Fatty acid composition of adipose tissue and colorectal cancer: a case-control study

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ABSTRACT
Background: Unlike experimental results, epidemiologic studies that used dietary questionnaires were not convincing as regards the relations between dietary fatty acids (FAs) and risk of colorectal cancer (CRC). The FA composition of adipose tissue, which is considered to be an indicator of dietary intake over 2–3 y because of the slow turnover rate, appears promising but has so far been rarely used to explore the relation between CRC and exogenous or endogenously produced FAs.
Objective: In this case-control study, we aimed to investigate associations between risk of CRC and the FA composition of subcutaneous adipose tissue and product-to-precursor ratios as indexes of enzymatic activities.
Design: From 2008 to 2011, we recruited 203 cases with newly diagnosed CRC and elective surgery with a curative intent and 223 control subjects with planned abdominal surgery for benign disease and no history of CRC or polyp resection. During surgery, abdominal subcutaneous adipose tissue samples were optimally collected, stored, and analyzed by using high-performance gas chromatography. Multivariate logistic regression was used to estimate ORs for CRC in relation to individual FAs divided into tertiles according to the FA distribution in controls.
Results: After adjustment, significant positive associations with CRC risk were observed in highest compared with lowest tertiles of 16:1n−9 (OR: 1.75; 95% CI: 1.00, 3.06; P-trend = 0.045), 20:3n−6 (OR: 1.79; 95% CI: 1.01, 3.17; P-trend = 0.038), 22:5n−3 (OR: 1.82; 95% CI: 1.06, 3.12; P-trend = 0.023), and the ratio of 18:2n−6 to 18:3n−3 (OR: 2.34; 95% CI: 1.37, 3.98; P-trend = 0.001). Significant inverse associations were observed for 18:3n−3 (OR: 0.48; 95% CI: 0.29, 0.81; P-trend = 0.007). Several product-to-precursor ratios showed significant differences between cases and controls in particular ratios that reflected elongase 2/5 activity.
Conclusions: CRC patients presented higher concentrations of some FAs but lower concentrations of α-linolenic acid in their subcutaneous adipose tissue than did controls. These results may reflect both dietary patterns and altered FA metabolism but require mechanistic explorations. This study was registered at clinicaltrials.gov as NCT01966081.

Keywords biomarkers, colorectal cancer, desaturase, elongase, fatty acids

INTRODUCTION
Colorectal cancers (CRCs) are the third most-common cancers worldwide (~1.2 million cases recorded in 2008) and the fourth most-common cause of death from cancer (1). Variations in international incidence rates (2), especially in migrants (3, 4), suggested that lifestyle factors such as diet play a role in the cause of the disease, and their modification could result in the prevention of 70% of colon cancer cases (5). High intakes of red and processed meat increase risk of CRC, whereas high intakes of dietary fiber decrease it (6). Conversely, epidemiologic studies that directly linked fat intake with CRC were inconsistent (6–8). Indeed, individuals usually consume a wide range of foods, the composition of which, in different kinds of fatty acids (FAs) (each with a specific biological role), can vary according to cooking methods and industrial processing (9). The accurate and precise measurement of each FA by food-frequency questionnaires used in the majority of epidemiologic studies has been difficult, partly because food-composition tables are incomplete with regard to the diversity of FAs. Moreover, once consumed, dietary FAs are more or less absorbed and metabolized.
through enzymatic pathways, resulting in a variable individual exposure to exogenous and endogenously produced FAs. Technical advances now allow sensitive and precise analyses of an increasing number of different FAs present at very low concentrations in adipose tissue triglycerides and plasma or erythrocyte phospholipids (10, 11). These FAs can be considered biological markers of FA dietary intake and metabolism and constitute a more-objective measurement of an individual’s exposure to bioavailable FAs, irrespective of the source and quality of the food, than data from questionnaires. Because of different rates of FA turnover in these tissues (12), FA concentrations in human serum or in erythrocyte membranes reflect intakes of the preceding days or weeks (13–15), whereas FA concentrations in adipose tissue are a valid index of the habitual dietary intake over the past 2–3 y (16, 17). Therefore, to investigate the association between FA concentrations and cancer risk, adipose tissue seems particularly pertinent.

Few epidemiologic studies on CRC risk have used biomarkers of FAs measured in biological tissues possibly because of the complexity in obtaining samples. To our knowledge, 5 studies used serum, 5 studies used erythrocytes (18–23), and only 3 studies used adipose tissue with comparisons of the FA composition between tissues (23–25). These 3 studies presented inconsistent results possibly because of their limited statistical power and insufficient controls for confounders. Therefore, by using a comprehensive assessment of FAs in subcutaneous adipose tissue, our aim was to investigate the association between FA profiles and risk of CRC in a large study carried out in well-phenotyped CRC and control patients.

