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Original article

Low eicosapentaenoic acid and gamma-linolenic acid levels in breast adipose tissue are associated with inflammatory breast cancer

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ABSTRACT

Objective: Since it is thought that breast adipose tissue could influence breast cancer clinical presentation, we wanted to characterize specifically the relationship between breast adipose tissue fatty acid profile and Inflammatory Breast cancer (IBC).
Methods: Two hundred thirty-four women presenting with breast cancer were managed in our centre between January 2009 and December 2011. Breast adipose tissue specimens were collected during breast surgery. We established the biochemical profile of adipose tissue fatty acids (FA) by gas chromatography and assessed whether there were differences in function of the presence of breast inflammation or not.
Results: We found that IBC was associated with decreased levels in breast adipose tissue of eicosapentaenoic acid (EPA), one of the two main polyunsaturated n-3 fatty acids (n-3 PUFA) of marine origin, but also with decreased levels of Gamma Linolenic acid (GLA). Inversely, an increase in palmitic acid levels was associated with IBC.
Conclusion: These differences in lipid content may contribute to the occurrence of breast cancer inflammation.

Introduction

Inflammatory breast cancer (IBC) is considered an aggressive form of locally advanced cancer. It is a rare type of breast cancer (2–5% of all breast cancer cases) that develops rapidly, characterized by diffuse dermatologic erythema and edema making the affected breast red, swollen and tender [1]. IBC is designated as T4d in the American Joint Committee on Cancer (AJCC) Tumor, Node, Metastasis (TNM) staging system [2,3]. All of the following criteria must be met for its diagnosis: 1) Rapid onset of breast erythema, edema and/or peau d'orange, and/or warm breast, with or without an underlying palpable mass. 2) Duration of history no more than six months. 3) Erythema occupying at least one-third of the breast. 4) Histologic confirmation of invasive carcinoma. Despite IBC can be associated with any breast cancer subtypes, this is most common with hormone receptor-negative or HER2-positive disease [4–6]. Multimodality therapy is standard for non-metastatic IBC disease and includes neoadjuvant chemotherapy followed by mastectomy and post mastectomy radiation. IBC is associated with a worse prognosis, due to higher risk of relapse and shorter survival compared to non-inflammatory disease [4,7].

Mechanisms underlying breast inflammation remain unclear. However, strong overexpression of angiogenesis-related genes and eicosanoid-linked COX-2 enzyme, up-regulation of Rho C GTPase (involved in cytosqueletal organization or regulation of inflammatory, angiogenic factors) or abnormal E-cadherin retention despite EMT phenotype have been described to be associated with IBC [8–10].

In the breast epithelial cells are embedded within a fat environment. There is a growing interest in potential metabolites or substrates that could be released during adipose lipolysis process thereby contributing to tumor progression [11,12]. Thus, characterizing the relationship between breast adipose tissue fatty acid

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profile and IBC is particularly important.

We hypothesized that a specific adipose tissue fatty acid composition may be associated to the onset of IBC. We studied the fatty acid profile of samples of breast adipose tissue and investigated whether this composition differed in relation with breast inflammation or not.

Materials and methods

Study population

In this retrospective study, we analyzed data of 234 consecutive women treated for an invasive breast cancer between January 2009 and December 2011 in our Breast Cancer Care unit.

Patients with invasive breast cancer treated between January 2009 and December 2011 with an available breast adipose tissue sample stored in liquid nitrogen were included in the analysis.

Patients diagnosed with ductal carcinoma in situ (DCIS) alone were excluded. Data about baseline patients' characteristics were retrieved from the electronic medical records at our Institution.

Methods

The population of patients was from Central France. Our pathologist (FA) with expertise in breast pathology reviewed the histology specimen's slides and reports.

Samples details

Samples were excised during surgery from the external (tumor-free) region of the lumpectomy or mastectomy. Samples were stored in liquid nitrogen to minimize degradation.

