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Total body aromatization in postmenopausal breast cancer patients is strongly correlated to plasma leptin levels☆

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Abstract

The adipocytokine leptin has recently been shown to enhance the expression of aromatase via promoter II and I.3 using an AP-1 motif. Thus, we evaluated the correlation between plasma leptin concentrations and total body aromatization (TBA) as well as plasma levels of estrone (E_1), estradiol (E_2) and estrone sulfate (E_1S) in postmenopausal breast cancer patients. Twenty-two postmenopausal women with metastatic breast cancer, participating in tracer studies for the measurement of total body aromatization (TBA) *in vivo*, were available. In addition, blood samples for plasma estrogens and leptin measurements were available from another 22 breast cancer patients and 114 healthy postmenopausal women participating in the mammography-screening program.

Values for TBA varied from 1.46 to 4.72% while plasma leptin levels ranged from 1.83 to 95.51 ng/ml in the same group of patients. All plasma estrogen levels were in the normal range expected for postmenopausal women. We found a significant correlation between pretreatment leptin levels and TBA (r_s 0.452, $P = 0.01$). In contrast, basal levels of TBA did not correlate to body mass index (BMI) in the same group of patients. Plasma leptin levels correlated to plasma levels of estradiol (r_s 0.659, $P = 0.007$), and estrone sulfate (r_s 0.562, $P = 0.01$) in the group of breast cancer patients ($n = 44$) as well as in the group of healthy postmenopausal women (estradiol, r_s 0.363, $P \leq 0.001$, estrone sulfate r_s 0.353, $P \leq 0.001$).

In conclusion, we found plasma leptin levels to correlate to TBA in breast cancer patients and to plasma levels of estradiol and estrone sulfate in breast cancer patients as well as in healthy postmenopausal females. These findings suggest that leptin may influence on aromatase activity *in vivo*, providing a possible link between body weight and plasma estrogen levels as well as breast cancer risk.

Keywords: Breast cancer; Leptin; Estrogens; Aromatase; Androgens

1. Introduction

Obesity is a well-established risk factor for breast cancer development in postmenopausal women [1–5]. Although adipose tissue is one of the major sites for the conversion of androgen precursors into estrogens ("aromatization") in postmenopausal women, and elevated plasma estrogen levels have been associated with an increased breast cancer risk [6–8], the direct link between obesity and breast cancer has not yet been identified. Recently, a variety of adipocyte-derived polypeptides ("adipocytokines") [9] like leptin, adiponectin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), heparin-binding epidermal growth factor-like growth factor (HB-EGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) have been suggested to exert paracrine and endocrine effects on normal and neoplastic breast tissue [10].

The current publication focuses on leptin, a 16 kda protein encoded by the *LEP* (previously called *ob* for obesity)

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gene [11]. The main source of leptin is white adipocytes [11], with minor additional contributions from alternative sources like placenta, stomach and skeletal muscle [12]. Leptin has been suggested to control food intake and energy balance by signals to the hypothalamus [13–15] and to participate in the regulation of multiple processes, like hematopoiesis, immune responses, puberty, pregnancy and lactation [16,17]. Leptin receptors [18] are expressed in a diversity of tissues, including normal mammary epithelial cells and human breast cancer [19–23]. Subsequent to leptin binding to its receptors (obR) an increasing number of signal transduction pathways like the JAK/STAT pathway and the MAPK pathway have been suggested to be involved [24]. Polymorphisms in LEP and LEPR genes have been shown to be associated with increased breast cancer risk as well as disease progress [25]. Human obesity has been associated with increased leptin levels [26,27] and a recent publication suggested plasma leptin levels to be associated with overall survival in postmenopausal breast cancer patients [28]. *In vitro* studies with the human breast cancer cell line MCF-7 have shown that leptin induces the functional activation of estrogen receptor α [29].

Leptin has been shown to enhance the expression of aromatase via promoter II and I.3 using a AP-1 motif [30] suggesting an influence on estrogen synthesis. Targeted disruption of the aromatase gene causes accumulation of excess adipose tissue and elevated plasma leptin levels in a mouse model ("ArKO-mouse") raising the possibility of a feedback mechanism [31]. In humans, a significant association between plasma sex hormone levels and plasma leptin levels has been reported [32,33]. Concerning breast cancer patients, elevated leptin expression has been reported in breast cancer biopsies followed by significantly increased plasma levels of leptin in breast cancer patients compared to controls [34].

Based on the evidence summarized above, we hypothesised that leptin may influence aromatase activity *in vivo*, providing a link between body weight, estrogen levels and the risk for breast cancer development as well as poor prognosis associated with obesity in postmenopausal breast cancer patients.

2. Materials and methods

2.1. Patients

Forty-four postmenopausal breast cancer patients participated in this study (age range: 55–79 years). Twenty-two participated in protocols evaluating the basal level of total body aromatization before and following treatment with novel aromatase inhibitors and inactivators by the use of tracer techniques [35,36]. Another 22 postmenopausal breast cancer patients provided blood samples prior to the initiation of systemic therapy. All participating breast cancer patients were postmenopausal and had an ER and/or PgR positive diseases. ER or PgR positivity was given when more than 10% of the tumor cells stained positive for the individual receptor. In addition, 114 postmenopausal, healthy women (age range: 55–72 years) involved in the local breast cancer screening program provided fasting plasma samples for plasma leptin and estrogen measurements (Table 1). All protocols were approved by the Regional Ethics Committee at the University of Bergen and written informed consent was given by all participating patients prior to enrollment.