SUBJECTS AND METHODS

Study population

The AGARIC (acides gras polyinsaturés, métabolisme du tissu adipeux et risque de cancer colorectal) study was a case-control study conducted in 5 university hospitals located in Northeastern France (Besançon, Dijon, Nancy, Reims, and Strasbourg). Between June 2008 and June 2011, subcutaneous adipose tissue samples were obtained from cases and controls recruited by surgeons in digestive surgery departments in patients aged ≥45 y who were admitted for elective abdominal surgery.

Cases were patients with newly diagnosed primary CRC but without known distant metastases before surgery (except for a priori resectable liver metastases) who were consecutively admitted for elective surgery with a curative intent. To avoid any possible effect of radiotherapy and chemotherapy on the FA composition of tissues, CRC patients with preoperative treatments were excluded from the AGARIC study. Controls were defined as subjects free of any history of CRC or polyp resection and admitted for benign abdominal surgery or an endoscopic procedure under general anesthesia. Exclusion criteria for both groups were as follows: known familial adenomatous polyposis or hereditary nonpolyposis CRC syndrome, history of inflammatory bowel disease, another malignancy within the previous 5 y, significant changes in dietary habits within the previous 3 mo, and a serious concomitant organic or psychic disorder that would prevent the understanding of the study protocol.

We recruited 224 cases with CRC and 252 controls (105 patients with hiatus or inguinal hernia, 75 patients with incisional hernia, 45 patients with diverticulitis, and 27 patients with other benign diseases) who met the eligibility criteria. For the purpose of the current study, patients with a missing adipose tissue sample or nonanalyzable sample were excluded (21 cases and 29 controls).

The calculation of the number of subjects required was based on the assumption that a difference between cases and controls of 25–30% of the SD would be relevant. With a risk of 5% and a statistical power of 80%, the number of subjects required to detect a between-group difference of a 25% or 30% SD (bilateral test) was 252 or 175 subjects/group, respectively.

All patients provided signed informed consent. The study was carried out in accordance with the principles of the Helsinki declaration and was approved by the local ethics committee (CPP Est 1, Dijon, France) and the National Commission for Data Processing and Liberties. This study was registered at clinicaltrials.gov as NCT01966081.

Biological sample collection and measurement

In each study center, preoperative blood samples (15 mL) were collected after overnight fasting and immediately stored at 4°C. After a common protocol, samples were processed, separated into aliquots of plasma, serum, and erythrocytes, and frozen separately at −80°C within a maximal delay of 4 h. During the surgery, 2 samples of ≥50 mg of abdominal subcutaneous adipose tissue were taken by the surgeon from the area of the surgical incision in the abdominal wall. The sampling site was the periumbilical area in all cases and the majority of controls, except for controls with an inguinal hernia for whom the sampling site was the iliac fossa or the groin area. Although a recent article showed differences in structural and functional properties in 2 layers of abdominal subcutaneous adipose tissue, no attempt was made to precisely determine the sample depth by using imaging technologies because of the relatively large numbers of patients across 5 centers (26).

Samples were immediately stored at 4°C in the surgical unit and later processed and frozen at −80°C within a maximal delay of 4 h. Frozen samples were regularly transported to the central biobank (Biological Resource Center Ferdinand Cabanne BB-0033-00044) and either dispatched to 2 central biological laboratories for planned analyses (Institut national de la santé et de la recherche médicale Unité mixte de recherche 866, Dijon, France; Institut des Corps Gras) or stored at −80°C in the biobank.

All samples were analyzed by trained staff blinded to the case-control status. Control and case samples were mixed in batches of ≥50 samples. For quality control, we added, in random order, 2 aliquots of a standard pool to each batch of samples analyzed.

Total lipids were extracted from adipose tissue by using 20 vol chloroform:methanol (2:1, vol:vol) and several washes according to the method of Folch et al. (27). FA methyl esters were prepared for additional gas chromatography analysis according to the method of Kramer et al. (28), which improves the recovered quantity of conjugated linoleic acid (CLA). The FA methyl ester composition of adipose tissue was determined by using high-resolution capillary gas chromatography with a Trace Ultra chromatograph (Thermo Electron Corp.) equipped with a flame
ionization detector kept at 280°C. FA methyl esters were sepa-
rated on a capillary column (BPX 120, 120-m × 0.25-mm i.d.,
0.25-μm film thickness; SGE Ltd.) by using helium as the
carrier gas (inlet pressure: 120 kPa). The split ratio was 1:70.
The column temperature was first fixed at 170°C for 55 min,
programmed from 170 to 225°C at 2°C/min, and held at 225°C
until completion of the analysis (135 min). The injection port
was maintained at 250°C. Gas chromatography peaks were in-
tegrated by using an SP 4400 integrator (Spectra Physics).

