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Relationship of body mass index with aromatisation and plasma and tissue oestrogen levels in postmenopausal breast cancer patients treated with aromatase inhibitors

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Abstract ***Background:*** Recent data have raised concern about the clinical efficacy of aromatase inhibitors in overweight and/or obese breast cancer patients. We report *in vivo* aromatase inhibition and plasma and tissue oestrogen levels in relation to body mass index (BMI) status among breast cancer patients treated with different aromatase inhibitors.
Methods: We compared data on *in vivo* aromatase inhibition (64 patients) as well as plasma and tissue oestrogen levels from patients participating in our studies to BMI values.
Results: We found a weak positive correlation between pretreatment aromatisation level and BMI ($n = 64$; $R = 0.236$; $p = 0.060$) but no correlation between on-treatment aromatisation levels or percentage aromatase inhibition and BMI within patient subgroups treated with any of a panel of aromatase inhibitors. Pre-treatment levels of plasma estradiol ($p < 0.001$), estrone ($p = 0.001$) and estrone sulphate ($p = 0.002$) correlated to BMI. While on-treatment levels of plasma estrane sulphate correlated to BMI in patients on letrozole ($R = 0.601$; $p = 0.001$; $n = 25$ for all) or anastrozole ($n = 12$; $R = 0.611$; $p = 0.035$) therapy, letrozole suppressed plasma estrone sulphate more than anastrozole independent of BMI. No correlation between on-treatment tumour oestrogen levels and BMI was recorded.

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Conclusions: Our unique data do not support a lack of effective aromatase inhibition in overweight patients or therefore a need for alternative therapy. The higher levels of estrogens in overweight postmenopausal breast cancer patients before and during aromatase inhibition may be due to effects of BMI on oestrogen metabolism rather than aromatisation.

1. Introduction

Obesity is associated with a significantly elevated breast cancer risk [1] and a poor breast cancer prognosis in postmenopausal women [2]. While the mechanisms are not fully understood, the fact that obesity has been associated with elevated plasma estradiol levels [3,4], a known risk factor for postmenopausal breast cancer [1], has suggested elevated estradiol (E_2) could be partly responsible for these effects.

Aromatase inhibitors, applied as either monotherapy or sequentially to tamoxifen have become standard adjuvant endocrine therapy for postmenopausal women [5]. However, conflicting evidence has questioned the benefit of aromatase inhibitors in overweight/obese patients. In the Austrian ABCSG-12 trial randomising premenopausal breast cancer patients between treatment with goserelin plus either tamoxifen or anastrozole, Pfeiler and colleagues found a significantly higher relapse rate among overweight premenopausal women treated with zoladex plus anastrozole as compared to those treated with zoladex with tamoxifen [6]. In the ATAC study, Sestak et al. [7] found women with a BMI >35 to have a poor prognosis compared to lean women independent of treatment arm (tamoxifen versus anastrozole); however, there was a non-significant trend indicating a reduced benefit for anastrozole as compared to tamoxifen among obese individuals. In contrast, analysing data from the BIG 1–98 study, Ewertz et al. [8] found the benefit for letrozole as compared to tamoxifen to be independent of BMI value.

Importantly, clinical superiority for aromatase inhibitors versus tamoxifen has been shown for the so-called third-generation compounds; anastrozole, exemestane and letrozole. In contrast, first- and second-generation aromatase inhibitors like aminoglutethimide, 4-hydroxyandrostenedione and fadrozole revealed anti-tumour efficacy resembling but not superior to the efficacy of conventional therapies [9]. While first-and second-generation aromatase inhibitors reduce *in vivo* aromatisation by 70–90% [9], anastrozole, exemestane and letrozole each inhibit *in vivo* aromatisation by >98% [10–12]. These data indicate a dose–response relationship between the degree of aromatase inhibition and anti-tumour efficacy related to treatment with aromatase inhibitors [13]; thus, even a moderate increase in oestrogen levels among overweight patients may potentially reduce the efficacy of aromatase inhibitors.