Table 1
Patients' characteristics and plasma measurements

	Breast cancer patients ($n = 44$)	Healthy postmenopausal women ($n = 114$)
Age	69.9 (64.2–76.0)	64.6 (63.8–65.5)
Body mass index	25.1 (21.9–28.8)*	25.1 (24.4–25.8)
Plasma leptin (ng/ml)	27.9 (18.9–41.2)	25.0 (22.3–28.0)
Estrone (pmol/l)	70.5 (56.9–87.3)	66.1 (62.2–70.3)
Estradiol (pmol/l)	14.8 (11.5–19.2)	10.6 (9.6–11.8)
Estrone sulfate (pmol/l)	319.2 (220.7–461.6)	328.4 (280.1–384.9)
Androstenedione (ng/ml)	1.1 (0.7–1.7)**	0.8 (0.7–0.9)

All values are given as geometric mean values with 95% confidence intervals of the mean; * $n = 12$; ** $n = 22$.

2.2. Plasma estrogen levels

Blood samples for estrogen measurements were obtained into heparinized vials (two vials containing 10 ml each) immediately before each tracer injection after an overnight fast. In breast cancer patients not participating in tracer studies, fasting blood samples were drawn in the morning before starting therapy with any antihormonal agent. Plasma was separated by centrifugation and stored at −20 °C until analysed. Estradiol (E_2) and estrone (E_1) were determined by radioimmunoassay as reported elsewhere [37,38]. Plasma levels of estrone sulfate (E_1S) were determined by a novel, highly sensitive assay involving purification and derivatization into E_2 and radioimmunoassay analysis using E_2-6-carboxy-methyloximine-[2-^{125}I]iodohistamine as tracer ligand [39]. The detection limits for plasma levels of E_1, E_2 and E_1S were 6.3, 2.1, and 2.7 pmol/l, respectively.

2.3. Plasma androstenedione levels

Plasma levels of androstenedione were measured following an overnight fast using commercial kits provided by Diagnostic Systems Laboratories Inc., Webster, TX, USA (DSL-3800 ACTIVE® Androstenedione Coated-Tube Radioimmunoassay). The detection limit for androstenedione using this RIA was 0.03 ng/ml.

2.4. Plasma leptin levels

Blood samples for leptin measurement were obtained into heparinized vials in the morning (between 08:00 and 09:00 h), following an overnight fast for 8–12 h. Leptin plasma levels were measured using a coated tube immunoradiometric assay (IRMA) provided by Diagnostic Systems Laboratories Inc.,

Webster, TX, USA (DSL-23100i). The detection limit of the assay was 0.1 ng/ml.

2.5. Measurement of total body aromatization in vivo

Total body aromatization of androstenedione (A) to estrone (E_1) was measured *in vivo* following a bolus injection of [^{3}H]androstenedione (500 μCi) and [^{14}C]estrone (5 μCi) dissolved in 50 ml of saline containing 8% ethanol (w/w) [40]. Aliquots of the isotopes in the injection mixture were taken to calculate the ^{3}H/^{14}C ratio. Urine was collected for a period of 96 h, pooled and kept frozen at −20 °C until processing. We finally calculated the percentage of total body aromatization from the equation:

$$\%\,\text{Aromatization} = \frac{[^3\text{H}]E_{ur}/[^{14}\text{C}]E_{ur}}{[^3\text{H}]A_{inj}/[^{14}\text{C}]E_{1inj}} \times 100$$

where $[^3\text{H}]E_{ur}/[^{14}\text{C}]E_{ur}$ is the mean value of the ratio of ^{3}H- to ^{14}C-labelled E_1 and E_3 in the urine and $[^3\text{H}]A_{inj}$ and $[^{14}\text{C}]E_{inj}$ are the amounts of ^{3}H-labelled A and ^{14}C-labelled E_1 injected into the patient. A recent assessment of the sensitivity of this method revealed that inhibition of whole body aromatization up to 99.1% is detectable [41]. This very time and manpower consuming research method has been used to evaluate *in vivo* inhibition of total body aromatization in postmenopausal breast cancer patients during treatment with the currently established drugs like anastrozole [42], letrozole [36,41], and exemestane [35]. The 22 postmenopausal breast cancer patients available for additional measurements of leptin of leptin and evaluation of correlation to total body aromatization came from two of these studies [36,42].

2.6. Statistical analysis

Previous work by our group has shown plasma estrogen levels in postmenopausal women to be well-fitted to a log-normal distribution [38]. This has also been shown for plasma leptin levels [43]. Thus, all estrogens, androgens and leptin values are given as their geometric mean values with 95% confidence intervals (CI) of the mean. Whenever estrogen or androgen levels below the sensitivity limits were found, the corresponding sensitivity limits of the assays were used for statistical analysis. Spearman's correlation was calculated using log-transformed data and the software package SPSS V.11.0. All results are given as Spearman's rho (r_s).

3. Results

Plasma leptin levels varied from 1.83 to 95.51 ng/ml in the group of postmenopausal women with breast cancer ($n = 22$) evaluated by our tracer method for total body aromatization (TBA) (Table 1). The percentage of TBA in the same group of patients varied between 1.46 and 4.72%. Body mass index was 25.1 (21.9–28.8) in the group of breast cancer patients and 25.1 (24.4–25.8) in the healthy postmenopausal women (geometrical mean with 95% confidence intervals of the mean).