The peak identification and quantitative precision were eval-
uated by using weighted individual and model mixtures of known
FA methyl esters. A total of 33 FAs were identified and expressed
as a percentage of total FAs. Interassay CVs were, on average, 5% 
and 10% for FAs present at concentrations ≥1% and <1%,
respectively.

Blood glucose was measured on a Vista Dimension analyzer
(Siemens Healthcare Diagnostics) with dedicated reagents
(hexokinase and bromocresol green). Ultrasensitive C-reactive
protein, albumin, and prealbumin were quantified by using
immunonephelometry. Insulin was quantified by using a chem-
iluminesent method on an Immulite analyzer (Siemens).

Collection of clinical data

In each study center, the same medical staff and dedicated
research assistants collected the following clinical data from
surgical and pathological reports for both cases and controls:
cancer site, invasion depth [pathologic T factor of the American
Joint Committee on Cancer Tumor Node Metastasis classifica-
tion, maximal tumor size, lymph node metastasis, and distant
metastasis. The presurgical treatment (transfusion, anticoagulant
drugs, and antiplatelet drugs) was also recorded. The following
measurements were assessed in the preoperative period: systolic
and diastolic blood pressures, weight, and waist and hip cir-
cumferences in standing patients breathing normally. Height and
weight histories were reported by patients.

In each center, the same research assistant interviewed cases
and controls about their personal medical history, family history
of CRC, marital status, educational level, physical activity,
smoking, and alcohol consumption. Medications and dietary
supplements during the month preceding the inclusion were
obtained from reports of anesthesiologists and prescriptions from
physicians and completed by interviews with patients.

Alcohol intake was divided into the following 3 categories
according to the approximate median in drinkers: no alcohol
intake, <5 drinks/wk (reference category), and ≥5 drinks/wk.
Smoking status was classified into 3 categories as never smoker,
former smoker, and current smoker. Leisure time physical ac-
tivity (e.g., walking, biking, sports activities, do-it-yourself ac-
tivities, and gardening) was defined as high if patients had an
intensive physical activity (leading to sweating or breathless-
ness) ≥2 h/wk, low if patients had no intensive physical activity
or only moderate activity (not leading to sweating or breath-
lessness) <1 h/wk, and medium in other situations. BMI (in kg/
$m^2$) was calculated as weight divided by height squared. A high
waist-to-hip ratio (WHR) was defined according to the classi-
fications of the WHO as >0.85 for women and >0.90 for men.
Type 2 diabetes was defined as self-reported, a physician’s di-
agnosis of diabetes, or fasting plasma glucose ≥126 mg/dL, or
current treatment of diabetes. The HOMA-IR was calculated as

\[
\text{Fasting glucose (mmol/L) × fasting insulin (mU/L)} ÷ 22.5
\]

Waist and hip circumferences were not available for 11% of
patients. Missing values were handled for men and women
separately with the use of conditional mean imputation by linear
regression with age, smoking status, alcohol consumption, sys-
tolic blood pressure, HOMA-IR, triglyceride, cholesterol, recent
weight loss, and BMI as predictor covariates. The distribution
of WHRs and their correlations with other variables were preserved
after imputation.

In addition to individual FAs, we calculated adipose tissue
concentrations of total SFAs, MUFAs, and total n–3 and n–6
PUFAs. The ratio of n–6 to n–3 PUFAs was also determined.
The FA composition of biological tissues is influenced by the
diet but also by enzymes involved in the elongation or desatu-
ration of FAs. The enzymatic activities were estimated by
product-to-precursor FA ratios. The ratio of 20:4n–6 to 20:3n–6
reflects δ5-desaturase activity and 18:3n–6:18:2n–6 reflects δ6-
desaturase activity. The ratio of 20:3n–6 to 18:2n–6 is an indicator of combined δ6-desaturase and elongase activity.
Elongase 2/5 activity was estimated by the ratios of 22:4n–6 to
20:4n–6 and 22:5n–3 to 20:5n–3. Adipose tissue FA desatura-

d indexes (desaturation index for n–7 equals the ratio of
16:1n–7 to 16:0, and the desaturation index for n–9 equals the
ratio of 18:1n–9 to 18:0) were calculated as an indicator of the
activity of the rate-limiting enzyme stearoyl-CoA desaturase
(SCD). SCD-1, which is the main isoform in humans, transforms
some saturated FAs into MUFAs (29, 30). The ratio of 16:1n–9

to 18:1n–9 is an indicator of the peroxisomal β-oxidation level.

Statistical methods

Continuous variables were expressed as medians with their
IQRs and categorical variables as percentages. Univariate
comparisons between cases and controls were performed by
using chi-square or Fisher’s exact tests, when appropriate, for
qualitative variables and the Kruskal-Wallis test for quantitative
variables.