While postmenopausal estrogens are synthesised by peripheral aromatisation, plasma levels may be influenced by several factors, like androgen substrate level and oestrogen clearance rate [14]. Thus, it is of importance to directly determine *in vivo* aromatase inhibition in overweight patients treated with aromatase inhibitors. Here, we took the opportunity to determine potential correlations between BMI and the level of *in vivo* aromatisation, assessed by *in vivo* tracer injections, prior to commencing on and during treatment with different aromatase inhibitors. For this purpose, we included all patients participating in our previous tracer studies for whom data on BMI were available. In addition, we correlated BMI to plasma and tissue oestrogen levels before treatment and during therapy with the third-generation non-steroidal compounds letrozole and anastrozole, and to androgen levels in a cohort of healthy postmenopausal women.

2. Methods

2.1. Studying effect of BMI on in vivo aromatisation

An overview of patients included from different previous studies is presented in Table 1.

To evaluate potential correlation between *in vivo* aromatisation of androstenedione into estrone and BMI, we obtained data from six trials [11,12,15–18] enrolling a total of 64 patients. As for the on-therapy data, due to the fact that different aromatase inhibitors express different potency, on-treatment data obtained on each aromatase inhibitor were analysed separately. In addition, data obtained during treatment with exemestane [11] and during treatment with anastrozole from the cross-over study [12] were pooled, considering these two drugs to be of similar biochemical efficacy. On the contrary, we did not analyse data obtained on letrozole treatment in the same patients crossing-over between anastrozole and letrozole [12]. The main reason for this was the fact that all patients obtained aromatase inhibition >99.1%, the formal detection limit of the assay, during letrozole treatment.

The protocol for aromatase assessment was identical in all the studies. In brief, each patient received a bolus injection of [^{3}H] androstenedione (500 μ CI) and [^{14}C]estrone (5 μ CI) dissolved in 8% ethanol (W/W) followed by 96 h of urine collection. The percentage aromatisation was calculated from the $^3H/^{14}C$ isotope ratio in the oestrogen metabolites in the urine.

Table 1
Different studies providing material for the analysis performed here.

No	Body mass index (BMI) range	Setting	Drug treatment	Parameter addressed	Reference
In vivo aromatisation					
10	19.5–42.0	Metastatic	Exemestane	Pre/on treat % aromatisation	[11]
12	18.3–32.5	Metastatic	Anastrozole*	Pre/on treat % aromatisation	[12]
7	19.5–34.9	Metastatic	Aminoglutethimide (AG)	Pre/on treat % aromatisation	[18]
5	13.9–29.4	Metastatic	Rogletimide	Pre/on treat % aromatisation	[18]
7	20.3–32.7	Metastatic	i.m. 4-OHA	Pre/on treat % aromatisation	[15]
10	23.5–32.0	Metastatic	i.m. 4-OHA + AG	Pre/on treat % aromatisation	[17]
13	20.4–35.6	Metastatic	Oral 4-OHA	Pre-treat*** % aromatisation	[16]
Plasma oestrogen levels					
12	18.3–32.5	Metastatic	Anastrozole	Pre/on treat plasma oestrogen levels	[20]**
12	18.3–32.5	Metastatic	Letrozole	Pre/on treat plasma oestrogen levels	[20]**
13	22.3–37.2	Primary	Letrozole	Pre/on treat plasma oestrogen levels	[20]
Tissue oestrogen levels					
13	22.3–37.2	Primary	Letrozole	Pre/on treat tissue oestrogen levels	[20]

* Cross-over study in which patients were crossed over between anastrozole and letrozole. Because all patients obtained aromatase inhibition below detection limit on letrozole (>99.1% inhibition), only on-treatment data on anastrozole are used for BMI correlations.
** The plasma oestrogen values presented here are based on re-analysis of samples obtained from reference 12 with the novel improved radioimmunoassay described.
*** Only pretreatment levels are used for BMI comparison. The reason for this is that 4-OHA administered by the oral route is an insufficient aromatase inhibitor; thus, to correlate on-treatment aromatisation to BMI may be misleading. 4-OHA = 4-hydroxyandrostenedione.

