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 (E1), estradiol (E2) and estrone sulfate (E1S) 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 = 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 = 0.659, P = 0.007), and estrone sulfate (r = 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 = 0.363, P ≤ 0.001, estrone sulfate r = 0.353, P ≤ 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).
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 cancer risk as well as disease progress [25]. Human obesity genes have been shown to be associated with increased breast cancer risk as well as poor prognosis associated with obesity 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 hypothesized 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 disease. 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

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

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 (E2) and estrone (E1) were determined by radioimmunoassay as reported elsewhere [37,38]. Plasma levels of estrone sulfate (E1S) were determined by a novel, highly sensitive assay involving purification and derivatization into E2 and radioimmunoassay analysis using E2-6-carboxy-methyloximine-[2-125I]iodohistamine as tracer ligand [39]. The detection limits for plasma levels of E1, E2 and E1S 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.,
2.5. Measurement of total body aromatization in vivo

Total body aromatization of androstenedione (A) to estrone (E1) was measured in vivo following a bolus injection of [3H]androstenedione (500 μCi) and [14C]estrone (5 μCi) dissolved in 50 ml of saline containing 8% ethanol (w/w). Aliquots of the isotopes in the injection mixture were taken to calculate the 3H/14C 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:

$$\% \text{ Aromatization} = \frac{[3H]\text{Eur}/[14C]\text{Eur}}{[3H]\text{Ainj}/[14C]\text{E1inj}} \times 100$$

where [3H]Eur/[14C]Eur is the mean value of the ratio of 3H- to 14C-labelled E1 and E3 in the urine and [3H]Ainj and [14C]E1inj are the amounts of 3H-labelled A and 14C-labelled E1 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).

We found a significant correlation between plasma leptin levels and the percentage of total body aromatization in postmenopausal breast cancer patients ($n = 22$).

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

Fig. 1. Spearman’s correlation between plasma leptin levels and percentage of total body aromatization in postmenopausal breast cancer patients ($n = 22$).
reaching a level of statistical significance for E₂ and E₁S (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 E₁, E₂, 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].

### Table 2

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

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>TBA (%)</th>
<th>Leptin</th>
<th>BMI</th>
<th>Plasma E₁</th>
<th>Plasma E₂</th>
<th>Plasma E₁S</th>
<th>Plasma A</th>
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<tr>
<td><strong>Total body aromatization (%)</strong></td>
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<tr>
<td>BC</td>
<td>0.452** (n = 22)</td>
<td>0.252* (n = 12)</td>
<td>0.317* (n = 12)</td>
<td>0.299* (n = 22)</td>
<td>0.153* (n = 22)</td>
<td>-0.470** (n = 22)</td>
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<tr>
<td>Plasma Leptin</td>
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<tr>
<td>BC</td>
<td>0.452** (n = 22)</td>
<td>0.737** (n = 114)</td>
<td>0.182* (n = 114)</td>
<td>0.363*** (n = 114)</td>
<td>0.353*** (n = 114)</td>
<td>0.562** (n = 44)</td>
<td>-0.104* (n = 22)</td>
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<td>BMI</td>
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<td>0.252* (n = 12)</td>
<td>0.566* (n = 12)</td>
<td>0.524* (n = 44)</td>
<td>0.659*** (n = 44)</td>
<td>0.562** (n = 44)</td>
<td>0.500* (n = 12)</td>
<td>0.031* (n = 113)</td>
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<tr>
<td>BC</td>
<td>0.317* (n = 22)</td>
<td>0.182* (n = 114)</td>
<td>0.510* (n = 12)</td>
<td>0.747*** (n = 114)</td>
<td>0.782*** (n = 44)</td>
<td>0.620*** (n = 114)</td>
<td>0.403*** (n = 113)</td>
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<tr>
<td>BC</td>
<td>0.299* (n = 22)</td>
<td>0.363*** (n = 114)</td>
<td>0.354*** (n = 114)</td>
<td>0.747*** (N = 114)</td>
<td>0.728*** (n = 114)</td>
<td>0.872*** (n = 44)</td>
<td>0.258* (n = 22)</td>
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<tr>
<td>BC</td>
<td>0.153* (n = 22)</td>
<td>0.353*** (n = 114)</td>
<td>0.370*** (n = 114)</td>
<td>0.620*** (n = 114)</td>
<td>0.728*** (n = 114)</td>
<td>0.170* (n = 113)</td>
<td>0.31* (n = 22)</td>
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<tr>
<td>BC</td>
<td>-0.470** (n = 22)</td>
<td>0.013* (n = 113)</td>
<td>0.031* (n = 113)</td>
<td>0.403*** (n = 113)</td>
<td>0.278** (n = 113)</td>
<td>0.170* (n = 113)</td>
<td>0.31* (n = 22)</td>
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</table>

**Abbreviations**: TBA, total body aromatization in %; E₁, estrone; E₂, estradiol; E₁S, 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; *Spearman’s rho, 2-tailed: *P > 0.05; **P < 0.05; ***P < 0.001; number of patients given in parentheses.
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.
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.

Acknowledgements

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References