To investigate associations between case-control status and
adipose tissue concentrations of FAs, unconditional logistic re-
gression analyses stratified by study center were used. Individual
FAs, ratios, or sums were divided into tertiles on the basis of the
distribution in controls. ORs and 95% CIs were calculated by
using the lowest tertile as the reference category. Tests for linear
trends across tertiles of FAs were performed by assigning the
median value to each tertile and modeling this as a continuous
variable in separate regression models.

Models were systematically adjusted for age, sex, BMI, family
history of CRC, and alcohol intake. To select other potential
confounding factors, all characteristics associated with CRC with
\( P < 0.20 \) in the univariate analysis were tested, and \( P < 0.10 \)
was chosen to keep them in the final model. WHR, prealbumin,
recent weight-loss, and regular statin use in the past month were
retained after backward selection. In a second stage, diabetes,
which is a known risk factor for CRC, was forced into the
model.

In a sensitivity analysis, 47 cases and 3 controls were excluded
because they had received oral supplementation during the week.
before digestive surgery with an immunutrition (Oral Impact; Nestlé). In addition to amino acids, carbohydrates, ribonucleic acids, minerals, and vitamins, one 74-g sachet of this supplementation contains 8.3 g fats, of which there are 4.6 g saturated fats, and 1 g EPA and DHA. We also conducted sensitivity analyses without controls enrolled for surgery because of diverticulitis and without patients with recent weight loss (46 cases and 26 controls) consecutively.

All statistical analyses performed with SAS software release 9.3 (SAS Institute Inc.) were 2-sided, and \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Table 1 gives baseline characteristics of 426 patients involved in the present study (203 CRC cases and 223 controls with available adipose tissue samples). Cases were more likely than controls to have a high WHR \( (P = 0.03) \), have experienced weight loss >5 kg during the previous 3 mo \( (P = 0.002) \), and be heavy drinkers \( (P = 0.01) \). Cases also showed lower blood concentrations of prealbumin and albumin and a higher C-reactive protein concentration \( (P < 0.001) \). Although differences were NS, CRC patients tended to be older \( (P = 0.09) \) and present a lower median BMI \( (P = 0.09) \) than did controls. A family history of CRC \( (P = 0.08) \) and statin use \( (P = 0.12) \) also tended to be more frequent in CRC patients. Sex distribution, waist and hip circumferences, smoking status, physical activity, and the use of aspirin or nonsteroidal anti-inflammatory drugs did not differ significantly between cases and controls.

Of 203 cases with CRC, 44 patients (21.7%) had tumors located in the rectum, 76 patients (37.4%) had tumors located in left colon, 75 patients (37%) had tumors located in right colon, and 8 patients (3.9%) had tumors located in multiple locations. The distribution of Tumor Node Metastasis classification stage was 32% of patients with stage I or in situ CRC \( (n = 65) \), 34% of patients with stage II \( (n = 69) \), 27.1% of patients with stage III \( (n = 55) \), and 6.9% of patients with stage IV \( (n = 14) \).

Mean percentages of adipose tissue FAs in cases and control patients are presented in Table 2. MUFAs accounted for the

### TABLE 1

Baseline characteristics of colorectal cancer cases and controls in the AGARIC study

<table>
<thead>
<tr>
<th></th>
<th>Cases ( (n = 203) )</th>
<th>Controls ( (n = 223) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>69.5 (59.8–75.8(^2))</td>
<td>66.8 (58.1–75.6)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Women, n (%)</strong></td>
<td>75 (37.0)</td>
<td>97 (43.5)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>BMI, kg/m(^2)</strong></td>
<td>26.2 (23.5–30.1)</td>
<td>27.1 (24.4–30.8)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>98.5 (90.5–109.0)</td>
<td>98.0 (89.0–108.0)</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Hip circumference, cm</strong></td>
<td>104.0 (96.0–111.0)</td>
<td>104.0 (97.0–111.5)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>High waist-to-hip ratio, n (%)</strong></td>
<td>170 (83.7)</td>
<td>168 (75.3)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Recent loss of weight, n (%)</strong></td>
<td>46 (22.7)</td>
<td>26 (11.7)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Years of education, n (%)</strong></td>
<td>&lt;12</td>
<td>95 (46.8)</td>
<td>91 (40.8)</td>
</tr>
<tr>
<td><strong>Family history of CRC, n (%)</strong></td>
<td>26 (12.8)</td>
<td>21 (9.4)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Alcohol intake, n (%)</strong></td>
<td>37 (18.2)</td>
<td>43 (19.3)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Smoking status, n (%)</strong></td>
<td>62 (30.5)</td>
<td>96 (43.1)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Physical activity, n (%)</strong></td>
<td>104 (51.2)</td>
<td>84 (37.7)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Low intensity</strong></td>
<td>61 (30.1)</td>
<td>57 (25.6)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Medium intensity</strong></td>
<td>97 (47.8)</td>
<td>123 (55.2)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>High intensity</strong></td>
<td>45 (22.2)</td>
<td>43 (19.3)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Statin use</strong></td>
<td>46 (22.7)</td>
<td>46 (20.6)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>62 (30.5)</td>
<td>53 (23.8)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Glycemia(^4)</strong></td>
<td>45 (22.2)</td>
<td>38 (17.0)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>5.0 (4.5–5.9)</td>
<td>5.0 (4.5–5.6)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/L</strong></td>
<td>6.9 (0.4–2.1)</td>
<td>0.9 (0.4–2.0)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Prealbumin, g/L</strong></td>
<td>4.5 (1.5–12.4)</td>
<td>2.0 (0.9–5.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Albumin, g/L</strong></td>
<td>0.2 (0.2–0.3)</td>
<td>0.3 (0.2–0.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\)Chi-square tests or Fisher-exact tests were used for categorical variables. The Kruskal-Wallis test was used for quantitative variables. AGARIC, acides gras polyinsaturés, métabolisme du tissu adipeux et risque de cancer colorectal; CRC, colorectal cancer.