2.2. Plasma oestrogen levels and BMI

Over the years, our radioimmunoassays for plasma oestrogen analysis have gradually been improved aiming at obtaining the lowest sensitivity limits. Thus, for the purpose of the analysis presented here, only plasma oestrogen levels analysed by our most recently developed methods [19] were used. As for samples collected from studies conducted prior to implementation of this technique [12], these plasma samples had all been re-analysed as part of a later study [20] using our novel assay [19]. In brief, ^{3}H-labelled estrone, estradiol and estrone sulphate (about 2000 dpm each) were added to plasma samples (2 mL) as internal recovery standards. Unconjugated estrogens (estrone and estradiol) were removed by ether extraction followed by chromatographic separation on LH-20 columns using dichloromethane: ethyl acetate: methanol (97:5:1 by vol) as solvent. Estradiol was analysed using ^{125}I-estradiol (estradiol-6-(*O*-carboxymethyl)-oximino-2-(2-[^{125}I]-iodohistamine) with a specific activity of about 2000 Ci/mmol as tracer and the ER 150 (Sorin Biomedica) antibody. To obtain maximum sensitivity, estrone, following separation, was converted into estradiol [21] and analysed according to the same procedure as for estradiol.

Subsequent to extraction of the unconjugated estrogens, ethanol was added to the residual water fraction. Conjugated estrogens were extracted, and estrone sulphate hydrolysed into unconjugated estrone with use of the S-9754 sulphatase enzyme followed by extraction, column purification, conversion into estradiol and radioimmunoassay as described above. Final values for each of the estrogens were corrected for procedural loss through assessing the amount of ^{3}H-labelled compound in each fraction (estradiol, estrone and estrone sulphate separately), and final values corrected for the amount of ^{3}H-labelled steroid included. Detection limits for plasma estradiol, estrone and estrone sulphate were 0.67, 1.14 and 0.55 pmol/L, respectively.

To correlate plasma oestrogen levels to BMI, we used plasma oestrogen levels from a study [20] evaluating plasma and tissue oestrogen levels before and during letrozole administered as primary medical therapy for locally advanced breast cancer (Table 1). In addition, samples obtained from the patients during treatment with letrozole or anastrozole from the 2002 cross-over study [12] were re-analysed with our novel assay [19] in 2008 [20]. As data from both studies were analysed in parallel, we found it feasible to combine pretreatment plasma estrogen levels from the two data sets for BMI correlation analysis. As letrozole and anastrozole have been shown to differ with respect to potency as *in vivo* aromatase inhibitors [12], oestrogen levels during treatment with either anastrozole or letrozole were correlated with BMI separately.

2.3. Breast cancer tissue oestrogen levels and BMI

To compare potential correlation between BMI and breast cancer oestrogen levels before and during treatment, data from the 13 patients [20] treated with letrozole from whom breast cancer tissue was collected prior to commencing treatment and at surgery after 3 months on letrozole therapy were available.

2.4. Plasma androgen levels in respect to BMI

Finally, to look for potential correlations between plasma androgen and BMI levels, we compared plasma

androgen values to BMI data in a group of $n = 114$ healthy women previously reported [22].

2.5. Ethical considerations

Each individual study was conducted in accordance with the Helsinki declaration and approved by the Ethics Committee at the Royal Marsden Hospital or the regional ethics committee at the University of Bergen. All patients provided written informed consent to participate in these studies.

2.6. Statistical analysis

Previous work by our group has shown plasma oestrogen and androgen levels in postmenopausal women to be well fitted to a lognormal distribution [23]. Thus, all hormone values and percentage aromatisation were analysed after lognormal transformation. Statistical analysis was conducted with use of the SPSS version 20.0 software. *R*-values were calculated according to the Spearman formula, and all *p*-values are reported as two-tailed.

3. Results

3.1. In vivo aromatisation and BMI

Combining the results from the six tracer studies (Table 1 and Fig. 1a), a non-significant correlation between BMI and the pre-treatment percentage aromatisation was observed ($n = 64$; $R = 0.236$; $p = 0.060$). Excluding the single outlier with a BMI of 42.0 slightly improved the correlation ($n = 63$; $R = 0.281$; $p = 0.026$).

Correlation between on-treatment percentage aromatisation during treatment with the different compounds and BMI is presented individually in Fig. 1b–h with their respective *R*- and *p*-values. As may be observed, no significant correlation between on-treatment aromatisation values and BMI was recorded. In addition, no correlation between BMI and individual percentage aromatase inhibition was observed (data not shown).

