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The lipidome as a composite biomarker of the modifiable part of the risk of breast cancer

Philippe Bougnoux *, Nawale Hajjaji, Charles Couet

INSERM U921 Nutrition, Croissance et Cancer, HS Kaplan Cancer Center - CHU Bretonneau, 2 bis Boulevard Tonnellé, 37044 Tours, France

A B S T R A C T

The potential for dietary fat to prevent breast cancer makes identification of defined molecules a mandatory step. In order to circumvent the limitations and/or bias of dietary exposure assessment tools, we have used the fatty acid composition of white adipose tissue as biomarker of past lipid intake. When considered separately, candidate fatty acids identified as favourable on the basis of their association with breast cancer risk have usually led to inconsistent results in dietary intervention studies carried out in rats. This inconsistency indicates that any approach based on a single fatty acid should be abandoned for an integrated view over the complex lipid interactions, which finally determines the lipidome, the lipid profile that is found in individuals. We reappraised the role of the complete lipid profile through a comprehensive study of adipose tissue fatty acids obtained in patients with benign or malignant breast tumors. Rather than a single fatty acid, a composite indicator combining elevated monounsaturates and low *n*-6/*n*-3 fatty acid ratio was associated with decreased breast cancer risk. The lipidome may provide the opportunity to quantify the modifiable part of the risk of breast cancer. The lipidome may be used as a template for designing proper dietary modifications in order to delay the occurrence of breast cancer. Which dietary modifications should be undertaken in order to bring a pertinent change to the lipidome with respect to the risk of breast cancer is currently unknown. The lipidome may allow the individualization of a high risk population of women, who may be targeted for a dietary prevention of breast cancer. The setting and validation of a high-throughput lipidomic station with analytical capabilities fitted to the need of mass screening is required. These two locks must be resolved before a primary prevention of breast cancer by diet could be contemplated.

1. Introduction

Cancer is the clinical expression of cellular disorders resulting mostly from the acquired gene alterations which accumulate with age. A large body of evidence from observational epidemiological studies and from migrant population studies has indicated that environmental factors can influence cancer expression [1]. Diet might account for 25–40% of the preventable causes of cancer [2], particularly breast cancer. Among food components, lipids may influence breast cancer incidence rate. Although ecologic association and animal studies support a direct effect of dietary lipids on the development of breast cancer, results of epidemiologic studies have been inconclusive. Recently reported findings from the Women's Health Initiative (WHI) provide indications that modification of the lipid part of the diet can modify the risk of breast cancer. This major dietary intervention study randomized 48,835 postmenopausal women into a controlled dietary lipid reduction of 20 g/d for 5 years [3]. Although the diet was non selective in terms of which components of lipid were reduced, although the population was not selected-beside the menopausal status-in terms of risk factors for breast cancer, and although the follow-up was shorter and the reduction in fat intake in the intervention group, smaller than originally planned, there was a nearly significant reduction in breast cancer incidence in the dietary group compared to the control group. Thus, diet as a potentially modifiable risk factor for the risk of breast cancer remains of great interest, provided pertinent lipids are identified and provided women at high risk for breast cancer for dietary reasons are individualized.

1.1. How to identify the pertinent lipids?

Analytical epidemiology based on estimates of the past intake of lipid nutrients has provided essentially non consistent results. Considered together, there has been no specific effect of saturated fatty acids, non consistent results with monounsaturates, weak and contradictory results for *n*-6 polyunsaturated fatty acids (PUFA) on breast cancer risk. With respect to *n*-3 PUFA,

* Corresponding author. Tel.: +33 2 4747 8265; fax: +33 2 4747 6012/33 247 478065/33 247 366226.
E-mail address: bougnoux@med.univ-tours.fr (P. Bougnoux).

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contradictory results have been reported for alpha-linolenic acid (ALA) and a trend towards a protective effect was observed with long-chain *n*-3 PUFA. Diet assessment methods and other potential factors that may contribute to these heterogeneous and inconsistent results have been thoroughly analyzed in a recent review [4]. Therefore, there is a need for an approach based on a biomarker of past dietary intake.