\(^2\)Median; IQR in parentheses (all such values).

\(^3\)Twenty-six missing values.

\(^4\)Five missing values.

\(^5\)Eight missing values.
largest proportion in the adipose tissue in both control and CRC group. In both groups, the 5 most-abundant individual FAs were 18:1n-9 (oleic acid), which represented >45%, 16:0 (palmitic acid), which represented ~23%, 18:2n-6 [linoleic acid (LA), which was the most-abundant PUFA], which represented 11%, 16:1n-7 (palmitoleic acid), which represented 5%, and 18:0 (stearic acid), which represented 4.3%.

Overall, associations between FA concentrations in adipose tissue and CRC risk were only marginally affected by adjustment (Table 3). The only exception was the disappearance of the inverse association with 14:0 (myristic acid) and the 2 trans FAs 18:1 9t and 18:1 11t (elaidic and vaccenic acids, respectively) in multivariate analyses. SFAs were not associated with CRC risk. In MUFAs, adipose tissue concentrations of 16:1n-9c18 were significantly higher in cases than controls [for highest compared with lowest tertiles, i.e., OR tertile 3 compared with tertile 1 (ORT3vsT1): 1.75; 95% CI: 1.00, 3.06; P-trend = 0.045]. No association was shown with the 18:2 9c11n-7 (CLA) and the sum of n-6 PUFAs. However, a positive association was observed between CRC risk and 20:3n-6 (di-homo-γ-linolenic acid) (ORT3vsT1: 1.79; 95% CI: 1.01, 3.17; P-trend = 0.038) and 22:4n-6 (ORT3vsT1: 1.76; 95% CI: 0.99, 3.14; P-trend = 0.055) although the significance was borderline. The sum of n-3 PUFAs tended to be lower in cases than controls. The essential FA of the n-3 family, 18:3n-3 (α-linolenic acid; ALA), was inversely associated with CRC risk (ORT3vsT1: 0.48; 95% CI: 0.29, 0.81; P-trend = 0.045). In contrast, cases had accumulated more 22:5n-3 (docosapentaenoic acid) in their adipose tissue than did controls (ORT3vsT1: 1.82; 95% CI: 1.06, 3.12; P-trend = 0.023). The ratio of 18:2n-6 to 18:3n-3 (LA:ALA), which are n-6 and n-3 essential FAs, was clearly higher in cases than controls (ORT3vsT1: 2.34; 95% CI: 1.37, 3.98; P-trend = 0.001). Finally, some markers of enzymatic function showed differences between cases and controls. Elongase 2/5 activity estimated with the ratio of 22:4n-6 to 20:4n-6 and 22:5n-3 to 20:5n-3 was positively associated with CRC risk. ORs of lowest compared with highest tertiles were as follows: ORT3vsT1 of 1.62 (95% CI: 0.95, 2.76; P-trend = 0.039) and ORT3vsT1 of 1.96 (95% CI: 1.14, 3.36; P-trend = 0.026), respectively. This elevated enzymatic activity combined with the δ-6-desaturase activity was also highlighted by a higher 20:3 n-6 to 18:2 n-6 ratio in CRC cases (ORT3vsT1: 2.43; 95% CI: 1.37, 4.31; P-trend = 0.003). The association between CRC risk and peroxisomal β-oxidation (estimated by the ratio of 16:1n-9 to 18:1n-9) did not reach the significance level after adjustment (ORT3vsT1: 1.71; 95% CI: 0.97, 2.99; P-trend = 0.09).