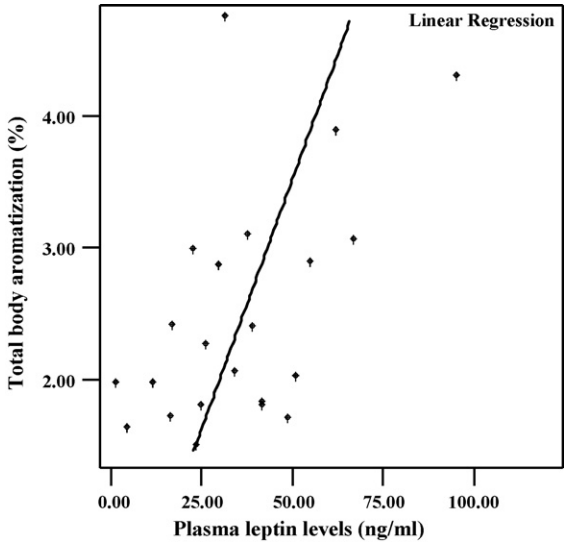

Fig. 1. Spearman's correlation between plasma leptin levels and percentage of total body aromatization in postmenopausal breast cancer patients ($n = 22$).

We found a significant correlation between plasma leptin levels and the percentage of TBA (r_s 0.452, $P = 0.01$) (Fig. 1 and Table 2). In contrast, basal level of TBA was not correlated to body mass index (BMI) in the same group of patients (r_s 0.252, $P = 0.43$). The correlation between body mass index and plasma leptin levels in this group of breast cancer patients was of borderline significance (r_s 0.566, $P = 0.055$). All plasma estrogen concentrations were in the normal range expected for postmenopausal women. The mean plasma levels of leptin, estrone, estradiol and androstenedione were found to be slightly higher in the group of breast cancer patients compared to the healthy postmenopausal women, but none of them reached a level of statistical significance (Table 1). Plasma leptin levels were correlated to plasma estradiol (r_s 0.659, $P = 0.007$) and estrone sulfate (r_s 0.562, $P = 0.01$; Fig. 2A–C) with a weaker association to plasma estrone (r_s 0.524). The plasma levels of E_2 and E_1S were also found to be significantly correlated with BMI in the group of breast cancer patients (r_s 0.643 and 0.580, respectively, $P < 0.05$ for both; Table 2). Plasma androstenedione levels were in the normal range for postmenopausal women and found to be negatively correlated to total body aromatization (r_s −0.104, $P = 0.025$). Moreover, plasma androstenedione levels were positively correlated to both plasma E_1 and E_2 levels in healthy postmenopausal women but not in breast cancer patients (Table 2).

In the group of 114 healthy postmenopausal women recruited from the mammography-screening program at our hospital, we found plasma estrogen and androgen levels as well as plasma leptin levels within the normal range in all patients (Table 1). Again, plasma levels of E_1, E_2 and E_1S were correlated with the plasma levels of leptin, (Fig. 3)

Table 2
Correlations[a] between total body aromatization, leptin, BMI, plasma estrogens and androstenedione in postmenopausal women with (BC) or without breast cancer (N)

		TBA (%)	Leptin	BMI	Plasma E_1	Plasma E_2	Plasma E_1S	Plasma A
Total body aromatization (%)	N		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	BC		**0.452**** (n = 22)	0.252* (n = 12)	0.317* (n = 22)	0.299* (n = 22)	0.153* (n = 22)	**-0.479**** (n = 22)
Plasma Leptin	N	n.a.		**0.737***** (n = 114)	0.182* (n = 114)	**0.363***** (n = 114)	**0.353***** (n = 114)	0.013* (n = 113)
	BC	**0.452**** (n = 22)		0.566* (n = 12)	0.524* (n = 44)	**0.659***** (n = 44)	**0.562***** (n = 44)	-0.104* (n = 22)
BMI	N	n.a.	**0.737***** (n = 114)		0.182* (n = 114)	**0.354***** (n = 114)	**0.370***** (n = 114)	0.031* (n = 113)
	BC	0.252* (n = 12)	0.566* (n = 12)		0.510* (n = 12)	**0.643**** (n = 12)	**0.580**** (n = 12)	0.476* (n = 12)
Plasma E_1	N	n.a.	0.182* (n = 114)	0.182* (n = 114)		**0.747***** (n = 114)	**0.620***** (n = 114=	**0.403***** (n = 113)
	BC	0.317* (n = 22)	0.524* (n = 44)	0.510* (n = 12)		**0.782***** (n = 44)	**0.787***** (n = 44)	0.281* (n = 22)
Plasma E_2	N	n.a.	**0.363***** (n = 114)	**0.354***** (n = 114)	**0.747***** (N = 114)		**0.728***** (n = 114)	**0.278**** (n = 113)
	BC	0.299* (n = 22)	**0.659***** (n = 44)	**0.643**** (n = 12)	**0.782***** (n = 44)		**0.872***** (n = 44)	0.258* (n = 22)
Plasma E_1S	N	n.a.	**0.353***** (n = 114)	**0.370***** (n = 114)	**0.620***** (n = 114)	**0.728***** (n = 114)		0.170* (n = 113)
	BC	0.153* (n = 22)	**0.562***** (n = 44)	**0.580**** (n = 12)	**0.787***** (n = 44)	**0.872***** (n = 44)		0.331* (n = 22)
Plasma A	N	n.a.	0.013* (n = 113)	0.031* (n = 113)	**0.403***** (n = 113)	**0.278**** (n = 113)	0.170* (n = 113)	
	BC	**-0.479**** (n = 22)	-0.104* (n = 22)	0.476* (n = 12)	0.281* (n = 22)	0.258* (n = 22)	0.331* (n = 22)	

Abbreviations: TBA, total body aromatization in %; E_1, estrone; E_2, estradiol; E_1S, estrone sulfate; A, androstenedione; BMI, body mass index; n.a., not available; N, normal postmenopausal women in the mammographic screening program; BC, postmenopausal breast cancer patient; [a]Spearman's rho, 2-tailed: $^{*}P > 0.05$; $^{**}P < 0.05$; $^{***}P < 0.001$; number of patients given in parentheses.

reaching a level of statistical significance for E_2 and E_1S (Table 2). All in all, the correlation between plasma estrogen levels and plasma leptin was weaker compared to the group of breast cancer patients (see Table 2 for details). Plasma leptin levels correlated strongly to BMI (r_s 0.737, $P < 0.001$).

