and Alzheimer's disease [76] will increase with age. Recently, some reports showed that these diseases could be prevented after HRT [77,78]. However, Marjoribanks *et al.* reported that HRT could prevent only postmenopausal osteoporosis [79,80]. HRT usually involves three methods: estrogen, EPT, and progestin. In this meta-analysis, all of the PW who underwent treatment with EPT had LC values that did not change after HRT.

We were very cautious when comparing absolute LC values across studies by Nagy and Gower almost 10 year ago [81]. This conclusion was confirmed by two recent meta-analyses [49,82] and our meta-analysis. Many sources of analytical variation will affect the accuracy of the standardization of LC measurement. Additionally, we analyzed the total LC in PW, but leptin mediates its effects after it binds to its receptors in tissues, so free leptin has not elicited its effects [83,84]. An internationally accepted reference LC does not exist currently because of detection from different populations and different methods of analyses.

In conclusion, the present meta-analysis provides solid evidence in different population groups that LC values in PW are positively associated with the BMI and not associated with HRT.

Supporting Information

Checklist S1   Checklist of our manuscript.
(DOC)

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Author Contributions

Conceived and designed the experiments: ZL XZ. Performed the experiments: XZ ZL YLC. Analyzed the data: XZ KC YYY. Contributed reagents/materials/analysis tools: ZL. Wrote the paper: XZ ZL.

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