Additional adjustment for diabetes did not affect these associations except for the positive association with 22:4n-6, which exceeded the significance level (ORT3vsT1: 1.92; 95% CI: 1.09, 3.38; P-trend = 0.023). The inverse association between 18:3n-3 (ALA) and CRC risk remained significant after adjustment for 18:2 9c11n-7 (CLA) as a proxy of red meat intake (ORT3vsT1: 0.46; 95% CI: 0.27, 0.79; P-trend = 0.005).

The exclusion of patients with immunornutrition (Oral Impact) confirmed the associations between adipose tissue FA concentrations and CRC risk presented in Table 3. The inverse association with δ5-desaturase activity (ratio of 20:4n-6 to 20:3n-6) exceeded the significance level (ORT3vsT1: 0.57; 95% CI: 0.33, 1.00; P-trend = 0.039) as did the negative effect of 22:4n-6 (ORT3vsT1: 2.16; 95% CI: 1.15, 4.06; P-trend = 0.016). In contrast, associations with the sum of trans FAs and with 16:1n-9 were no longer significant [ORT3vsT1 of 0.59 (95% CI: 0.31, 1.12; P-trend = 0.111) and ORT3vsT1 of 1.57 (95% CI: 0.86, 2.88; P-trend = 0.117), respectively].

The exclusion of control patients with diverticulitis did not change the associations described in Table 3 except for the positive associations between CRC risk and 20:3n-6 and the activity index of elongase 2/5 (ratio of 22:5n-3 to 20:5n-3), which no longer reached the significance level [ORT3vsT1 of 1.77 (95% CI: 0.96, 3.29; P-trend = 0.058) and ORT3vsT1 of 1.84 (95% CI: 1.03, 3.30; P-trend = 0.067), respectively]. The exclusion of patients with recent weight loss did not affect the results presented in Table 3.

DISCUSSION

In this case-control study, subcutaneous adipose tissue concentrations of 16:1n-9, 20:3n-6, and 22:5n-3 were significantly higher in CRC patients than controls after adjustment for known risk factors. In contrast, we showed a lower concentration of 18:3n-3 and a higher value for the ratio of essential PUFAs (18:2n-6 to 18:3n-3). The calculated ratios, regarded as putative indexes of 6-desaturase and elongase 5 activity, were positively associated with CRC risk. Although differences in FA
## Table 3

ORs (95% CIs) for colorectal cancer according to tertiles of adipose tissue fatty acids (percentage of total fatty acids) in 203 cases and 223 controls.

<table>
<thead>
<tr>
<th>Fatty acid name</th>
<th>Total FAs, %</th>
<th>Cases/controls, n</th>
<th>OR (95% CI)</th>
<th>P-trend OR (95% CI)</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SFAs</strong></td>
<td></td>
<td></td>
<td>Crude Adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of SFAs</td>
<td>16.44–29.87</td>
<td>86/74</td>
<td>1.00 (reference)</td>
<td>0.204</td>
<td>1.00 (reference)</td>
</tr>
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<td>14:0 Myristic acid</td>
<td>0.96–2.67</td>
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<td>0.67 (0.40, 1.12)</td>
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<td>15:0 + 17:0</td>
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<td>16:0 Palmitic acid</td>
<td>13.2–22.19</td>
<td>80/74</td>
<td>0.60 (0.38, 0.97)</td>
<td>0.045</td>
<td>0.67 (0.40, 1.12)</td>
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<td>18:0 Stearic acid</td>
<td>3.85–4.77</td>
<td>67/75</td>
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<td>Sum MUFAs</td>
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<td>16:1n–7 Palmitoleic acid</td>
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<td>16:1n–9 Oleic acid</td>
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<td>18:1n–7 Conjugated linoleic acid</td>
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<td>18:2 9c11t n–7 Conjugated linoleic acid</td>
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<td>20:3 n–6 Di-homo-γ-linolenic acid</td>
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<td>20: 4 n–6 Arachidonic acid</td>
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(Continued)
### TABLE 3 (Continued)

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<th>Fatty acid name</th>
<th>Total FAs, %</th>
<th>Cases/controls, n,n</th>
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<th>P-trend</th>
<th>OR (95% CI)</th>
<th>P-trend</th>
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<tr>
<td>Docotetraenoic acid</td>
<td>0.150–0.221</td>
<td>66/75</td>
<td>1.35 (0.83, 2.22)</td>
<td>1.33 (0.76, 2.32)</td>
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<td>22:4 n–6</td>
<td>0.222–0.58</td>
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<td>1.00 (reference)</td>
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<td>1.33 (0.76, 2.32)</td>
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<td>1.76 (0.99, 3.14)</td>
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</tr>
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concentrations expressed as a percentage of total FAs in samples seemed negligible, they were close to those shown in the literature (31) and could have represented a nonnegligible quantity at the level of an individual. A small variation in adipose tissue storage could correspond to greater differences in the amount of FAs consumed, metabolized, or converted into biologically active molecules.