3.2. Plasma oestrogen levels and BMI

Pretreatment plasma oestrogen levels in relation to BMI are shown in Fig. 2a–c. We detected statistical significant correlations between BMI and pre-treatment plasma levels of E_2 ($R = 0.757$; $p < 0.001$), E_1 ($R = 0.628$; $p = 0.001$) and E_1S ($R = 0.590$; $p = 0.002$); in addition, on-treatment values of plasma E_1S during treatment with letrozole (Fig. 3a) as well as during treatment with anastrozole (Fig. 3b) correlated to BMI as well ($n = 25$; $R = 0.601$; $p = 0.001$ and $n = 12$; $R = 0.611$; $p = 0.035$). Notably, plasma levels of E_1S during anastrozole treatment were higher as compared to values on letrozole (Fig. 3b and c) independent of BMI.

3.3. Intratumour tissue oestrogen levels and BMI

Intratumour tissue levels of E_2, E_1 and E_1S before and during treatment with letrozole are depicted in Fig. 4a–c. While we observed a positive albeit non-significant correlation between pretreatment intratumour levels of E_1 ($R = 0.451$, $p = 0.12$) and BMI, no correlation between BMI and intratumour levels of E_2 or E_1S or on-treatment levels of any of the estrogens was recorded.

3.4. Plasma androgen levels and BMI

In postmenopausal women, estrogens are synthesised in different body compartments from plasma androgens taken up from the circulation [14]. No correlation between either plasma levels of androstenedione ($R = 0.006$; $p > 0.4$) or testosterone ($R = –0.142$; $p > 0.10$) and BMI was recorded, excluding elevated androgen precursor levels as a potential cause of elevated oestrogen levels in overweight women.

4. Discussion

Previous studies by our groups [23–26] as well as others [1] have revealed positive correlations between plasma and/or normal breast tissue oestrogen levels and BMI. Using highly sensitive radioimmunoassays [19], we confirmed a significant association between plasma levels of E_2 as well as E_1 and E_1S and BMI in patients prior to commencing endocrine treatment. In addition, we found a significant correlation between on-treatment levels of plasma E_1S and BMI during treatment with the third-generation aromatase inhibitors letrozole but also anastrozole. A potential correlation between on-treatment levels of plasma E_1 and E_2 during treatment and BMI could not be addressed due to the fact that 22 and 18 out of a total of 25 patients revealed plasma E_2 and E_1 levels below detection limit on letrozole treatment, respectively. With anastrozole, five out of 12 patients revealed plasma E_2 levels below the detection limit. For patients treated with exemestane, pre- and on-treatment plasma oestrogen levels had to be analysed by a special procedure including pre-purification with use of HPLC due to cross-contamination from drug metabolites in conventional radioimmunoassays [27]. Accordingly, these results could not be pooled with results from other studies for joint analysis.

To the best of our knowledge, two previous studies only have addressed plasma oestrogen levels in relation to BMI in patients on treatment with an aromatase inhibitor. In a previous study, some of us [26] revealed low E_2 and E_1S levels in patients during treatment with aromatase inhibition; yet, there was a positive correlation between on-treatment plasma levels of E_2 as well as E_1S and BMI during treatment with letrozole and a non-significant trend during anastrozole therapy. For

