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DIET, CANCER AND THE LIPIDOME

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Summary :

The potential for dietary fat to interfere with the development of breast cancer by delaying its occurrence makes identification of defined molecules a mandatory step in cancer prevention. In order to circumvent the limitations and/or bias of dietary exposure assessment tools, biomarkers of past lipid intake such as the fatty acid composition of white adipose tissue have been used. When considered separately, candidate fatty acids identified as favorable on the basis of their association with breast cancer risk have usually led to inconsistent results in animal intervention studies. 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. This article presents a reappraisal of the role of the lipid profile through a comprehensive reanalysis of adipose tissue fatty acid composition obtained in patients with benign or malignant breast tumors as well as in experimental animals during dietary interventions. Rather than a single fatty acid, a composite indicator combining elevated monounsaturates and low ω6/ω3 fatty acid ratio was associated with breast cancer protection. This lipidome may become the template for identifying breast cancer risk related to diet, and for designing proper dietary modifications to delay the occurrence of breast cancer, although the universality of the findings cannot be assessed from a single study.

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Numerous epidemiological studies have established striking geographic differences in the rate of incidence of breast cancer, and those conducted in migrant populations strongly support a role for environmental exposure including diet in the variation of breast cancer rates across countries [1]. Indeed, it has been suggested that 20 to 60 % of cancer, depending on the anatomic localization of the tumor, may be avoidable by altering the diet [2]. Among several dietary components that could modulate breast cancer risk, fat has been extensively examined with little evidence for a promoting effect of total fat intake independent of the fat contribution to total energy intake [3], total energy intake which has been recently shown to be positively associated with breast cancer risk in Chinese women [4]. Ecological, cohort, and case-control studies aimed at evaluating the association between usual or past diet assessed by either dietary recall, dietary record, dietary interview, food frequency questionnaire on the one hand, and breast cancer risk on the other hand have led to discordant conclusions [5]. The discrepancies between studies are mainly due to the limitations and/or bias of dietary exposure assessment tools, including subjectivity, unintentional inaccuracy, underreporting, and dieting behavior which alter the quality of dietary data collected in free-living populations [6]. Beyond the uncertainties that are common to any study of nutritional epidemiology, there are specific limitations in evaluating the intake of some dietary fatty acids which are partly or totally excluded from food composition tables currently available. This is usually the case for the fatty acids quantitatively considered as minor, but which are biologically important, including long-chain polyunsaturated fatty acids (PUFA) of the ω3 and ω6 series, trans fatty acids, and conjugated linoleic acid, for instance.

Lessons learned from the use of biological markers of dietary exposure

In order to circumvent these limitations, biological markers of dietary exposure such as the fatty acid composition of serum phospholipids, membrane phospholipids and/or white adipose tissue (WAT) triglycerides have been extensively used in population-based studies with special

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emphasis on ω3 and ω6 PUFA. Briefly, studies dealing with the ω3-ω6 PUFA composition of either serum or erythrocyte membrane phospholipids and breast cancer risk were inconclusive [7]. It should be stressed that plasma and membrane phospholipid composition vary according to recent dietary intakes which might be altered by the occurrence of cancer. Conversely, the fatty acid composition of WAT triglycerides best reflects past dietary intake for the essential fatty acids (linoleic acid, α-linolenic acid, and long-chain ω3 PUFA) [8-9], and appears to be a more relevant marker of lasting exposure with respect to the breast cancer risk and fat intake relationships. This marker has been used in several studies aiming to investigate the relation between exposure to ω3 and ω6 PUFA and breast cancer. In our case-control study published in 2002 [10] and conducted in 241 patients with invasive breast carcinoma and 88 control patients with benign breast disease, we found significant inverse association between individual levels of α-linolenic acid and docosahexaenoic acid (DHA) in breast adipose tissue and the risk of breast cancer, with the strongest inverse association found for the ratio of long-chain ω3 PUFA to ω6 PUFA. The results of other studies have been reviewed recently with the overall conclusion that the protective effect of ω3 PUFA on breast cancer risk depends on background levels of ω6 PUFA [7].