Very few studies have investigated the association between different adipose tissue FA concentrations and risk of tumors, mostly of breast (32–35) and prostate cancers (36, 37). This lack can be explained by the great difficulty in obtaining such samples in controls, even in a hospital setting. To our knowledge, only 3 studies concerned CRC, but their small sample sizes prevented any adjustment for confounding factors. Neoptolemos et al. (24) showed no significant differences between 41 CRC patients matched with 34 controls for the 7 FAs investigated, whereas Okuno et al. (23) only showed a higher concentration of arachidonic acid in cases. However, they selected patients without body weight loss, diabetes, or hyperlipidemia and without n–3 PUFA supplements or statins. In our study, patients with such characteristics were included, and statistical analyses were adjusted for these factors. Recently, Giuliani et al. (25) observed a lower concentration of 18:3n–3 but higher concentration of 20:3n–6 in colon cancer patients than controls. These 3 previous studies, however, did not consider putative markers of the endogenous enzymatic production of some FAs.

The higher concentration of 20:3n–6 (di-homo-γ-linolenic acid) we showed in CRC patients has previously been reported in advanced prostate cancer (38), gastric adenocarcinoma (39), CRC (18, 25), and colorectal adenoma (40). The main dietary source of 20:3n–6 is oil seeds, but its concentration is probably increased by the great difficulty in obtaining such samples in controls, even in a hospital setting. To our knowledge, only 3 studies concerned CRC, but their small sample sizes prevented any adjustment for confounding factors. Neoptolemos et al. (24) showed no significant differences between 41 CRC patients matched with 34 controls for the 7 FAs investigated, whereas Okuno et al. (23) only showed a higher concentration of arachidonic acid in cases. However, they selected patients without body weight loss, diabetes, or hyperlipidemia and without n–3 PUFA supplements or statins. In our study, patients with such characteristics were included, and statistical analyses were adjusted for these factors. Recently, Giuliani et al. (25) observed a lower concentration of 18:3n–3 but higher concentration of 20:3n–6 in colon cancer patients than controls. These 3 previous studies, however, did not consider putative markers of the endogenous enzymatic production of some FAs. Supported by our results that showed an increased concentration of the other surrogate marker of elongase 2/5 activity, i.e., the 22:4n–6 to 20:4n–6 ratio, in CRC cases. Although the approach of enzymatic activities by the product-to-precursor ratio is attractive and informative, it is difficult to disentangle exogenous sources and endogenous metabolism. Additional experimental studies are necessary to show the influence of enzyme activities on colorectal carcinogenesis.

n–3 PUFAs are considered protective nutrients, although some studies yielded disturbing positive associations between ALA and cancers (mainly prostate cancer) (42–45). Regarding CRC, consistent inverse associations with ALA, as in our study, have been shown in previous studies that used biomarkers of FA intake (19, 22, 23). The mechanisms put forward to explain the protective role of n–3 PUFAs are decreased bile acid excretion, the activation of peroxisome proliferator activated receptor α and γ, decreased nuclear transcription factor κB activity, the inhibited production of cicosanoids, altered protein kinase C activity, inhibition of ornithine decarboxylase, and decreased nitric oxide production (46, 47). Another explanation often given is the competition of n–3 and n–6 PUFAs for the same enzymes and transport systems, e.g., elongase 5 and cyclooxygenase 2. The elevated ratio of 18:2n–6 to 18:3n–3 we showed in CRC cases is in agreement with these hypotheses. This ratio between the 2“essential PUFAs suggests a dietary imbalance, which has been largely pinpointed in the worrying increasing incidence of obesity and cardiovascular disease. Thus, given the influence of PUFAs on inflammation through membrane fluidity, lipid raft formation, and receptor function (48, 49), an altered ratio of n–3 to n–6 PUFAs can markedly alter the tissue composition and functions in humans. However, how such PUFAs mechanisms are involved in the associations observed between specific FA storage in subcutaneous adipose tissue and carcinogenesis remains an interesting domain to explore.

Our results showed a higher concentration of 16:1n–9 in CRC cases, which contrasts with the lack of any difference for 18:1 n–9 mainly brought by the diet. Abundant amounts of MUFA are present in cancer cells (50). The expression of SCD-1, which is an enzyme that transforms SFAs into MUFA shown in adipose tissue (18:1n–9 and 16:1n–9), is elevated in several human cancers (51, 52). However, the present study showed that CRC risk was not related to surrogate markers of SCD-1 activity but markers of peroxisomal β-oxidation. Therefore, we can hypothesize