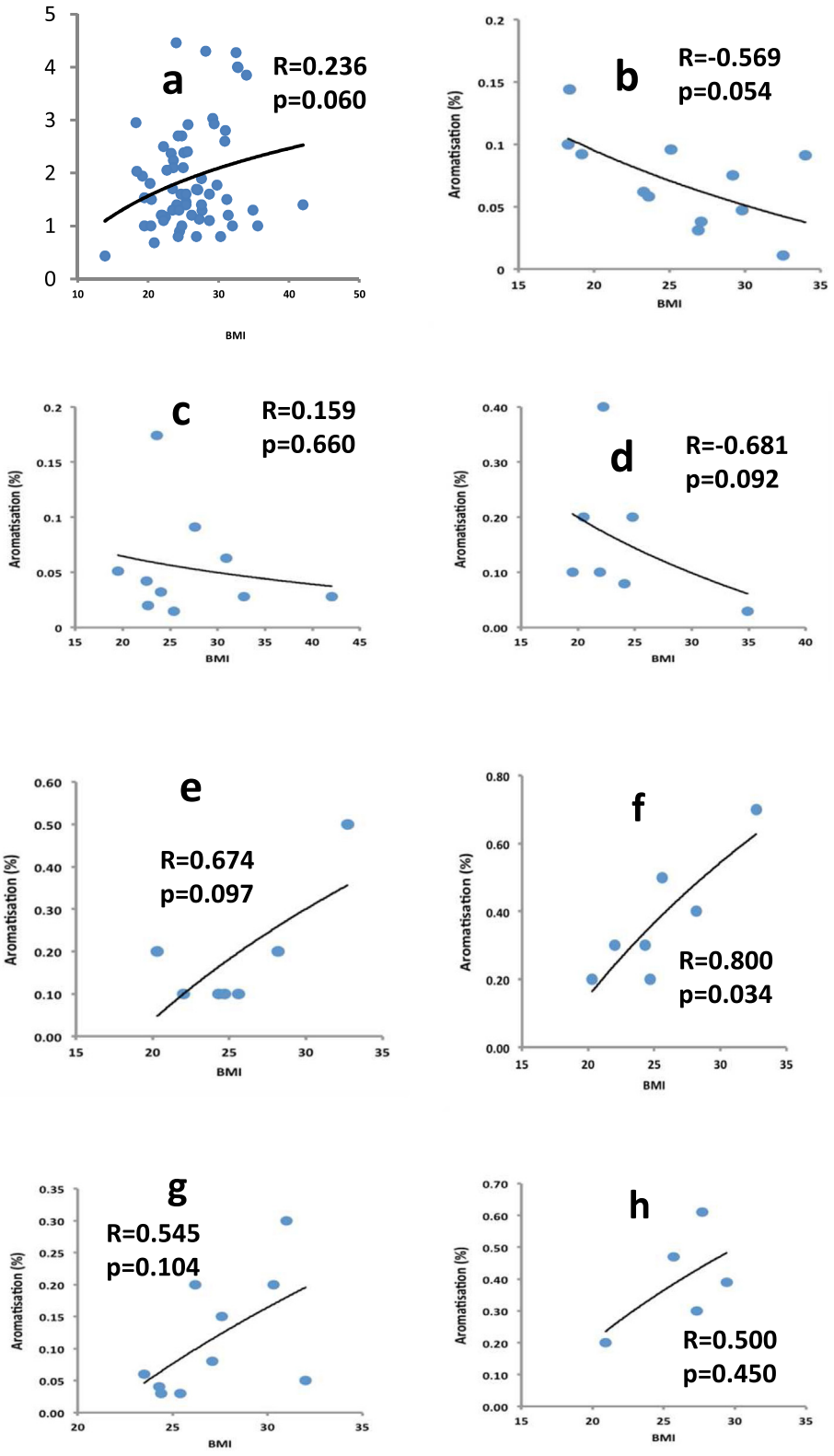

Fig. 1. *In vivo* aromatisation in relation to body mass index (BMI) before treatment with different aromatase inhibitors (pooled data; a) and during therapy with anastrozole (b), exemestane (c), aminoglutethimide (AG) (d), 4-hydroxy androstenedione (4OHA) 500 mg i.m. (e), 4-hydroxy androstenedione 250 mg i.m. (f), AG and 4OHA in combination (g) and rogletimide (h). Correlation lines are drawn based on log-normal distribution of the data.

all patients, independent of BMI, plasma oestrogen levels were higher on anastrozole as compared to on letrozole treatment. These findings resemble the results reported here. In contrast, Diorio and colleagues [28]