Intervention studies targeted on single fatty acids : a repeated failure

To fully interpret the information that the biomarkers (i.e., the WAT content of α-linolenic acid, DHA and ω6 to ω3 PUFA ratio) can provide with respect to cancer risk and possible prevention in humans, dietary interventions targeted at either α-linolenic acid or DHA were set up in NMU and DMBA-induced mammary tumor models in rats with inconsistent results [7]. Whereas some studies appeared to support the epidemiological evidence concerning the importance of the ratio of ω6 to ω3 PUFA in mammary tumor growth [11-13], others failed [14-16], perhaps because of the inhibitory effect of ω3 PUFA on mammary tumor growth, which depends upon background levels of ω6 PUFA and also on antioxidants levels [17]. An experimental study on rats showed that addition of vitamin E to a 15% linseed oil diet rich in α-linolenic acid led to an increase in tumor

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growth compared to controls without vitamin E, whereas addition of a prooxidant compound (sodium ascorbate/2-methyl-1,4-naphtoquinone) led to a decrease in tumor growth [18]. Thus, despite the identification through epidemiological studies of a few WAT fatty acids as a biomarker of breast cancer risk in humans, a more complex picture is emerging from the experimental dietary intervention studies focusing on those fatty acids.

Breast cancer risk varies depending on the WAT fatty acid level considered

To get a more integrated view of the complex lipid interactions which, in turn, give rise to the individual lipid profile in WAT triglycerides, we retrospectively reconsidered the statistical analysis of hundreds of WAT fatty acid composition previously obtained from French women with either breast cancer or benign breast tumor [10], using principal component analysis. Secondly, we re-examined the conclusions drawn from our previous dietary intervention studies in rodents [16,20] in the light of the new and more sophisticated analysis of human data.

Figure 1 shows the adjusted odds ratios of breast cancer for the fatty acids of white adipose tissue sampled from 329 women and presented by lipid class. A decreased risk of breast cancer was associated with higher content of ω3 PUFA, either α-linolenate or the long chain ω3 fatty acids EPA or DHA. In contrast, a high content of ω6 PUFA was associated with either a trend (linoleic acid, 18:2ω6) or an increased risk (20:2ω6). Cis-monounsaturates were all protective, whereas trans-monounsaturates were not (*t*-16:1ω7), or were strongly associated with an increased risk (elaidic acid, *t*-18:1ω9). No association with breast cancer risk was detected for saturates.

WAT fatty acid levels are not independent variables

The significance of such associations is unclear when one considers the common metabolic pathways shared by numerous fatty acids which link the content of individual fatty acid to each other. Figure 2 presents the correlation coefficients found between two fatty acids of each PUFA family and the other fatty acids of WAT triglycerides. For instance, linoleic acid demonstrates a

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strong commensurate and positive correlation with its long-chain derivatives according to the number of metabolic steps involved in their biosynthesis, and a weak positive correlation with ω3 PUFA (Figure 2a). In contrast, linoleic acid inversely correlates with saturates and monounsaturates, with the exception of trans-monounsaturates. Positive and inverse associations are observed for arachidonic acid, alpha-linolenic acid, DHA (Figures 2b, 2c, and 2d) and for many others (data not shown). Consequently, a single fatty acid can not be considered as an independent biomarker of breast cancer risk, and there is a need to simplify this complex system of correlations into a smaller number of dimensions.