### TABLE 3 (Continued)

<table>
<thead>
<tr>
<th>Fatty acid name</th>
<th>Total FAs, %</th>
<th>Cases/controls, n,M</th>
<th>OR (95% CI)</th>
<th>P-trend</th>
<th>OR (95% CI)</th>
<th>P-trend</th>
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<td>22:5n–3 to 20:5n–3</td>
<td>Elongase 2/5</td>
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<td>0.416–0.511</td>
<td>52/75</td>
<td>0.95 (0.58, 1.58)</td>
<td>0.015</td>
<td>1.00 (0.47, 1.41)</td>
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<tr>
<td>0.512–1.21</td>
<td>96/75</td>
<td>1.72 (1.07, 2.76)</td>
<td>0.026</td>
<td>1.00 (0.95, 2.76)</td>
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<td>0.46–3.2436</td>
<td>41/74</td>
<td>1.00 (reference)</td>
<td>0.005</td>
<td>1.00 (reference)</td>
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<td>3.2439–5.14</td>
<td>72/74</td>
<td>1.71 (1.03, 2.84)</td>
<td>0.029</td>
<td>1.61 (0.94, 2.78)</td>
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<tr>
<td>5.15–74.5</td>
<td>90/75</td>
<td>1.99 (1.22, 3.27)</td>
<td>0.024</td>
<td>1.96 (1.14, 3.36)</td>
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</table>

1ALA, α-linolenic acid; DPA, docosapentaenoic acid; SCD-1, enzyme stearoyl-CoA desaturase 1.
2Unconditional logistic regression models were stratified by study center and adjusted for age, sex, BMI, family history of colorectal cancer, alcohol intake, waist-to-hip ratio, prealbumin, recent weight loss, and statin use.
3Ranges (all such values).
that increased CRC risk linked to a high concentration of 16:1n–9 in adipose tissue could be mostly related to β-oxidation of 18:1n–9 in the peroxisome rather than to synthesis from 16:0 via SCD-1.

Because the origin of trans FAs varies according to isomers, i.e., 18:1n–11t (vaccenic acid), which is naturally present in fat from ruminant animal meat and milk, and 18:1n–9t (elaidic acid), which is unnaturally synthesized in industrially hydrogenated fats (53, 54), it is important to distinguish isomers of trans FAs in a nutritional epidemiologic study. In the current study, the association between CRC risk and trans FAs 18:1n–9t or 18:1n–11t disappeared after adjustment. Indeed, the WHR and alcohol intake were strongly associated with concentrations of elaidic or vaccenic acid in adipose tissue.

Several limitations of the current study should be considered. First, CRC patients recruited in university hospitals may not be representative of all CRC patients. The small number of patients with advanced CRC or with rectal cancer could explain the absence of any difference in FA concentrations according to tumor or cancer location (data not shown). Controls could have had CRC at an asymptomatic stage. However, such a misclassification would have led to an underestimation of the associations identified. We could not exclude that some of the results were due to chance alone because of the high number of tests performed. Moreover, we were unable to adjust our results for total energy intake or the consumption of specific foods. Last, the case-control design did not allow temporal links to be established, although the use of biomarkers of long-term intake (2–3 y) counterbalanced this point.

In contrast, this study had the advantage of including relatively large samples of well-characterized cases and controls, which allowed potential confounders to be taken into account. The results relied on a centralized and blinded analysis by a specialized lipidomic laboratory of blood samples stored in optimal conditions. The major strength was the use of adipose tissue FAs as biomarkers of both long-term dietary intake and endogenous metabolism. In particular, the investigation of surrogate markers of specific enzymatic activities highlighted the importance of studying enzymatic dysfunction in CRC risk.

In conclusion, we observed a different FA profile in subcutaneous adipose tissue of CRC patients than controls. Although this result suggests a possible effect of unbalanced dietary intake, changes in FA metabolism, and adipose tissue storage in carcinogenesis, no causal links to CRC can be made at this stage. Mechanisms that underlie fat storage and enzymatic activities in adipose tissue in relation to carcinogenesis need to be explored more thoroughly in experimental studies.

We thank the members of the AGARIC Study Group for their contribution to the inclusion of patients, data collection and biological analyses. Members of the AGARIC study group are as follows—Coordinating center: A Felin and E Niot and L Loiodice (Dijon-F), N Combe and C Vaysse (ITERG, Bordeaux-F). Local research assistant staff: M-L Asensio (Inserm CIE 1, Dijon-F); D Da Costa-Souihel (Inserm CBT 506, Besançon); N Valentin (Inserm CIE 6, Nancy-F); F Hardy (CHU, Reims-F); N Derrijid-Ait Younes, G Larderet, E Richer (CHU, Strasbourg-F).

The authors’ responsibilities were as follows—CB-K and NC: study concept and design; CV, NC, M-LS, PO-D, ZL, J-BD, and SD-L: acquisition of data; VC: statistical analysis; VC, CV, NC, and CB-K: interpretation of data; VC and CB-K: drafting of the manuscript; and all authors: critical revision of the manuscript and reading and approval of the final manuscript. None of the authors reported a conflict of interest related to the study. Sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript.

REFERENCES


