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Effect of Sampling Site on Fatty Acid Composition of Human Breast Adipose Tissue

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Abstract

The effect of sampling site and proximity of malignant tumor on the relative fatty acid composition of human breast adipose tissue was studied in 10 cases of breast cancer. The four anatomic quadrants of breast did not statistically significantly differ from each other in relation to any of the 30 fatty acids studied. Proximity of the malignant tumor did not affect the relative fatty acid composition of fat when compared with more distant sampling sites. Representative samples of breast adipose tissue for fatty acid composition analysis can be obtained from tissue adjacent to the tumor.

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Introduction

Animal studies have demonstrated a causal relationship between high levels of dietary fat and mammary tumors (1-5). However, the evidence generated by epidemiological studies is more equivocal: ecological studies reveal a strong correlation between dietary fat and breast cancer incidence or mortality (6-9), whereas cohort and case-control studies are only weakly suggestive of an association between dietary fat and breast cancer risk (10-14).

Dietary surveys are a reliable tool for estimating total fat intakes for group comparisons (15,16), but they measure individual intakes of various fats less accurately because of large intraindividual variation (17-20). Fatty acid composition of adipose tissue, on the other hand, has been shown to be a valid indicator of dietary fatty acid content over the preceding one to three years (20-22).

The purpose of this study was to discover whether concentrations of 30 different fatty acids vary at different anatomic locations in the breast and particularly whether fatty acid concentrations in adipose tissue samples adjacent to the tumor differ from those at more

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distant sites. This information would be most useful in prospective epidemiological studies of breast cancer risk, where only a small sample of adipose tissue from the healthy breast or from one with a benign tumor can be obtained.

Materials and Methods

Human breast adipose tissue samples were obtained from 10 ablations performed because of a malignant breast tumor. The samples were removed from the unfrozen tissue block immediately after the operation. Sampling was done by one of our authors. Adipose tissue free of gross blood and debris was taken from each breast at five anatomic locations: upper medial, upper lateral, lower medial, lower lateral quadrant, and adjacent to the tumor. After sampling, the adipose samples were immediately transported in an ice chest to the laboratory. Each was manually homogenized with a surgical knife and stored in 0.50-g portions at -50°C until chemical analysis (within 4 months of sampling).

The extraction of lipids from tissue samples (0.3–0.5 g) utilized the dry column method developed by Maxwell and co-workers (23). Lipids were extracted with a dichloromethane-methanol (9:1) solution.

The reliability of the extraction method was tested with repeated measurements of two tissue samples with different content of lipids. The coefficient of variation of the lipid recovery was 0.002 if the tissue sample contained 80% lipids and 0.012 if the lipid content was 45%.

Fatty acids were analyzed as fatty acid methyl esters, which were prepared by the alkaline transesterification method (24). Fatty acid methyl esters were separated on an OV-351 silica capillary column and were detected with a flame ionization detector in a Micromat HRGC 412 gas chromatograph (Orion Analytica, Finland). The esters were identified with a retention index monitoring technique and were quantified as area percentages of the total peak area of the fatty acid methyl esters.

Two identical analytic runs were done for each of the samples, and the final results were calculated as means of the two runs.

The reliability of the analytic runs was tested by comparing the results of the two identical runs of each sample. When the area percent of the fatty acid methyl ester was more than 10%, the coefficient of variation (CV) was less than 0.01, and when the area percent was 3–10%, the CV was less than 0.035.

Results

In terms of relative concentrations of all 30 of the fatty acids studied, samples from the four anatomic sampling sites did not differ statistically significantly from each other or from the tumor-adjacent sample. The mean levels of the quadrants not containing the tumor were thus aggregated and compared with those of the tumor-adjacent sample (Table 1).

No statistically significant differences were observed between sample concentrations adjacent to the tumor and those at the more distant quadrant sites. The results were analyzed with the Kruskal-Wallis analysis of variance.

Discussion

The fatty acid composition of adipose tissue depends on the fatty acid content of the diet (17,21,22), which implies that the fatty acid composition of adipose tissue varies between populations with dissimilar dietary habits. Therefore, study results from different populations must be compared and interpreted carefully.