4. Discussion

For reasons unexplained, obesity is associated with elevated breast cancer incidence as well as inferior prognosis for women in the postmenopausal but not for those in the premenopausal age range [3,44], suggesting an endocrine explanation to these findings. However, the precise mechanisms linking obesity to elevated plasma estrogen levels in postmenopausal women are unknown.

In postmenopausal women, estrogens are synthesised in different tissue compartments followed by a passive diffusion to the plasma [45,46]. Thus, plasma estrogen measurements have been used as surrogate parameters to estimate the basal estrogen production in peripheral tissues. The level of total body aromatization in postmenopausal women varies in the range from 1 to 4% in individual postmenopausal women [47], but the underlying mechanisms controlling aromatase activity *in vivo* are incompletely understood [48–50].

In the present study, we found normal mean levels of leptin and all plasma estrogens in both groups of patients (Table 1). However, our results showed a trend toward higher plasma levels of E_1, E_2, and leptin in postmenopausal breast cancer patients compared to the group of healthy women. Thus, our findings tend to support observations published by others [34] suggesting higher plasma leptin concentrations in postmenopausal breast cancer patients. Our finding of a tight correlation between plasma estrogen levels and plasma leptin levels has been reported by other groups as well [33]. In premenopausal women, leptin has been suggested to play a role as a mediating signal of nutrition to affect the gonadotropin release by modulating the pulsatile GnRH secretion in the hypothalamus [51]. Lin et al. reported a simultaneous increase of leptin and GnRH following exogenous estrogen administration in premenopausal women indicating that leptin might be involved in feedback mechanisms following changes in the hormonal status [52]. Other factors that may influence on the interplay between leptin and plasma estrogen levels like changes in the adrenal function, etc. have been discussed and summarized in the literature [53–55].

