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 plasticity in 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].
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 $z_r=\frac{1}{2}\ln\frac{1+r}{1-r}$, 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 $r=\frac{e^{2Z_r} - 1}{e^{2Z_r} + 1}$, a variance of $V_{z_r}=\frac{1}{n-3}$ for the fixed-effect and $V_{Z_r}=\frac{1}{n-3}+\tau^2$ 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 the 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/m²; 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.
(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 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  |
| Hoffner 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         | 0.47  | RIA    |
| Hadji et al. [26]            | 216    | Fasting         | Morning         | 0.41  | RIA    |
| Blain et al. [27]            | 167    | Fasting         | Morning         | 0.5745| RIA    |
| Cagnacci et al. [43]         | 148    | Fasting         | Morning         | 0.356 | ELISA  |
| 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    | –               | –               |       | RIA    |
| Shaarawy et al. [33]         | 90     | Fasting         | Morning         |       | RIA    |
| Ushioyama 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    |
| Soniet et al. [46]           | 200    | Fasting         | –               |       | RIA    |

#### 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                          |
| Douchiet et al. [28] | 1.8                         | 48.3                        |
| Munoz et al. [31]   | 5.2                         | 63.6                        |
| Lambrinoudaki et al. [38] | 2                          | 54                          |

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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% 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 ($p = 0.004$)                          |
| Hadjie 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 [26,41,43,47] and the other 2 studies found LC was lower in between them [23,28,48], 4 studies found that LC was higher in [23,26,28,29,36,40,41,47,48]. Three studies found no difference LC.

Publication bias

For excluding heterogeneity, we tested only the ten studies that detected LC in FS in the morning. The funnel plot and Egger’s linear regression test detected 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 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, and can 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 gluconeogenesis [10]. However, different LC values in the FS could not be ascertained.

Leptin administration for the obese population has 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–adrenaland hypothalamic–pituitary–thyroid axes, and is involved in 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

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**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. doi:10.1371/journal.pone.0072734.g003
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, Flier et al. found that radioiodinated leptin preferentially reaches the hypothalamic arcuate nucleus 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. [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 progesterin. In this meta-analysis, all of the PW who underwent hormone replacement therapy (HRT) 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 years 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: XZ ZL. Performed the experiments: XZ ZL. Analyzed the data: XZ KC YYY. Contributed reagents/materials/analysis tools: XZ. Wrote the paper: XZ ZL.

References

1. Cheung WM, Mao P (2012) Recent advances in obesity: genetics and beyond. ISRN Endocrinol 2012: 536905.
2. Stunkard AJ, Foch TT, Hrubec Z (1986) A twin study of human obesity. JAMA 256: 51–54.
3. Hjelmberg J, Fagnani C, Silventoinen K, McGue M, Karhula M, et al. (2008) Genetic influences on growth traits of BMI: a longitudinal study of adult twins. Obesity (Silver Spring) 16: 847–852.
4. Scherer T, Lehner H, Hallemich M (2013) Brain insulin and leptin signaling from animal research to clinical application. Endocrinol Metab Clin North Am 42: 109–123.
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, et al. (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432.
6. Pralong FP, Gonzales C, Voirel MJ, Palmer RD, Branner HR, et al. (2002) The neuroendocrine Y1 receptor regulates leptin-mediated control of energy homeostasis and reproductive functions. FASEB J 16: 712–714.
7. Carlton ED, Demas GE, French SS (2012) Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors. Horm Behav 62: 272–279.
8. Proacccini C, Lourenco EV, Mateare G, La Cava A (2009) Leptin signaling: A key pathway in immune responses. Curr Signal Transduct Ther 4: 22–30.
9. Oswal A, Yeo G (2010) Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. Obesity (Silver Spring) 18: 221–229.
10. Leibel RL (2002) The role of leptin in the control of body weight. Nutr Rev 60: S15–19; discussion S68–84, 85–17.
11. Shandley DJ, Irving AJ, Harvey J (2001) Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. J Neurosci 21: RC186.
12. Ray A (2012) Adipokine leptin in obesity-related pathology of breast cancer. J Biosci 37: 289–294.
13. Hedges LV, Vevea JL (1998) Fixed-and random-effects models in meta-analysis. Psychol Methods 3: 486.
14. Thompson KJ, Lau KN, Johnson S, Martinie JB, Iannitti DA, et al. (2011) Leptin inhibits hepatocellular carcinoma proliferation via p38MAPK-dependent signalling. HEP (Oxford) 13: 225–233.
15. Wang SN, Lee KT, Ker CG (2010) Leptin in hepatocellular carcinoma. World J Gastroenterol 16: 5801–5809.
16. Saxena NK, Sharma D, Ding X, Lin S, Marra F, et al. (2007) Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res 67: 2497–2507.
17. Piak A, Kołaczkowska E, Gregoreszczuk EL (2012) Leptin stimulation of cell cycle and inhibition of apoptosis gene and protein expression in OVCA-3 ovarian cancer cells. Endocrinology 153: 394–403.
51. Simon JA (2010) Postmenopausal hormone therapy: risks versus benefits. Nat Rev Endocrinol 6: 661–663.