Bearing in mind this effect of diet on the composition of adipose tissue, the results of this study agree very well with previous findings (22,25–35). In our investigation, a larger number

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Table 1. Relative Fatty Acid Composition of Adipose Tissue Samples Taken From Human Breasts Resected for Malignancy

Fatty Acid ^a		Quadrant Fatty Acids ^b	Tumor-Adjacent Fatty Acids ^c
Trivial name		Mean ± SEM, %	Mean ± SEM, %
10:0	Capric acid	0.2 (0.03)	0.3 (0.07)
12:0	Lauric acid	0.8 (0.04)	0.8 (0.07)
14:0	Myristic acid	5.2 (0.18)	5.1 (0.32)
14:1	Myristoleic acid	0.5 (0.03)	0.5 (0.06)
i15:0		0.1 (0.01)	0.1 (0.00)
ai15:0		0.1 (0.01)	0.1 (0.02)
15:0		0.5 (0.02)	0.5 (0.04)
i16:0		0.2 (0.01)	0.2 (0.02)
16:0	Palmitic acid	24.5 (0.25)	24.2 (0.32)
16:1 ω 9	Palmitoleic acid	0.5 (0.01)	0.5 (0.02)
16:1 ω 7		4.9 (0.26)	4.8 (0.53)
i17:0		0.3 (0.02)	0.3 (0.03)
ai17:0		0.3 (0.02)	0.3 (0.03)
17:0	Margaric acid	0.4 (0.02)	0.4 (0.03)
17:1		0.4 (0.03)	0.4 (0.04)
18:0	Stearic acid	6.7 (0.23)	6.8 (0.39)
18:1 ω 9	Oleic acid	39.4 (0.28)	39.6 (0.63)
18:1 ω 7	Vaccenic acid	3.0 (0.06)	3.0 (0.08)
18:2 ω 6	Linoleic acid	7.4 (0.62)	7.6 (1.17)
18:3 ω 3	Linolenic acid	0.6 (0.03)	0.6 (0.06)
18:4 ω 3		0.5 (0.02)	0.5 (0.05)
19:0		0.1 (0.02)	0.1 (0.03)
20:0	Arachidinic acid	0.2 (0.01)	0.2 (0.03)
20:1	Gadoleic acid	1.0 (0.06)	1.0 (0.11)
20:2 ω 6		0.1 (0.02)	0.1 (0.02)
20:3 ω 6		0.1 (0.01)	0.1 (0.02)
20:4 ω 6	Arachidonic acid	0.2 (0.01)	0.2 (0.02)
20:5 ω 3		0.1 (0.01)	0.1 (0.02)
22:5 ω 3		0.2 (0.02)	0.2 (0.05)
22:6 ω 3		0.2 (0.03)	0.3 (0.05)
SAFA		39.2 (0.53)	38.9 (0.86)
MUFA		49.6 (0.47)	49.8 (0.95)
PUFA		9.4 (0.65)	9.6 (1.20)
ω -3		1.6 (0.05)	1.6 (0.09)
ω -6		7.8 (0.64)	8.0 (1.19)

- a: Abbreviations are as follows: i, iso; ai, anteiso; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω -3, fatty acids of ω -3 series; ω -6, fatty acids of ω -6 series.
- b: Means of quadrant fatty acids are based on adipose tissue samples from 4 quadrants of breast so that in each case tumor-containing quadrant has been omitted. No. of tissue samples is 34.
- c: Means of tumor-adjacent fatty acids are based on adipose tissue samples taken from adjacent to tumor. No. of tissue samples is 10.

of fatty acids was analyzed than in the earlier studies. However, little is known of the importance of most fatty acids in human health.

All the cases in this study had a breast malignancy. The microenvironment of the breast with cancer is known to differ from normal (36). Also, the biosynthesis of lipids is known to be slightly altered in lipomas of the breast, although no essential differences from normal adipose tissue exist (26). To our knowledge there have been no reports of differences between

the composition of mammary fat and fat tissue from other parts of the body in cases of breast cancer, although two studies (26,33) found fatty acid composition of adipose tissue to be constant at different parts of the body and in different kinds of fat. Because we did not obtain adipose tissue samples from other body areas, we cannot contribute to this issue.

In this study, structural lipids were not separated from the stored lipids of adipose tissue. This also applies to most of the other studies of adipose tissue composition. Although the structural lipids have been shown to be more resistant to the effects of dietary fatty acids, their composition is also related to dietary fats (22). It can be argued that the importance of these two lipid classes in relation to malignancies is different and thus that their pooling is not justifiable. On the other hand, as long as comparison of fatty acid profiles takes place between different groups that have been studied by the same methods, pooling of the two lipid classes does not necessarily endanger the validity of the study. Pooling of structural and stored lipids would give misleading results if diet were to have an opposite effect on the concentrations of fatty acids in these two classes. However, the findings of Field and co-workers (22) do not support this possibility.

The results in this study indicate that proximity to a malignant tumor might not affect the fatty acid composition of breast adipose tissue to a great degree when compared with more distant sampling sites. A representative sample of breast adipose tissue could thus be obtained in a breast lumpectomy of practically any kind, which makes comparison of cases with different types of breast tumor possible. It also shows that even with a small adipose tissue sample from any part of the breast, a valid prospective study of risk factors for breast cancer might be feasible.

Acknowledgments and Notes

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