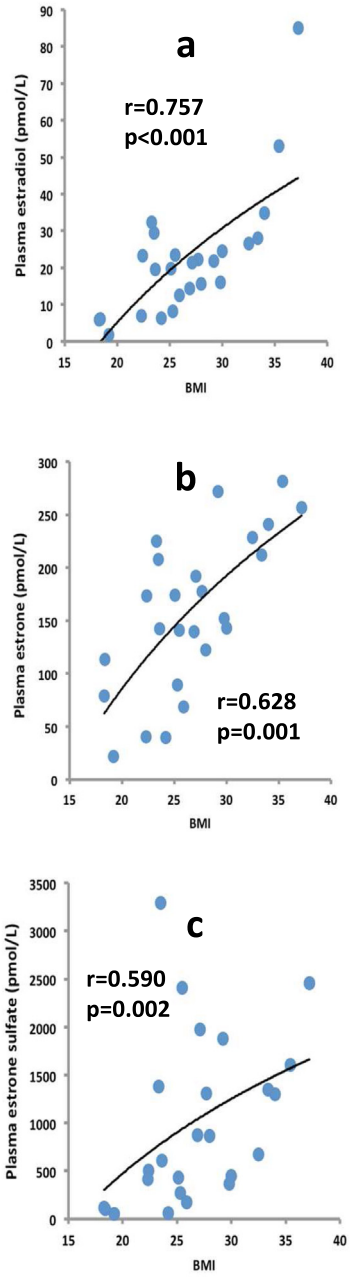

Fig. 2. Plasma oestrogen levels before treatment with anastrozole or letrozole based on data from Ref. [20] including data from Ref. [12] reanalysed and presented in [20] in relation to body mass index (BMI). (a) plasma estradiol, (b) plasma estrone and (c) estrone sulphate. Correlation lines are drawn based on log-normal distribution of the data.

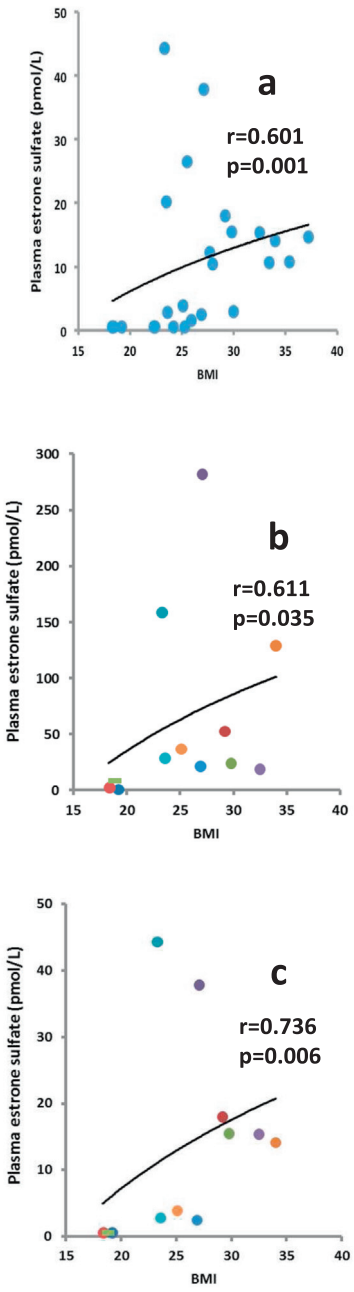

Fig. 3. Plasma levels of E_1S (obtained from Ref. [20] including data from Ref. [12] reanalysed and presented in Ref. [20]) during treatment with non-steroidal third-generation aromatase inhibitors in relation to body mass index (BMI). (a) pooled data ($n = 25$) during treatment with letrozole; (b) during treatment with anastrozole ($n = 12$; patients originally presented in reference [12]) and (c); data from the same individuals as reported in b during treatment with letrozole. Note that for each individual patient plasma E_1S suppression was more profound during letrozole as compared to during anastrozole treatment independent of BMI status. Correlation lines are drawn based on log-normal distribution of the data.

reported no correlation between on-treatment oestrogen levels and BMI. While most patients in their study revealed low concentrations of E_2 during treatment, several patients in their study revealed plasma levels of estradiol exceeding 10 pg/ml (37 pM) or, even, 20 pg/ml, values rarely observed with use of sensitive radioimmunoassays in any of our laboratories. The study by Diorio et al. also included a limited number of patients treated with exemestane; for these patients, the potential of cross-reactive metabolites in the radioimmunoassay should be considered [27].