52. Prentice RL, Manson JE, Lange RD, Anderson GL, Pettinger M, et al. (2009) Benefits and risks of postmenopausal hormone therapy when it is initiated soon after menopause. Am J Epidemiol 170: 12–23.

53. Banks E, Canfield K (2009) Invited Commentary: Hormone therapy risks and benefits—The Women’s Health Initiative findings and the postmenopausal estrogen timing hypothesis. Am J Epidemiol 170: 24–28.

54. van Staa TP, Cooper C, Barlow D, Leufkens HG (2008) Individualizing the risks and benefits of postmenopausal hormone therapy. Menopause 15: 374–383.

55. Manson JE (2013) The role of personalized medicine in identifying appropriate candidates for menopausal estrogen therapy. Metabolism 62 Suppl 1: S15–19.

56. Price TO, Farr SA, Yi X, Vinogradov S, Baratkov A, et al. (2010) Transport across the blood-brain barrier of pruritic leptin. J Pharmacol Exp Ther 333: 253–263.

57. Langendijk JG, Pijl H, Toornvliet AC, Burggraaf J, Frolich M, et al. (1998) Circadian rhythm of plasma leptin levels in upper and lower body obese women: influence of body fat distribution and weight loss. J Clin Endocrinol Metab 83: 1706–1712.

58. Koutkia P, Canavan B, Johnson ML, DePaoli A, Grinspoon S (2003) Characterization of leptin pulse dynamics and relationship to fat mass, growth hormone, cortisol, and insulin. Am J Physiol Endocrinol Metab 285: E372–379.

59. Weigle DS, Duzell FB, Connor WE, Stinner RA, Soules MR, et al. (1997) Effect of fasting, refeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab 82: 561–565.

60. Bergendahl M, Irmanansh E, Evans WS, Vahlhuth JD (2000) Short-term fasting selectively suppresses leptin pulse mass and 24-hour rhythmic leptin release in healthy midlife phase women without disturbing leptin pulse frequency or its entropy control (pattern orderliness). J Clin Endocrinol Metab 85: 207–213.

61. Hancox RJ, Landoulsi CE (2011) Correlation between measures of insulin resistance in fasting and non-fasting blood. Diabetol Metab Syndr 3: 23.

62. Yildiz BO, Haznedaroğlu IC (2006) Rethinking leptin and insulin action: therapeutic opportunities for diabetes. Int J Biochem Cell Biol 38: 820–830.

63. Friedman JM (2002) The function of leptin in nutrition, weight, and physiology. Nutr Rev 58: S1–14; discussion S68–84, S85–17.

64. Himm-Hagen J (1999) Physiological roles of the leptin endocrine system: differences between mice and humans. Crit Rev Clin Lab Sci 36: 573–655.

65. Fleury H, Schulz C, Lehner H (2006) Brain uptake of intranasally applied radioiodinated leptin in Wistar rats. Endocrinology 147: 2088–2094.

66. Chahal HS, Drake WM (2007) The endocrine system and ageing. J Pathol 211: 173–180.

67. Taylor GT, Barzczett M, Farr S, Womack S, Komitowski D, et al. (1993) Steroidal interactions in the ageing endocrine system: absence of suppression and pathology in reproductive systems of old males from a mixed-sex socially stressful rat colony. J Endocrinol 137: 113–122.

68. Coylewright M, Reckelhoff JF, Ouyang P (2000) Menopause and hypertension: an age-old debate. Hypertension 35: 952–959.

69. Rappelli A (2002) Hypertension and obesity after the menopause. J Hypertens 20: S26–28.

70. Britt K (2012) Menarche, menopause, and breast cancer risk. Lancet Oncol 13: 1071–1072.

71. Casadesus G, Kohlton KK, Wehrly KM, Atwood CS, Bowen RL, et al. (2008) Menopause, estrogen, and gonadotropins in Alzheimer’s disease. Adv Clin Chem 45: 139–153.

72. Dovey DA (2012) Update: estrogen and estrogen plus progesterin therapy in the care of women at and after the menopause. Womens Health (Lond Engl) 8: 169–184.

73. Hinds L, Price J (2010) Menopause, hormone replacement and gynaecological cancers. Menopause Int 16: 89–93.

74. Marjoribanks J, Farquhar C, Roberts H, Lethaby A (2012) Trial does not change the conclusions of Cochrane review of long term hormone therapy for perimenopausal and postmenopausal women. BMJ 343: c4144.

75. Marjoribanks J, Farquhar C, Roberts H, Lethaby A (2012) Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev 7: CD004145.

76. Nagy TR, Gosser BA, Trowbridge CA, Deenbergen C, Shawcush RM, et al. (1997) Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol Metab 82: 2148–2152.

77. Venner AA, Doyle-Baker PK, Lyon ME, Fung TS (2009) A meta-analysis of leptin reference ranges in the healthy paediatric prepubertal population. Ann Clin Biochem 46: 65–72.

78. Kelesidis T, Kelesidis I, Chou S, Mantzoros CS (2010) Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med 152: 93–100.

79. Brennan AM, Mantzoros CS (2006) Drug Insight: The role of leptin in human physiology and pathophysiology—emerging clinical applications. Nat Clin Pract Endocrinol Metab 2: 318–327.