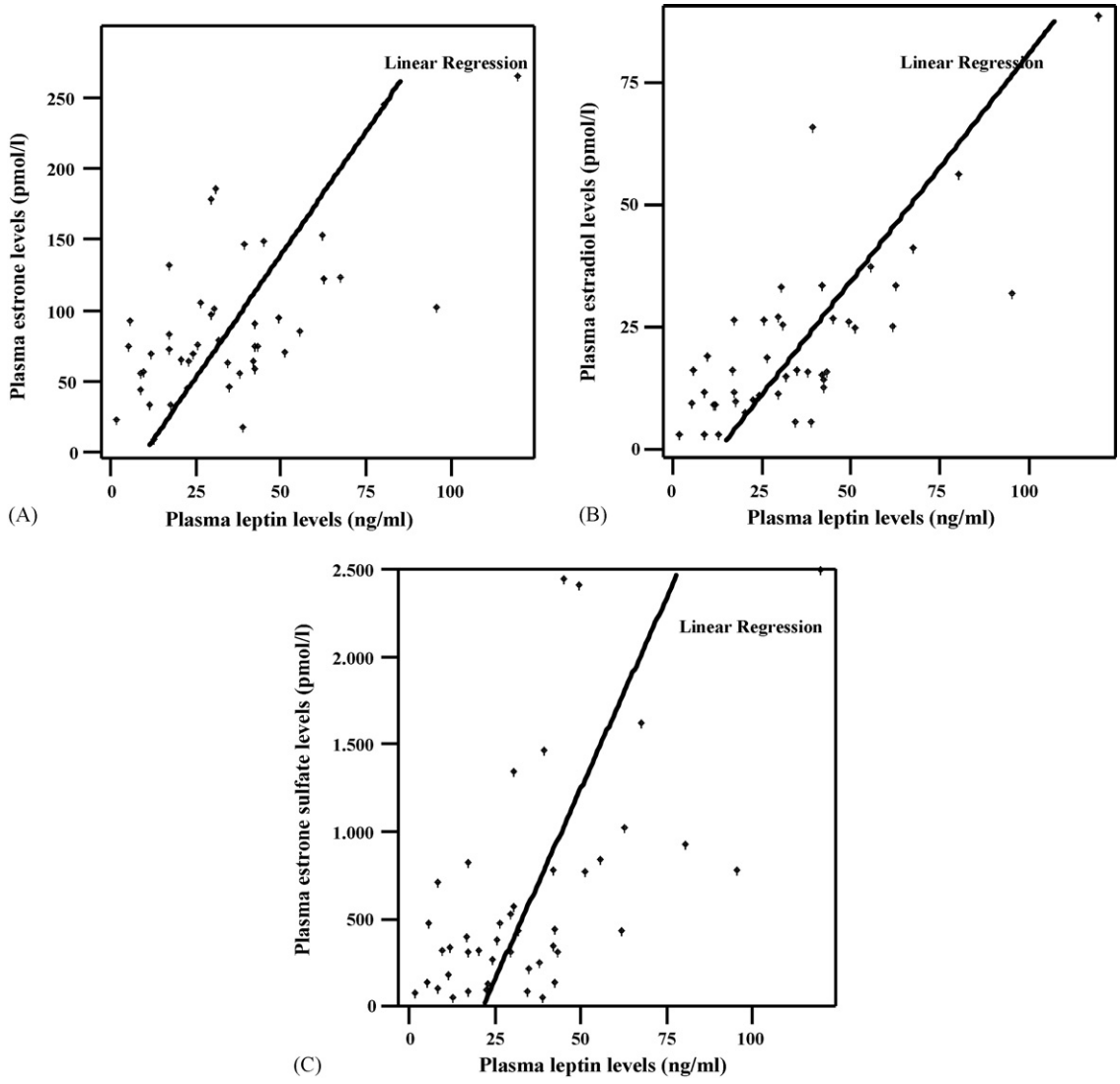

Fig. 2. (A–C) Spearman's correlation of plasma leptin levels and plasma levels of estrone (A), estradiol (B) and estrone sulfate (C) in postmenopausal breast cancer patients ($n = 44$).

Our group has performed a series of studies measuring TBA with a tracer method [40] as part of a program evaluating the biochemical efficacy of aromatase inhibitors and inactivators *in vivo* [35,36,41,42]. From these studies, plasma samples and levels of TBA from 22 postmenopausal breast cancer patients were available. Following reports suggesting leptin to interfere with aromatization *in vitro*, we measured plasma leptin levels in a group of postmenopausal breast cancer patients and in healthy controls.

We found a strong correlation between the basal levels of TBA and plasma leptin levels in the group of breast cancer patients. The fact that TBA was not correlated with BMI in the same group of breast cancer patients supports the hypothesis that leptin may influence on aromatase activity *in vivo* and may provide evidence that plasma leptin levels in breast cancer patients do not simply reflect BMI. This is underlined by the finding that plasma leptin levels in our breast cancer patients were statistically not correlated with BMI. In contrast, our findings confirmed the well-known tight correlation between BMI and plasma leptin levels in the group of healthy postmenopausal women. One might speculate whether the disappearance of this correlation in the group of breast cancer patients may be caused by other factors (like the IGF-system, etc.) following the establishment of a metastatic cancer disease. Similar to what has been recorded in the literature, we found plasma leptin levels and BMI to be correlated with plasma estrogen levels in healthy as well as in breast cancer patients. Due to the complexity of TBA measurements, we were able to assess the relationship between BMI and TBA in only 12 of the 22 postmenopausal breast cancer patients. Thus, it is possible that there may have been a significant relationship between TBA and BMI in the whole population that we were unable to assess. We cannot therefore entirely discount the possibility that the correlation between leptin and TBA is in part because of the correlation between leptin and BMI.