Our finding of a weak, borderline significant correlation between pre-treatment aromatisation levels and BMI is consistent with previous observations recorded

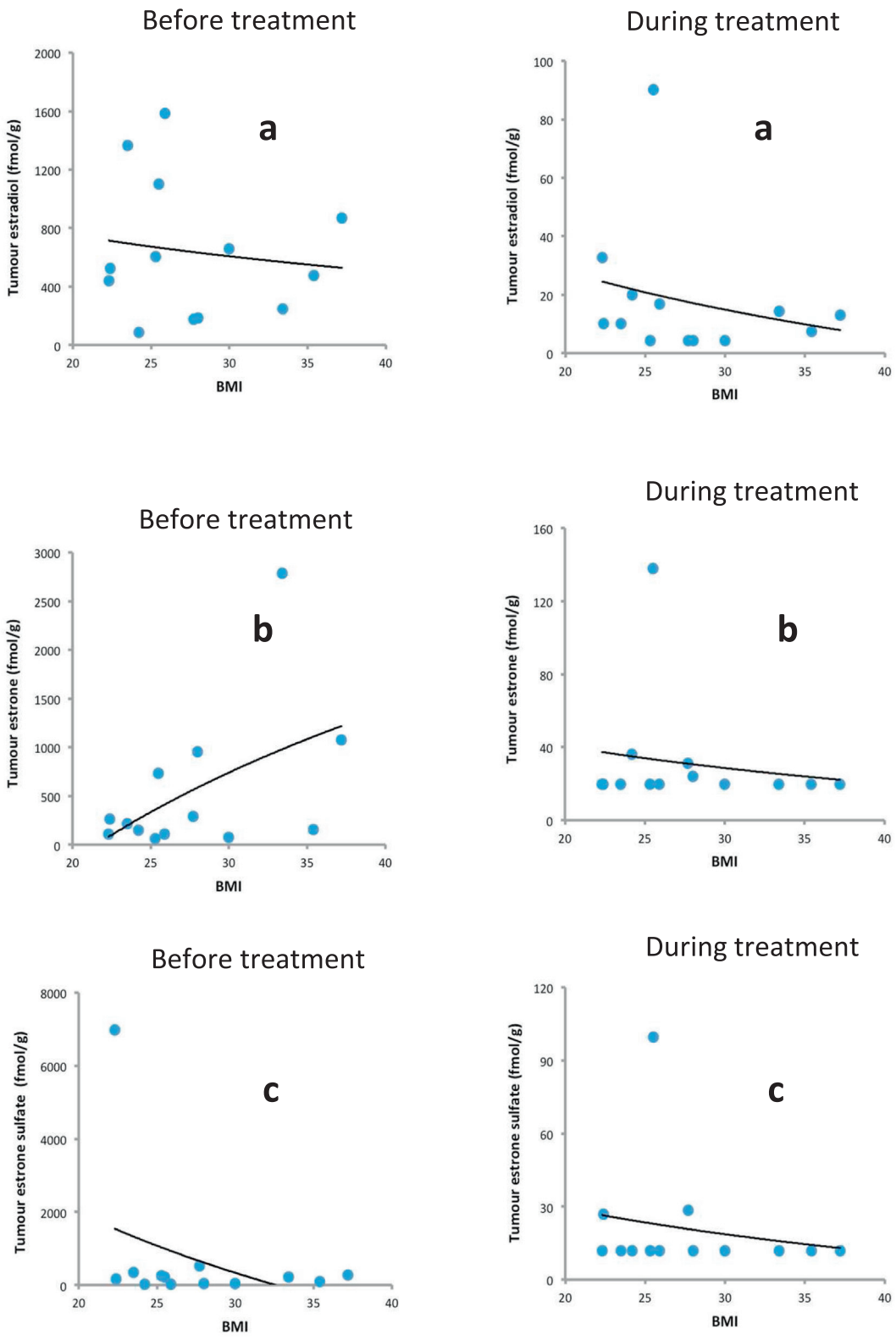

Fig. 4. Tumour tissue oestrogen levels before and during treatment with letrozole [25]. (a) estradiol, (b) estrone, and (c) estrone sulphate. Correlation lines are drawn based on log-normal distribution of the data.

by us two decades ago in a different set of patients [24]. Contrary to expectations, for patients treated with potent third-generation inhibitors we found a non-significant negative correlation between on-treatment percentage aromatisation and BMI (Fig. 1), consistent with a non-significant positive correlation between

percentage aromatase inhibition and BMI. While these moderate correlations may have occurred by chance, our findings argue against a hypothesis indicating lack of effective aromatase inhibition in overweight patients. This argument is further substantiated by the fact that all 13 patients investigated for *in vivo* aromatase inhibition on treatment with letrozole had total body aromatisation inhibited by >99.1% [12], which is the sensitivity limit of the assay.