Lipid analysis

From 10 mg frozen adipose samples, total lipids were extracted with 8 mL Methanol: Chloroform (2:1) and 2 mL water [13]. After vortexing and sonicating, the lower chloroform phase was withdrawn and evaporated under vacuum. One mg of total lipids were spotted on silica thin layer chromatography plate (Meckmillipore) and triglycerides were separated using development solvent as follows hexane:diethyl ether:glacial acetic acid 70:30:1. The triglycerides spots were scrapped and fatty acids were directly transmethylated with 14% boron trifluoride in methanol (BF3, Fluka, St. Quentin Fallavier, France) 30 min à 100 °C. After extraction with hexane, fatty acid methyl esters (FAME) were analyzed by capillary gas chromatography on a GC-2010 Plus chromatograph (Schimadzu, France) equipped with an AOC20i autosampler, an on-column injector, a flame ionization detector and a BPX70 column (60 m x id 0.25 mm, SGE, Courtaboeuf, France). Hydrogen was used as carrier gas at constant pressure (130 kPa). The oven program started with initial temperature 60 °C held for 5min, increased to 140 °C (rate of 5 °C/min) that it was held for 25min, increased to 145 °C (0.4 °C/min) held for 10min, increased to 180 °C (1.5 °C/min) held for 10 min, increased 190 °C (0.5 °C/min) held for 4 min, then increased to 220 °C (rate of 10 °C/min) held for 6min. FAME were identified by comparison of their retention time with those of authentic standards (Supelco, USA). Using GCSolution software (Shimadzu, France), peaks were integrated and each fatty acid was expressed as % of total peak area. The percentage of identified peaks was superior to 96%.

Statistical analysis

The categorical and numerical variables were analyzed using the Chi-square test and the Student t-test, respectively. P values < 0.05 were considered to denote significant differences. Data were managed with an Excel database (Microsoft, Redmond, WA, USA) and analyzed using R 2.15 software, available online.

Results

Breast adipose tissue samples were obtained from 234 patients who underwent surgery at our university teaching hospital. Characteristics of the population are described in Table 1. Among the 234 patients, twenty-one (8.9%) had IBC. There were no differences in age between the two groups. Body Mass Index (BMI) was higher (27.3 versus 25.3 kg/m^2, $p = 0.05$) in case of IBC when compared to women with non-inflammatory breast cancer.

Hormone receptor-negative subtypes were predominant in our population (71%), with a marked high proportion of HER2-positive subtype when compared to non-IBC (38.1% versus 12.2%).

The more frequent fatty acids found in breast adipose tissues are presented in Table 2, according to inflammation. Significant differences were found in the fatty acid profile in adipose tissue of patients with IBC as compared to patients with non-IBC. Total saturated fatty acids (SAFA) level tended to be increased in adipose environment of IBC ($p = 0.09$) and the increase was significant (+4.4%) for palmitic acid (16:0), the major SAFA.

No difference was observed for individual or total mono-unsaturated fatty acid levels.

Among PUFA, Gamma Linolenic acid (GLA) (C18:3n-6) and Eicosapentaenoic acid (EPA) (C20:5n-3) levels were lower in patients with IBC than in patients with non-IBC, with a decrease of 17% and 25%, respectively ($p = 0.009$, $p = 0.0003$). There were no significant differences with respect to other FA.

Discussion

The aim of this study was to evaluate whether alterations in the breast adipose tissue lipid composition are associated with IBC. We found a trend for increasing saturated FA levels, with a statistically significant increase in palmitic acid levels in case of IBC. We also found a significant association between low levels of EPA, and GLA in breast adipose tissue and IBC.

The aetiology of breast cancer is multifactorial and involves nutritional, endocrine and genetic factors [14]. As previously reported [4–6], we confirmed a higher prevalence of triple-negative and HER2-positive disease among cases of IBC. The molecular basis of the inflammatory process in IBC could be linked to regulation of pro-inflammatory and pro-angiogenic cytokines. This can be combined with pro-inflammatory eicosanoids synthesis, especially when tumours overexpress COX-2 enzyme [8]. However, adipose lipolysis during tumor progression and contribution of released components cannot be excluded [15].

Also, patients with IBC had higher BMI compared to patients with non-inflammatory disease. Moreover, obese women have higher chances of presenting with IBC ($p = 0.006$), large tumours ($p = 0.038$) and nodal involvement ($p = 0.03$) [16]. The association between adipose tissue inflammation and obesity is now well described in literature [17].

Our analysis shows that IBC is associated with increased SAFA levels. This pejorative effect of SAFA is in line with literature data describing higher specific mortality in women with breast cancer with high levels of SAFA [18,19]. As reported in a recent meta-analysis [20], breast cancer death was higher for women consuming diet with higher levels of saturated fat.

Fatty acid analysis showed also association between low GLA (18:3n-6) levels and IBC. In literature, anti-tumor effects by GLA enrichment remains controverted in vitro or in animal models

Table 1
Demographic and histological characteristics.