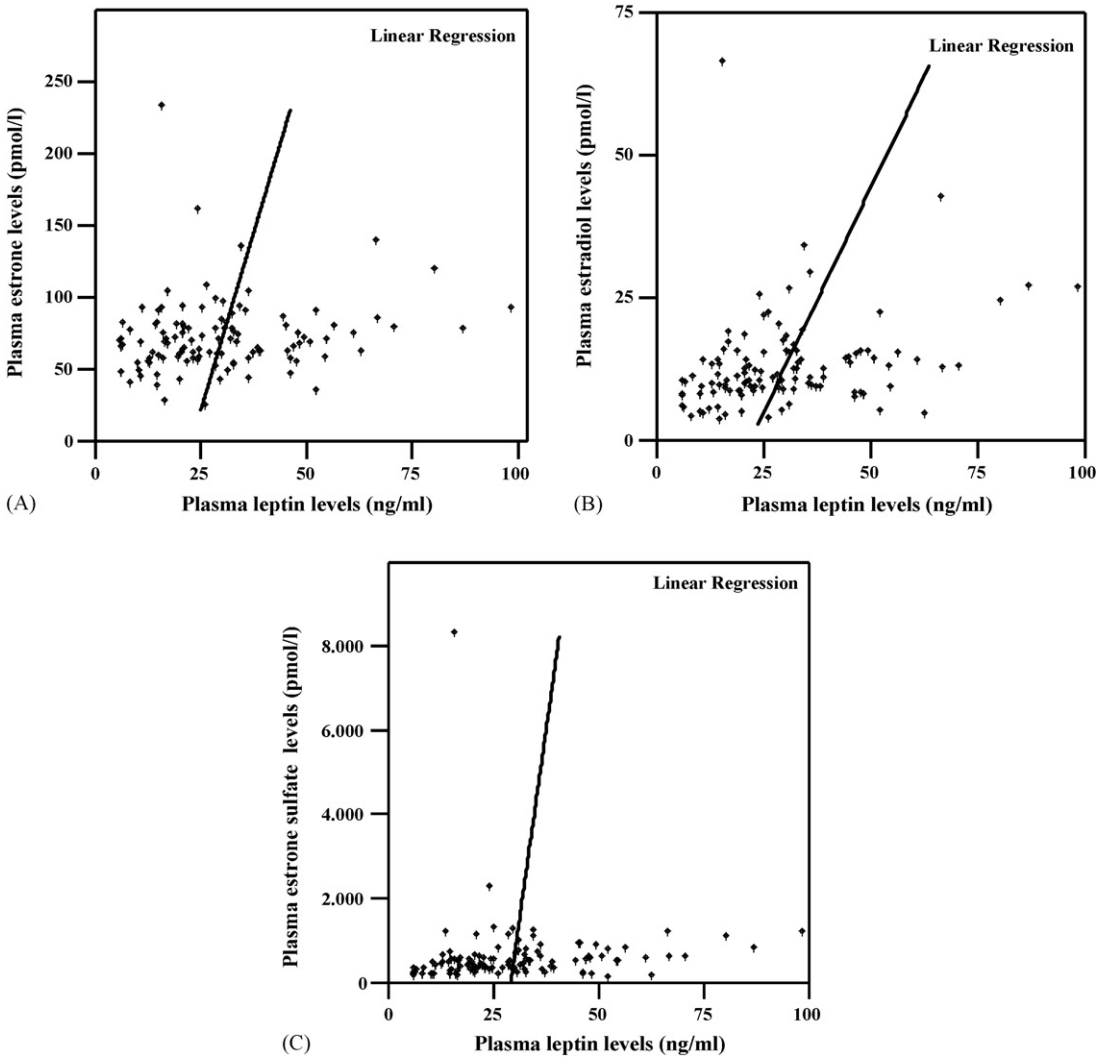

Fig. 3. (A–C) Spearman's correlation of plasma leptin levels and plasma levels of estrone (A), estradiol (B) and estrone sulfate (C) in healthy postmenopausal women ($n = 114$).

Together with strong evidence from recent *in vitro* findings suggesting leptin to induce aromatase expression from promoter II and I.3 [30] as well as the functional activation of the estrogen receptor-α by leptin [29] our findings suggest plasma leptin as a promising candidate for future research elucidating the mechanisms controlling the percentage of TBA and the concentration of estrogen plasma levels in postmenopausal women. However, tight correlations between leptin, BMI and plasma estrogens in healthy postmenopausal women make it difficult to define the precise role of leptin in estrogen production. The adipocytokine production rate in adipose tissue is directly proportional to the degree of obesity for all adipocytokines [10] with the exception of adiponectin [56], and plasma leptin concentrations in healthy women are positively correlated with both BMI and total body fat [57]. However, leptin is the only adipocytokine with documented effect on aromatization, the crucial step in postmenopausal estrogen production. Thus, additional studies (like leptin intervention trials) are warranted to elucidate the precise interaction between leptin and aromatase in peripheral tissues and the tumor level *in vivo*.

It is well known that obesity [58] as well as diet may influence on the outcome of breast cancer patients. The strong connection between plasma leptin levels to whole body aromatization may suggest that the positive effects of diet on overall survival following breast cancer treatment may partly be translated via a decrease in leptin plasma levels.

In conclusion, our findings show for the first time a strong correlation between plasma leptin levels and the basal levels of TBA in postmenopausal breast cancer patients. Thus, leptin appears to be a promising candidate for future research concerning factors controlling the level of aromatization *in vivo*. The well-known correlations between BMI and plasma leptin levels as well as plasma estrogen concentrations make the interpretation of data difficult. Although evidence for direct influences of leptin on aromatization exist from *in vitro* findings as well as from our present study *in vivo*, future studies are necessary to elucidate the precise interactions between

leptin and aromatase *in vivo*. The possible influence of plasma leptin levels on TBA may open new therapeutic strategies in breast cancer prevention and treatment in general.