Simplifying through principal component analysis

For this purpose, we performed a principal component analysis using our whole database of 329 patients (cases and controls). The principal component analysis was based on 23 fatty acids which belong to the four principal fatty acid classes as follows: saturates (14:0, 15:0, 16:0, 17:0, 18:0, 20:0), monounsaturates (14:1, 16:1c, 16:1t, 17:1, 18:1ω7, *c*-18:1ω9, *t*-18:1ω9, 20:1), ω6-polyunsaturates (18:2, 20:2, 20:3, 20:4 and 22:4), and ω3-polyunsaturates (18:3, 20:5, 22:5 and 22:6), on the basis of their level in adipose tissue or their carbon chain length (fatty acids with less than 14 carbons or with a level lower than 0.2% of total fatty acids were not included in the analysis). Because age and BMI are closely associated with risk of breast cancer, at least in postmenopausal women, and because they strongly correlate with long-chain PUFA levels in the WAT, fatty acids were considered through their residuals from the regression on age and BMI, thus preventing from artifact effects due to age and/or BMI. Principal component analysis is aimed at transforming a set of inter-correlated variables (the 23 fatty acids) into a set of uncorrelated variables, or principal component [19, http://www.statsoft.com/textbook/stfacan.html]. 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

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explained by the principal component. The interpretation of the principal components -i.e. the meaning of these new variables- is made in view of their correlation with the initial variables.

The results of this analysis are shown in Figure 3. The two principal components accounted for almost half (42.4%) of the information (inter-individual variability) borne by all 23 fatty acids. The first principal component (X axis) accounted for 24.4 % and the 2nd principal component (Y axis) for 18.0%. Fatty acids were not randomly located. Saturated fatty acid location was clustered into the left part of the scatter plot, monounsaturates (with the notable exception of *t*-18:1ω9) were in the upper part, long-chain polyunsaturates (both ω6 and ω3 PUFA) were on the right part while 18:2ω6 was on the lower part of the scatter plot. Therefore the X axis opposed saturates to long-chain polyunsaturates, and the Y axis opposed 18:2ω6 to monounsaturated fatty acids. In addition, we located the ω6/ω3 ratio as an illustrative variable –i.e., it was not used to establish the principal components. This ratio strongly correlated with the Y axis (Figure 3).

The two axes were then considered as independent covariates in the framework of a logistic regression model aimed at assessing the risk of breast cancer. The association was not significant for the X axis (Odds ratio, i.e. OR, associated to a decrease of one unit on the X axis: OR = 1.02, 95%CI = 0.92-1.14, p=0.661), and highly significant for the Y axis (OR associated to a decrease of one unit on the Y axis: OR=1.25, 95%CI = 1.10-1.42, p<0.001). The second principal component remained highly significantly associated with the risk of breast cancer after adjustment (OR=1.28, 95%CI = 1.11- 1.49, p=0.001). Considering the four quartiles associated to the second principal component, this association may be re-expressed as follows: $OR_{Quartile1/Quartile4}$=3.23, 95%CI = 1.37-8.07, $OR_{Quartile2/Quartile4}$=1.58, 95%CI=0.73-3.47 and $OR_{Quartile3/Quartile4}$=1.02, 95%CI=0.48-2.15. Thus, as shown in figure 3, a location in the left lower quadrant of the scatter plot is associated with an increased risk of breast cancer. Therefore a lipid profile of the WAT which comprises low linoleic acid or a low ω6/ω3 ratio along with elevated cis-monounsaturates is protective against the risk of breast cancer, independently of age and BMI.

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The lipidome as a new insight into the link between diet and breast cancer

Principal component analysis does not provide any indication of the inter-individual differences in the WAT content of each fatty acid. Figure 4 presents a lipid profile array of cases and controls. There are several differences in the pattern of colors between cases and controls. In controls, there is a spot of elevated values involving monounsaturates. In cases, more elevated values of ω6 PUFA are observed in the lower right corner compared to controls. The ratio of ω6 to ω3 fatty acids (shown in the lower panel) appears as a main distinctive feature between cases (left part) and controls (right part). Thus, similar to the profile array of transcripted genes which allowed the individualization of the combinations of gene alterations associated with the risk of death in breast cancer [21], the lipid profile array provides indication of the combinations of WAT fatty acid levels associated with the risk of breast cancer. By analogy with the proteome or genome, the word lipidome has been coined to characterize this lipid profile which may be altered through a dietary intervention.