An issue of controversy has been the potential role of local breast or breast cancer oestrogen production versus systemic delivery to intratumour oestrogen levels. While there is evidence in favour of elevated local breast aromatisation with obesity [29], recent studies by our groups [25,30,31] indicate local production may have limited effect on tissue oestrogen levels due to rapid equilibrium between plasma and tissue compartments. Rather, the reason for elevated tissue compared to plasma levels for E_1 and E_2 relates to lipophilicity of the steroidal compounds [32]. In addition, tumour E_2 levels may increase due to local ER binding [30]. Taken together, our finding of a positive correlation between intra-tumour pretreatment E_1 but not E_2 or E_1S to BMI is consistent with these previous observations. Similar, our finding of a non-significant negative correlation between each tumour oestrogen fraction (E_2, E_1 and E_1S) and BMI during aromatase inhibitor treatment argues against the hypothesis that obesity may be associated with elevated local oestrogen synthesis escaping aromatase inhibition.

While the findings in this study are consistent with a hypothesis indicating a moderate correlation between *in vivo* total body aromatisation and BMI, notably, plasma oestrogen levels are influenced by multiple factors in addition to degree of aromatisation. While we recorded no correlation between androgen precursor levels and BMI, variation in other parameters, including oestrogen metabolism, may contribute. Estrogens are metabolised by multiple CYPs in the liver influenced by exogenous as well as endogenous compounds, probably obesity as well [33–38].

The findings in this study provide information of clinical importance. First, our data provide no support for a positive correlation between residual *in vivo* aromatisation and BMI in patients on treatment with either a second-generation or a third-generation aromatase inhibitor. Second, plasma but also tissue oestrogen values detected during therapy were extremely low in all patients, arguing against systemic as well as local failure of aromatase inhibitors in overweight/obese patient. Third, as for patients treated sequentially with anastrozole and letrozole, letrozole consistently caused better plasma E_1S suppression as compared to anastrozole independent of BMI levels.

Previously, we found letrozole to be superior compared to anastrozole with respect to tissue oestrogen suppression as well [20]. While the aromatase inhibitor metaanalysis [5] did not reveal any preference for any of the three third-generation compounds (anastrozole, letrozole and exemestane), the findings presented here, in concert with the endocrine findings from the ALIQUOT study [26,39] and the clinical data of Pfeiler [6] and Sestak [7] argue for caution with respect to use of anastrozole and potential preference for letrozole in overweight and obese patients.

The negative impact of obesity recorded in the Austrian ABCSG 12 trial [6] was substantially stronger than what was observed in the ATAC study. There may be several potential explanations to these findings. Aromatase inhibitors, in contrast to tamoxifen, are ineffective in patients with any residual ovarian function [40]; thus, the data from the Austrian study raise the worrying question whether these findings may be due to zoladex failure in obese breast cancer patients. Notably, treatment with aromatase inhibitors may trigger the hypophyseal–gonadal axis [40]. Until more data are available, we suggest regular endocrine monitoring of all obese patients to be treated with an LHRH analogue with or without concomitant treatment with an aromatase inhibitor. As for such a purpose, direct radioimmunoassays that are able to discriminate between pre- and postmenopausal status, in concert with FSH and LH monitoring, may provide a crude assessment. To evaluate optimal suppression during treatment with an LHRH analogue and an aromatase inhibitor in concert, would require highly sensitive assays currently available for research purposes only. However, we believe studies evaluating oestrogen suppression in response to such combined treatment to be a significant priority, and blood samples for oestrogen analysis should be collected from such studies.

In conclusion, our unique data do not support a lack of effective aromatase inhibition in overweight patients or therefore a need for alternative therapy. The higher levels of estrogens in overweight postmenopausal breast cancer patients before and during aromatase inhibition may be due to effects of BMI on oestrogen metabolism rather than aromatisation.

Conflict of interest statement

Per Lønning has received speaker's honoraria and consultant fees from AstraZeneca, Pfizer and Novartis. Ben Haynes has no conflict of interest. Mitch Dowsett has received speaker's honoraria and research funding from AstraZeneca, Novartis and Glaxo-Smith-Kline.

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