	All patients n = 234		No breast inflammation n = 213		Breast inflammation n = 21		*p*
	Mean or n(%)	Range	Mean or n(%)	Range	Mean or n(%)	Range	
Age (years)	56.15	28–89	55.8	28–89	59	37–83	*0.45*
Post menopausal	140(59.8%)		87(40.8%)		5(23.8%)		*1*
HRT	37(26.4%)		33(37.9%)		4(19%)		*0.74*
BMI (Kg/m^2)	25.5	13–41	25.3	13–41	27.3	22–36	***0.05***
BMI categories							*0.11*
-Underweight	11(4.7%)		11 (5.2%)		0 (0%)		
-Normal	101(43.2%)		96 (45.1%)		5 (23.8)		
-Overweight	68(29%)		60 (28.2%)		8 (38.1%)		
-Obese	46(19.6%)		39 (18.3%)		7 (33.3%)		
- Unknown	8(3.4%)		7 (3.2%)		1 (4.7%)		
Histological size (mm)	26.5	3–210	25.2	3–210	42	5–70	*0.52*
Molecular phenotype							***0.002***
Luminal A	73 (31.2%)		72 (33.8%)		1 (4.8%)		
Luminal B	64 (27.3%)		59 (27.7%)		5 (23.8%)		
Triple negative	63 (26.9%)		56 (26.3%)		7 (33.3%)		
HER2	34 (14.5%)		26 (12.2%)		8 (38.1%)		
Grade							*0.34*
-Grade 1	22(9.4%)		21 (9.8%)		1 (4.7%)		
-Grade 2	100(42.7%)		93 (43.7%)		7 (33.3%)		
-Grade 3	109(46.6%)		96 (45.1%)		13 (61.9%)		
-Unknown	3(1.3%)		3 (1.4%)		0 (0%)		
Lymphovascular invasion	70(29.9%)		62 (29.1%)		8 (38.1%)		*0.12*
Axillary positive LN	97(41.4%)		86 (40.4%)		11 (52.4%)		*0.16*
Multifocality	57(24.3%)		49 (23%)		8 (38.1%)		*0.16*

HRT: hormone replacement therapy; BMI: Body mass index; LN: Lymph node.

Table 2
Gas chromatography assessment of fatty acid composition of breast adipose tissue according to breast inflammation.

Fatty acid		No breast inflammation		Breast inflammation		*p*
		Mean[a]	Range	Mean	Range	
Saturates						
Myristic acid	14:0	3.20	1.65–5.25	3.29	2.59–4.0	0.38
Palmitic acid	16:0	22.93	16.71–28.8	23.89	20.85–26.12	**0.007**
Stearic acid	18:0	5.48	1.99–8.33	5.44	3.04–8.57	0.88
	Total SFA	**32.53**	**23.42–40.62**	**33.55**	**28.82–38.58**	**0.09**
Monounsaturates						
Myristoleic acid	14:1	0.27	0.01–0.56	0.27	0.12–0.51	0.90
Palmitoleic acid	16:1	3.67	1.3–8.45	3.47	1.09–7.49	0.56
Oleic acid (OA)	18:1n-9c	43.5	36.59–50.57	43.3	37.62–47.26	0.73
Vaccenic acid	18:1n-7c	1.94	1.39–3.75	1.93	1.25–3.39	0.93
	Total MUFA	**50.69**	**43.42–59.59**	**50.22**	**42.02–55.94**	0.57
Polyunsaturates						
Linoleic acid (LA)	18:2n-6c	11.02	5.97–21.0	10.72	6.27–18.08	0.66
Gamma Linolenic acid (GLA)	18:3n-6	0.05	0.02–0.10	0.03	0.02–0.07	**0.009**
Arachidonic acid (AA)	20:4n-6	0.38	0.15–1.01	0.36	0.16–0.63	0.43
	Total n-6	**12.21**	**6.94–22.68**	**11.92**	**7.35–18.59**	0.67
Alpha Linolenic acid (ALA)	18:3n-3	0.60	0.20–1.20	0.59	0.30–1.30	0.86
Eicosapentaenoic acid (EPA)	20:5n-3	0.09	0.02–0.31	0.07	0.03–0.10	**0.0003**
Docosapentaenoic acid (DPA)	22:5n-3	0.25	0.05–0.52	0.25	0.07–0.44	0.99
Docosahexaenoic acid (DHA)	22:6n-3	0.21	0.03–0.54	0.19	0.05–0.42	0.31
	Total n-3	**1.19**	**0.60–2.15**	**1.13**	**0.65–1.83**	0.43
n-6/n-3	**n-6/n-3**	**10.85**	**5.49–27.08**	**10.97**	**6.33–18.97**	0.88

[a] Expressed as % area.