Failure of dietary interventions targeted on a single fatty acid in modifying favorably the lipidome in rats

On the basis of this new composite biomarker of a low risk for breast cancer –i.e., low ω6/ω3 ratio and high cis-monounsaturates- it was tempting to look back to the changes in fatty acid composition of WAT that we induced in our dietary interventions targeted at either α-linolenic acid or DHA in NMU-induced mammary tumor in rats. Table 1 summarizes the fatty acid composition of WAT triacylglycerols with or without dietary intervention over 26 weeks [16, 20]**.** In both diets, a significant increase in the specific ω3 fatty acid added to the control diet is obtained with a decrease in the ω6/ω3 ratio as expected. However, despite this change in the ω6/ω3 ratio, which seems to fit the appropriate direction with respect to breast cancer risk, a concomitant and significant drop in monounsaturates was induced. This drop is exactly in the opposite direction compared to the indications provided by the analysis of the lipidome. Thus, favorable dietary fatty

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acid exposure was not achieved in these animal studies, stressing the need for a composite dietary modification rather than for single nutrient intervention.

Conclusions and future prospects

It should be pointed out that the validation of any link between a biomarker and the emergence of any type of cancer requires parallel experimental animal and human studies. An appropriate animal model is usually set up to determine the role of the marker, whether associative or causal, in the disease pathway and to establish the dose-effect relationship. Thus, to fully interpret the informations provided by the analysis of the lipidome with respect to breast cancer risk, appropriate dietary intervention reproducing two prominent features of the Mediterranean diet, i.e., a low ω6/ω3 PUFA ratio and elevated monounsaturates levels, must be carried out in animal models and human pilot studies in the future. If successfully implemented in an appropriate model of breast cancer in rat, one may speculate that the Mediterranean-diet would bring as much comprehensive knowledge for dietary exposure and cancer relationships as did the “cafeteria” diet for the diet and obesity relationship. The reappraisal of the role of the environmental exposure to dietary fatty acids in breast cancer risk must be performed before public health applications based on the concept of cancer prevention by delay [22], either primary or secondary, can be considered. The lipidome may be the most appropriate mean to address this challenge. The use of the composite biomarker (OR) defined by the analysis of the lipidome provides a way to individualize women with a high risk of breast cancer due to dietary habits and to follow the impact of dietary interventions in those women. Such new approach would be even more opportune in women with genetic predisposition to breast cancer. However, the universality of our findings cannot be assessed from a single study and a confirmation is needed from studies in which the lipidome is derived from adipose tissue of women living in countries where the breast cancer incidence and dietary fat intake differs strongly from that of the French women.

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LEGEND TO FIGURES

Figure 1: Estimated associations of breast cancer and high level of white adipose tissue fatty acid content. Adipose tissue obtained at surgery from 241 patients with invasive, non metastatic breast cancer (cases) and from 88 patients with benign, non-proliferative tumors (controls) were analyzed for fatty acid composition. Prior to analysis standardization was performed for each fatty acid, thus allowing comparisons between fatty acids. For this purpose each individual fatty acid value was subtracted from the mean value of the group and divided by the standard deviation. Then, odds ratios were estimated for each fatty acid adjusted for BMI, height, age and menopausal status in the framework of logistic regression models [23]. Total saturates includes 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0; total cis-monounsaturates includes 14:1ω5, 16:1ω7, 17:1, 18:1ω7, 18:1ω9 and 20:1; total ω6 PUFA includes 18:2, 20:2, 20:3, 20:4 and 22:4 and total ω3 PUFA includes 18:3, 20:5, 22:5 and 22:6.

Figure 2. Coefficients of correlation obtained in adipose tissue between main fatty acids and either linoleic acid (panel a), arachidonic acid (panel b), alpha-linolenic acid (panel c), docosahexaenoic acid (panel d).