1.2. Need for a biomarker associated with breast cancer: the adipose tissue

Fatty acid composition of the adipose tissue reflects the past dietary fat intake over a several years period. Its low turnover makes the adipose tissue a biomarker insensitive to the influence of the last meal. We previously found, in a case-control study carried out in Central France, that lipids of the breast adipose tissue may predict the risk of breast cancer [5]. ALA and docosahexenoic acid (DHA) levels in the adipose tissue were inversely associated with the risk of breast cancer. A similar observation has also been made in several European countries [6] and in North America for long-chain *n*-3 PUFA [7], although other studies based on a similar design were not conclusive [8,9]. To investigate the potential causality of the inverse association of *n*-3 PUFA with breast cancer risk, i.e., whether dietary *n*-3 PUFA may interfere with tumor growth, we conducted several experimentations using a rodent model of autochtoneous, NMU-induced mammary tumors. To determine the effect of ALA (18:3*n*-3), we compared tumor incidence and tumor growth kinetics in rat were fed with a high-ALA-containing diet (rapeseed oil) to a control group fed peanut oil (low ALA content). No dietary ALA effect on tumor growth was detected [10]. The same experiments were performed with DHA, an experimental rat group receiving DHASCO (an algae-derived oil) and the control group palm oil. Effects on tumor growth were inconsistent. We observed that results were influenced by the antioxidant or pro-oxidant content. Addition of pro-oxidants (a REDOX mixture of naphtoquinone/ascorbate) to a high PUFA diet (>40% PUFA) reduced tumor growth, whereas the addition of anti-oxidants (vitamin E) reversed this effect and stimulated tumor growth. *n*-3 PUFA inhibited tumor growth by enhancing cell loss rate in the tumor, an effect that was abolished by antioxidant molecules. Therefore, the action of *n*-3 PUFA on mammary tumorigenesis depended on their interactions with other components of lipids. Such confounding factors probably account for inconsistent results observed with dietary interventions targeted on *n*-3 PUFA in rats. These results indicate that focusing on a particular lipid component is a simplistic and probably a misfit approach which should be abandoned for an integrated view over the complex lipid interactions which finally determines the lipid profile that is found in individuals.

1.3. The individual fatty acid approach is no longer appropriate: the need for a comprehensive study

We used the resources of the Tours case-control study to stress the role of interaction between fatty acids in breast cancer risk. The study was based on adipose breast tissue sampled at diagnosis during surgery for 241 patients with invasive, non metastatic breast cancer (cases) and 88 patients with benign, non proliferative tumor (controls). We examined the correlations between the different fatty acids in the adipose tissue. We observed that linoleic acid was positively correlated with the other *n*-6 fatty acids, the strength of the correlation being commensurate to the metabolic steps involved, and was inversely correlated with monounsaturates, and also with saturates, up to 16 carbons [11]. DHA was highly correlated with docosapentaenoate, but also positively correlated with long-chain *n*-6 PUFA such as arachidonic acid, and also with 22:1. Similar correlations were found for arachidonate, but not for ALA [11]. Thus, fatty acids are not independent variables. When we examined each individual fatty acid for their association with the risk of breast cancer (Table. 1), we found that all the *n*-3 fatty acids were significantly associated with a decreased risk of breast cancer. The same observation was made for cis monounsaturates, while elaidic acid (18:1w9 trans, a trans fatty acid derived from industrial processes) was significantly associated with an increased risk. Saturates and *n*-6 PUFA had no association, with the exception of 20:2w6. Thus, the risk of breast cancer can be estimated by adipose tissue fatty acids levels. There is a need to simplify this complex system of correlations into a smaller number of dimensions.

1.4. The lipidome: integration of interactions between fatty acids into a principal component analysis

To take into account interactions between fatty acids, we used a principal component analysis (Fig. 1). Such an analysis is aimed at transforming a set of inter-correlated variables (the fatty acids) into a set of uncorrelated variables or principal component. The first principal component accounts for as much as possible of the variability between patients, and each succeeding component accounts for as much as possible of the remaining variability. Thus, each principal component explains a fraction of the variance, which is the fraction of information explained by the principal component. The principal components are the best covariates to extract the maximum of the information carried by all the fatty acids according to their correlations. This analysis was based on 23 fatty acids of the whole population (cases and control). Since fatty acid level in the adipose tissue is strongly correlated to age and body mass index, standardization upon these two factors was made.

The lipidomic profile associated with a decreased risk of breast cancer (protective lipid signature) combined a low *n*-6/*n*-3 FA ratio with high level of monounsaturates (oleic acid).

Table 1
Estimated association of breast cancer and high level of fatty acid in adipose tissue

Fatty acid	OR	IC 95%	*p*
Saturates			
14:0	0.86	[0.64; 1.17]	0.334
16:0	0.89	[0.67; 1.20]	0.436
18:0	1.32	[0.95; 1.85	0.100
20:0	1.39	[1.03; 1.91]	0.035
Monounsaturates			
16:1*n*-7t	1.17	[0.83; 1.66]	0.381
18:1*n*-9t	2.44	[1.69; 3.64]	<0.001
14:1	0.66	[0.50; 0.87]	0.004
16:1*n*-7c	0.67	[0.49; 0.89]	0.009
18:1*n*-9c	0.59	[0.43; 0.81]	0.002
n-6 PUFA			
18:2*n*-6c	1.33	[0.98; 1.84]	0.074
20:2*n*-6	1.64	[1.17; 2.39]	0.007
20:3*n*-6	0.97	[0.67; 1.46]	0.879
20:4*n*-6	0.81	[0.56; 1.20]	0.281
n-3 PUFA			
18:3*n*-3	0.67	[0.49; 0.90]	0.009
20:5*n*-3	0.51	[0.36; 0.69]	<0.001
22:6*n*-3	0.66	[0.47; 0.91]	0.012

In order to allow comparisons between fatty acids, standardization was done for each fatty acid. ORs were estimated for each fatty acid adjusted for BMI, height, age and menopausal status.