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References

[1] D.P. Rose, E.M. Gilhooly, D.W. Nixon, Adverse effects of obesity on breast cancer prognosis, and the biological actions of leptin, Int. J. Oncol. 21 (2002) 1285–1292 (Review).
[2] G.D. Stephenson, D.P. Rose, Breast cancer and obesity: an update, Nutr. Cancer 45 (2003) 1–16.
[3] T.J. Key, P.N. Appleby, G.K. Reeves, A. Roddam, J.F. Dorgan, C. Longcope, F.Z. Stanczyk, H.E. Stephenson Jr., R.T. Falk, R. Miller, et al., Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women, J. Natl. Cancer Inst. 95 (2003) 1218–1226.
[4] P.H. Lahmann, K. Hoffmann, N. Allen, C.H. van Gils, K.T. Khaw, B. Tehard, F. Berrino, A. Tjonneland, J. Bigaard, A. Olsen, et al., Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC), Int. J. Cancer 111 (2004) 762–771.
[5] E.E. Calle, R. Kaaks, Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms, Nat. Rev. Cancer 4 (2004) 579–591.
[6] F. Berrino, P. Muti, A. Micheli, G. Bolelli, V. Krogh, R. Sciajno, P. Pisani, S. Panico, G. Secreto, Serum sex hormone levels after menopause and subsequent breast cancer, J. Natl. Cancer Inst. 88 (1996) 291–296.
[7] H.V. Thomas, T.J. Key, D.S. Allen, J.W. Moore, M. Dowsett, I.S. Fentiman, D.Y. Wang, A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey, Br. J. Cancer 76 (1997) 401–405.
[8] P.G. Toniolo, M. Levitz, A. Zeleniuch-Jacquotte, S. Banerjee, K.L. Koenig, R.E. Shore, P. Strax, B.S. Pasternack, A prospective study of endogenous estrogens and breast cancer in postmenopausal women, J. Natl. Cancer Inst. 87 (1995) 190–197.
[9] Y. Matsuzawa, T. Funahashi, T. Nakamura, Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances, Ann. N.Y. Acad. Sci. 892 (1999) 146–154.
[10] D.P. Rose, D. Komninou, G.D. Stephenson, Obesity, adipocytokines, and insulin resistance in breast cancer, Obes. Rev. 5 (2004) 153–165.
[11] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.
[12] R.S. Ahima, J.S. Flier, Leptin, Annu. Rev. Physiol. 62 (2000) 413–437.
[13] L.A. Campfield, F.J. Smith, Y. Guisez, R. Devos, P. Burn, Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks, Science 269 (1995) 546–549.
[14] M.A. Pelleymounter, M.J. Cullen, M.B. Baker, R. Hecht, D. Winters, T. Boone, F. Collins, Effects of the obese gene product on body weight regulation in ob/ob mice, Science 269 (1995) 540–543.
[15] J.L. Halaas, K.S. Gajiwala, M. Maffei, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.L. Lallone, S.K. Burley, J.M. Friedman, Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (1995) 543–546.
[16] D.S. Weigle, Leptin and other secretory products of adipocytes modulate multiple physiological functions, Ann. Endocrinol. (Paris) 58 (1997) 132–136.
[17] J. Himms-Hagen, Physiological roles of the leptin endocrine system: differences between mice and humans, Crit. Rev. Clin. Lab. Sci. 36 (1999) 575–655.
[18] L.A. Tartaglia, M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G.J. Richards, L.A. Campfield, F.T. Clark, J. Deeds, et al., Identification and expression cloning of a leptin receptor, OB-R, Cell 83 (1995) 1263–1271.
[19] M.N. Dieudonne, F. Machinal-Quelin, V. Serazin-Leroy, M.C. Leneveu, R. Pecquery, Y. Giudicelli, Leptin mediates a proliferative response in human MCF7 breast cancer cells, Biochem. Biophys. Res. Commun. 293 (2002) 622–628.
[20] K. Laud, I. Gourdou, L. Pessemesse, J.P. Peyrat, J. Djiane, Identification of leptin receptors in human breast cancer: functional activity in the T47-D breast cancer cell line, Mol. Cell. Endocrinol. 188 (2002) 219–226.
[21] K. Laud, I. Gourdou, L. Belair, D.H. Keisler, J. Djiane, Detection and regulation of leptin receptor mRNA in ovine mammary epithelial cells during pregnancy and lactation, FEBS Lett. 463 (1999) 194–198.
[22] N. O'Brien S, B.H. Welter, T.M. Price, Presence of leptin in breast cell lines and breast tumors, Biochem. Biophys. Res. Commun. 259 (1999) 695–698.
[23] M. Ishikawa, J. Kitayama, H. Nagawa, Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer, Clin. Cancer Res. 10 (2004) 4325–4331.
[24] G. Sweeney, Leptin signalling, Cell Signal. 14 (2002) 655–663.
[25] K. Snoussi, A.D. Strosberg, N. Bouaouina, S.B. Ahmed, A.N. Helal, L. Chouchane, Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma, BMC Cancer 6 (2006), doi:10.1186/1471-2407-1186-1138.
[26] M. Rosenbaum, M. Nicolson, J. Hirsch, S.B. Heymsfield, D. Gallagher, F. Chu, R.L. Leibel, Effects of gender, body composition, and menopause on plasma concentrations of leptin, J. Clin. Endocrinol. Metab. 81 (1996) 3424–3427.
[27] S. Klein, S.W. Coppack, V. Mohamed-Ali, M. Landt, Adipose tissue leptin production and plasma leptin kinetics in humans, Diabetes 45 (1996) 984–987.
[28] P.J. Goodwin, M. Ennis, I.G. Fantus, K.I. Pritchard, M.E. Trudeau, J. Koo, N. Hood, Is leptin a mediator of adverse prognostic effects of obesity in breast cancer? J. Clin. Oncol. 23 (2005) 6037–6042.
[29] S. Catalano, L. Mauro, S. Marsico, C. Giordano, P. Rizza, V. Rago, D. Montanaro, M. Maggiolini, M.L. Panno, S. Ando, Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells, J. Biol. Chem. 279 (2004) 19908–19915.
[30] S. Catalano, S. Marsico, C. Giordano, L. Mauro, P. Rizza, M.L. Panno, S. Ando, Leptin enhances, via AP-1, expression of aromatase in the MCF-7 cell line, J. Biol. Chem. 278 (2003) 28668–28676.
[31] M.E. Jones, A.W. Thorburn, K.L. Britt, K.N. Hewitt, M.L. Misso, N.G. Wreford, J. Proietto, O.K. Oz, B.J. Leury, K.M. Robertson, et al., Aromatase-deficient (ArKO) mice accumulate excess adipose tissue, J. Steroid Biochem. Mol. Biol. 79 (2001) 3–9.
[32] L. Tessitore, B. Vizio, D. Pesola, F. Cecchini, A. Mussa, J.M. Argiles, C. Benedetto, Adipocyte expression and circulating levels of leptin increase in both gynaecological and breast cancer patients, Int. J. Oncol. 24 (2004) 1529–1535.
[33] G. Paolisso, M.R. Rizzo, C.M. Mone, M.R. Tagliamonte, A. Gambardella, M. Riondino, C. Carella, M. Varricchio, F. D'Onofrio, Plasma sex hormones are significantly associated with plasma leptin concentration in healthy subjects, Clin. Endocrinol. (Oxf.) 48 (1998) 291–297.
[34] L. Tessitore, B. Vizio, O. Jenkins, I. De Stefano, C. Ritossa, J.M. Argiles, C. Benedetto, A. Mussa, Leptin expression in colorectal and breast cancer patients, Int. J. Mol. Med. 5 (2000) 421–426.
[35] J. Geisler, N. King, G. Anker, G. Ornati, E. Di Salle, P.E. Lonning, M. Dowsett, In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients, Clin. Cancer Res. 4 (1998) 2089–2093.
[36] J. Geisler, B. Haynes, G. Anker, M. Dowsett, P.E. Lonning, Influence of letrozole and anastrozole on total body aromatization and plasma

estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study, J. Clin. Oncol. 20 (2002) 751–757.
[37] M. Dowsett, P.E. Goss, T.J. Powles, G. Hutchinson, A.M. Brodie, S.L. Jeffcoate, R.C. Coombes, Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route, Cancer Res. 47 (1987) 1957–1961.
[38] P.E. Lonning, S.I. Helle, D.C. Johannessen, H. Adlercreutz, E.A. Lien, M. Tally, D. Ekse, T. Fotsis, G.B. Anker, K. Hall, Relations between sex hormones, sex hormone binding globulin, insulin-like growth factor-I and insulin-like growth factor binding protein-1 in postmenopausal breast cancer patients, Clin. Endocrinol. (Oxf.) 42 (1995) 23–30.
[39] P.E. Lonning, D. Ekse, A sensitive assay for measurement of plasma estrone sulphate in patients on treatment with aromatase inhibitors, J. Steroid Biochem. Mol. Biol. 55 (1995) 409–412.
[40] S. Jacobs, P.E. Lonning, B. Haynes, L. Griggs, M. Dowsett, Measurement of aromatisation by a urine technique suitable for the evaluation of aromatase inhibitors in vivo, J. Enzyme Inhib. 4 (1991) 315–325.
[41] M. Dowsett, A. Jones, S.R. Johnston, S. Jacobs, P. Trunet, I.E. Smith, In vivo measurement of aromatase inhibition by letrozole (CGS 20267) in postmenopausal patients with breast cancer, Clin. Cancer Res. 1 (1995) 1511–1515.
[42] J. Geisler, N. King, M. Dowsett, L. Ottestad, S. Lundgren, P. Walton, P.O. Kormeset, P.E. Lonning, Influence of anastrozole (Arimidex), a selective, non-steroidal aromatase inhibitor, on in vivo aromatisation and plasma oestrogen levels in postmenopausal women with breast cancer, Br. J. Cancer 74 (1996) 1286–1291.
[43] M. Rosenbaum, M. Nicolson, J. Hirsch, E. Murphy, F. Chu, R.L. Leibel, Effects of weight change on plasma leptin concentrations and energy expenditure, J. Clin. Endocrinol. Metab. 82 (1997) 3647–3654.
[44] P.A. van den Brandt, D. Spiegelman, S.S. Yaun, H.O. Adami, L. Beeson, A.R. Folsom, G. Fraser, R.A. Goldbohm, S. Graham, L. Kushi, et al., Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk, Am. J. Epidemiol. 152 (2000) 514–527.
[45] W.R. Miller, T.J. Anderson, D.B. Evans, A. Krause, G. Hampton, J.M. Dixon, An integrated view of aromatase and its inhibition, J. Steroid Biochem. Mol. Biol. 86 (2003) 413–421.
[46] J. Geisler, P.E. Lonning, Aromatase inhibition: translation into a successful therapeutic approach, Clin. Cancer Res. 11 (2005) 2809–2821.
[47] P.E. Lonning, M. Dowsett, T.J. Powles, Postmenopausal estrogen synthesis and metabolism: alterations caused by aromatase inhibitors used for the treatment of breast cancer, J. Steroid Biochem. 35 (1990) 355–366.
[48] A. Purohit, M.W. Ghilchik, M.P. Leese, B.V. Potter, M.J. Reed, Regulation of aromatase activity by cytokines, PGE2 and 2-methoxyoestrone-3-*O*-sulphamate in fibroblasts derived from normal and malignant breast tissues, J. Steroid Biochem. Mol. Biol. 94 (2005) 167–172.
[49] M.J. Reed, A. Purohit, Aromatase regulation and breast cancer, Clin. Endocrinol. (Oxf.) 54 (2001) 563–571.
[50] W.R. Miller, P. Mullen, P. Sourdaine, C. Watson, J.M. Dixon, J. Telford, Regulation of aromatase activity within the breast, J. Steroid Biochem. Mol. Biol. 61 (1997) 193–202.
[51] S. Nagatani, P. Guthikonda, R.C. Thompson, H. Tsukamura, K.I. Maeda, D.L. Foster, Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting, Neuroendocrinology 67 (1998) 370–376.
[52] K.C. Lin, N. Sagawa, S. Yura, H. Itoh, S. Fujii, Simultaneous increases of leptin and gonadotropin-releasing hormone following exogenous estrogen administration in women with normally menstrual cycle, Endocr. J. 52 (2005) 449–454.
[53] S.R. Bornstein, K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, W.A. Scherbaum, Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly, Diabetes 46 (1997) 1235–1238.
[54] G.Y. Cao, R.V. Considine, R.B. Lynn, Leptin receptors in the adrenal medulla of the rat, Am. J. Physiol. 273 (1997) E448–E452.
[55] P.J. Havel, Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance, Proc. Nutr. Soc. 59 (2000) 359–371.
[56] W.S. Yang, W.J. Lee, T. Funahashi, S. Tanaka, Y. Matsuzawa, C.L. Chao, C.L. Chen, T.Y. Tai, L.M. Chuang, Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin, J. Clin. Endocrinol. Metab. 86 (2001) 3815–3819.
[57] R.V. Considine, M.K. Sinha, M.L. Heiman, A. Kriauciunas, T.W. Stephens, M.R. Nyce, J.P. Ohannesian, C.C. Marco, L.J. McKee, T.L. Bauer, et al., Serum immunoreactive-leptin concentrations in normal-weight and obese humans, N. Engl. J. Med. 334 (1996) 292–295.
[58] R.T. Chlebowski, E. Aiello, A. McTiernan, Weight loss in breast cancer patient management, J. Clin. Oncol. 20 (2002) 1128–1143.