Figure 3 - Principal component analysis of adipose tissue fatty acids. In the scatter plot of the second principal component against first principal component, (where X axis represents the first principal component, Y axis the second principal component), the coordinates of each fatty acid equals the coefficients of correlation between the fatty acid and the principal components. The unity correlation circle drawn defines the limits in which the fatty acids locate: the closer a fatty acid to this unity circle, the higher its contribution to the definition of the principal components. The ω6/ω3 ratio is located as illustrative variables - i.e., it does not contribute to the definition of the principal components, but it is positioned in the scatter plot according to its correlation with the two principal

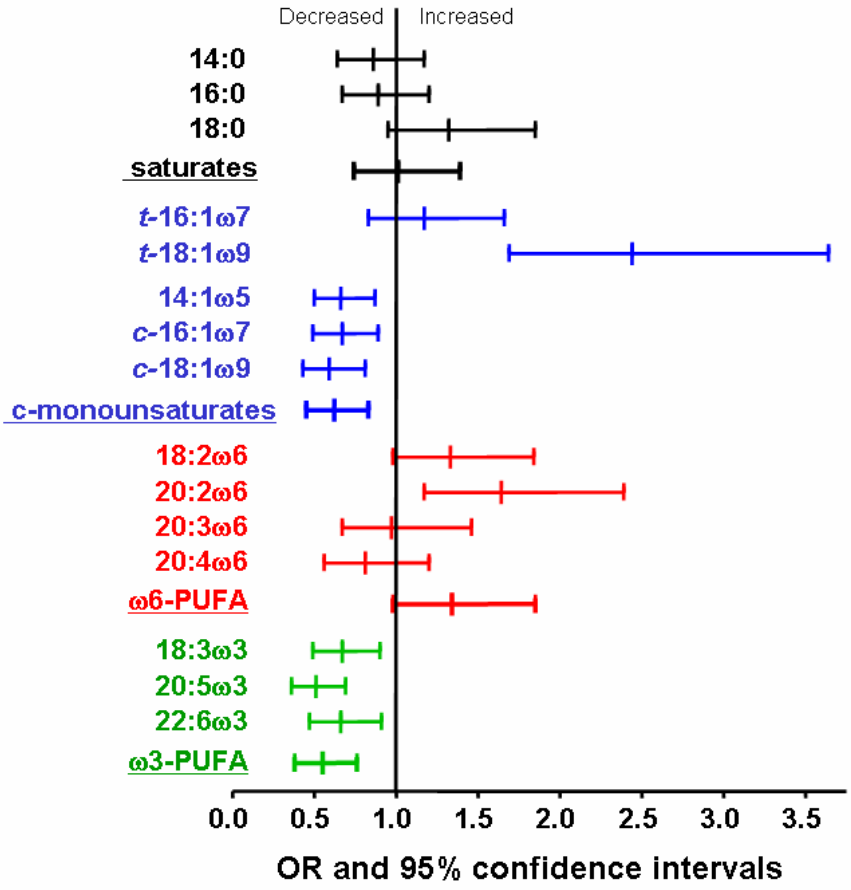
components. The red arrow, which takes into account the odds ratio associated with both the X and Y axis, indicates the increased risk of breast cancer, adjusted for BMI, age, menopausal status and height. The position of this arrow is almost superposed on the Y axis because the odds ratio associated with the first component is close to 1, while the odds ratio associated with the second principal component is 1.28 (95% CI=1.11-1.49, p=0.001).

Figure 4. Fatty acid level array in patients with benign (controls) or malignant (cases) breast tumors. In this representation, each lane represents a patient, sorted according to its position on the second principal component as shown in figure 3. Each line represents one fatty acid, according to its correlation with the second principal component. Fatty acid values are represented as different colors for each quartile, from green (low) to red (elevated). Lower panel represents the ω6/ω3 ratio of polyunsaturated fatty acids.