[21–23]. Whereas anti-tumor effects can be linked to fatty acid synthase or HER2 decrease on tumor cells [24,25], major ambivalence of GLA is performed by regulating quality/quantity of eicosanoids [23]. Although it belongs to n-6 PUFA family which is rather associated to tumor promotion, GLA can be converted by cyclooxygenase (such as COX-2) and lipooxygenase and produce pro-inflammatory but also anti-inflammatory eicosanoids [26,27]. This is linked to its prior metabolization to gamma-linolenate (20:3n-6) or arachidonic acid (20:4n-6). Whereas gamma-linolenate lead to 1-serie prostaglandins and 15HETrE with anti-inflammatory or anti-proliferative properties, arachidonic acid allow synthesis of 2-serie prostaglandins and leukotrienes and thromboxane. These latter support tumor growth and invasion and stimulate angiogenesis [12]. In our study, arachidonic acid level was not modified in IBC group. The impact of low level of GLA (18:3 n-6) have to be elucidated but decrease of anti-inflammatory eicosanoids may be compatible with IBC.

Our findings about EPS are consistent with previous literature. A low intake of n-3 PUFA of marine origin (EPA, C20:5n-3 and docosahexaenoic acid (DHA, C22:6n-3)) has been associated with a high risk of recurrence. Patterson et al. showed that high dietary intake of EPA and DHA is associated with a 25% reduction of breast

cancer-specific mortality [28]. Khankari et al. showed that a diet enriched with n-3 PUFA with low levels of n-6 PUFA could reduce breast cancer risk [29,30] and increase overall survival after breast cancer treatment [14]. Low levels of EPA and DHA are also associated with higher chances of multifocal breast cancer, which is a known poor prognostic feature [31]. A pilot study of n-3 PUFA supplementation along with chemotherapy documented improved treatment outcomes in metastatic breast cancer patients [32]. Protective effects of regimen enriched with n-3 PUFA are well documented in rodent models and are increasingly supported by evidence in humans [33,34]. Several molecular mechanisms, more and less interlinked, are proposed to explain the effects of n-3 PUFA. Among these it can be noticed a regulation of growth factor receptors by membrane fluidity, a regulation of gene and signal transduction via PPAR activation [35] or NF-kB, a decrease of angiogenesis [36], a regulation of immune system with decrease of inflammatory cytokines (IL-6, TNFa) or synthesis of anti-inflammatory EPA-derived eicosanoids (3-series prostaglandins, resolvins) at the expense of arachidonate-derived eicosanoids [33,37]. As suggested above, deficiency of EPA fatty acid is compatible with inflammatory phenotype expression.

Lipid composition of adipose tissue is considered as a long term biomarker of past dietary intake of fatty acids due to its slow turnover [38–40]. Our results suggest the possibility of a link between dietary habits and clinical presentation of breast cancer. Saturated fatty acids are particularly found in animal-derived food, butter or cheese and in vegetable oils such as palm oil. GLA can be obtained from green vegetables, walnuts, or vegetable GLA-rich primrose or borage oils. Major food sources of EPA are fatty cold-water fish/fish oil of marine origin. Endogenously synthetized fatty acids cannot be eluded, particularly for saturated fatty acids [39]. However, elongation and desaturation of PUFA remain low in humans [37] and strong correlation have been found between dietary EPA and DHA fatty acid supplements and breast adipose composition [41,42]. Thus, the low EPA level in this storage tissue could reasonably reflect a lower past intake of long chain FA of marine origin.

Since inflammation is linked to breast cancer prognosis [4,7], it is not surprising to find a similar association between lower n-3 PUFA levels and IBC. The low rate of both fatty acids in adipose tissue with potential anti-inflammatory properties could cooperate with IBC presentation, particularly whether IBC tumor overexpress COX-2. In association with findings of other studies, these present results reinforce the possibility of a link between dietary habits and the clinical expression of breast cancer. This hypothesis needs to be confirmed prospectively and with a study of dietary intervention.

Conflicts of interest

We declare that we have no conflict of interest.

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