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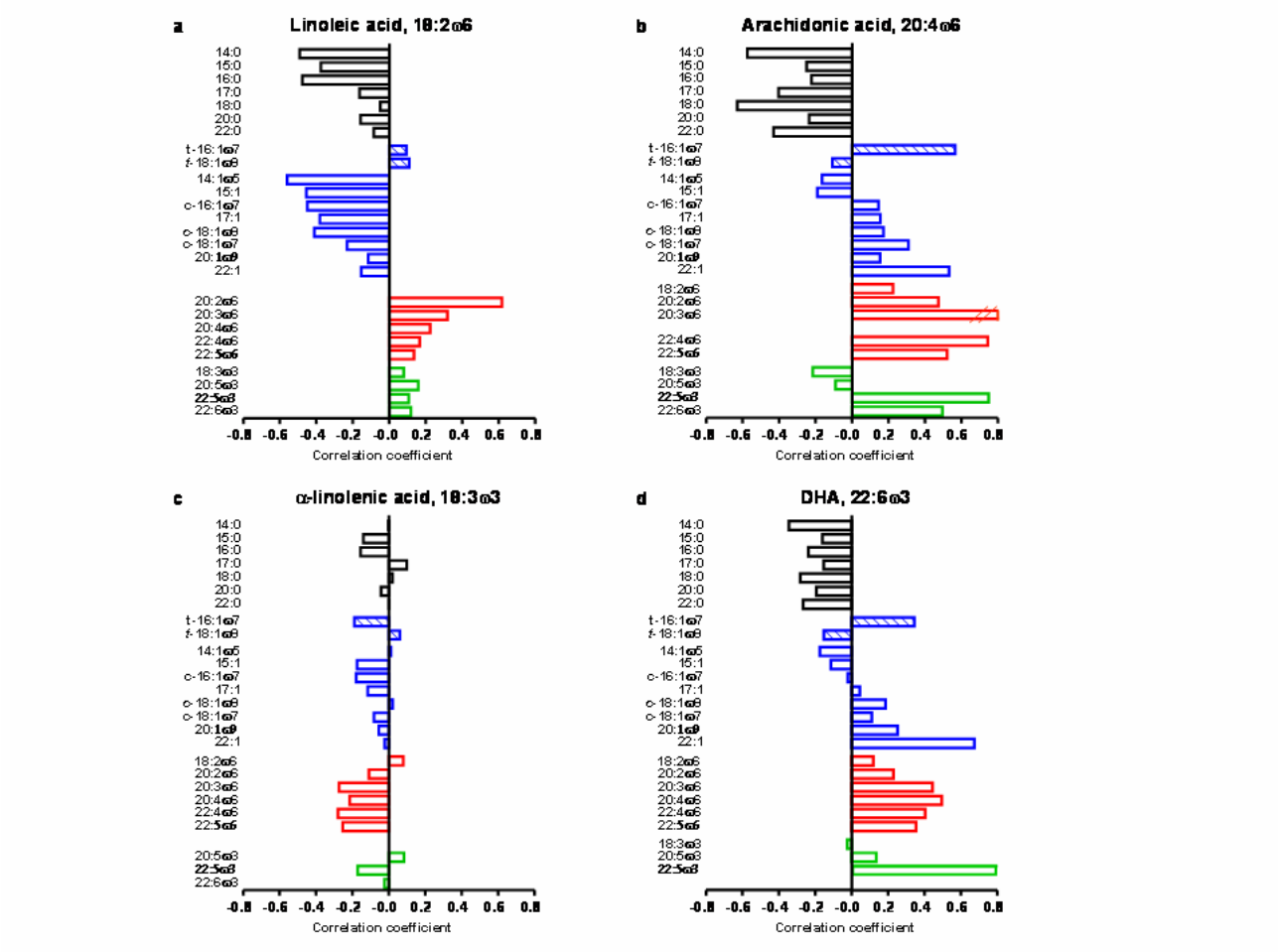
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Figure 1



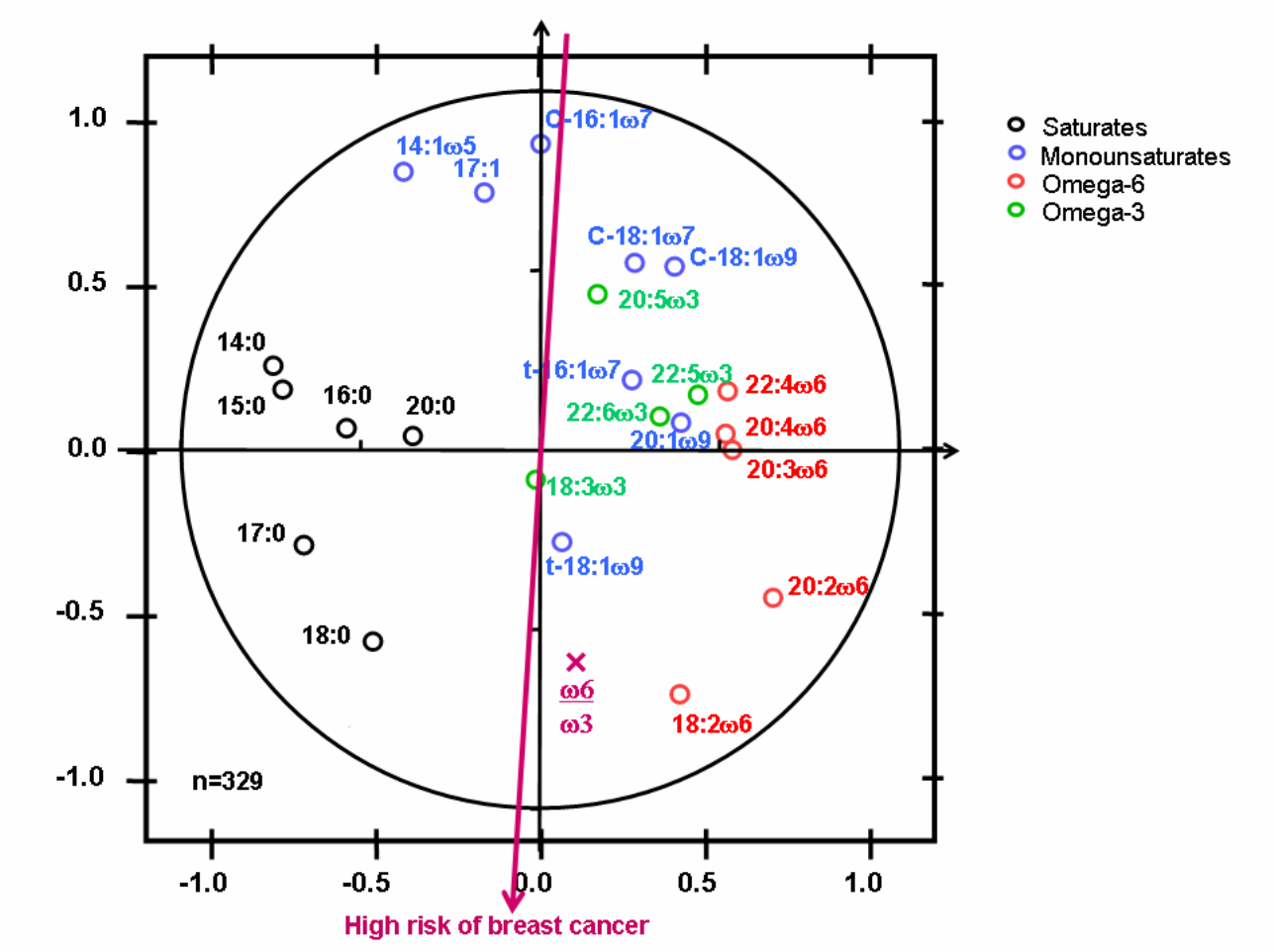
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Figure 2



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Figure 3



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Figure 4

