Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease1–3

Qi Sun, Jing Ma, Hannia Campos, and Frank B Hu

ABSTRACT

Background: The relation between dairy product intake and the risk of ischemic heart disease (IHD) remains controversial.

Objective: We aimed to explore biomarkers of dairy fat intake in plasma and erythrocytes and to assess the hypothesis that higher concentrations of these biomarkers are associated with a greater risk of IHD in US women.

Design: Among 32 826 participants in the Nurses’ Health Study who provided blood samples in 1989–1990, 166 incident cases of IHD were ascertained between baseline and 1996. These cases were matched with 327 controls for age, smoking, fasting status, and date of blood drawing.

Results: Among controls, correlation coefficients between average dairy fat intake in 1986–1990 and 15:0 and trans 16:1n−7 content were 0.36 and 0.30 for plasma and 0.30 and 0.32 for erythrocytes, respectively. In multivariate analyses, with control for age, smoking, and other risk factors of IHD, women with higher plasma concentrations of 15:0 had a significantly higher risk of IHD. The multivariate-adjusted relative risks (95% CI) from the lowest to the highest tertile of 15:0 concentrations in plasma were 1.0 (reference), 1.20 (1.20, 3.98), and 2.36 (1.16, 4.78) (P for trend = 0.03). Associations for other biomarkers were not significant.

Conclusions: Plasma and erythrocyte contents of 15:0 and trans 16:1n−7 can be used as biomarkers of dairy fat intake. These data suggest that a high intake of dairy fat is associated with a greater risk of IHD.


KEY WORDS

Dairy fat, plasma, blood cells, coronary disease, women

INTRODUCTION

Consumption of whole milk and other high-fat dairy products, such as cheese and butter, has been postulated to be associated with the risk of ischemic heart disease (IHD) because of the high saturated fat content (1). Although strong positive associations between the intake of dairy products with high fat content and IHD mortality have been indicated in ecologic studies (2–4), prospective cohort studies showed mixed results for these associations (5). Dairy products are complex with respect to their nutrient composition. Besides fat, dairy products also contain many nutrients, such as calcium, that may play different roles in the development of IHD (1). Dairy fat intake could be a more specific risk factor for IHD, because it is the fat content of dairy products that is suggested to be detrimental to coronary health. Few epidemiologic studies of the association between dairy fat intake and the risk of IHD have been published (6, 7). Measuring dairy fat intake can be challenging to epidemiologic studies that use food-frequency questionnaires (FFQs) and similar methods to estimate nutrient intake, because dairy fat exists in many foods, and fat content varies within these foods (8).

Biological markers are believed to be more objective than FFQs, because biomarkers do not rely on the precision of food composition databases and self-reports or the appropriateness of FFQ items (9, 10). So far, only a few studies have been conducted to determine valid biomarkers of dairy fat intake. The preliminary results showed that fatty acids (FAs) with odd-number carbon chains—ie, 15:0 and 17:0—in human adipose tissue may be used as biomarkers of dairy fat intake (11, 12). The possibility that other FAs exclusively existing in ruminant fats, such as certain trans isomers, could be used as valid biomarkers has not been assessed. Even fewer studies have been conducted to explore the associations between these biomarkers and the risk of IHD. In the current study, we evaluated the validity of odd-numbered FAs and explored other FAs in plasma and erythrocytes as biomarkers of dairy fat intake. We further examined the associations between these biomarkers and the risk of IHD in a prospective case-control study nested in the Nurses’ Health Study cohort.

SUBJECTS AND METHODS

Study population and blood collection

In 1976, >120 000 female registered nurses aged 30–55 y were enrolled in the Nurses’ Health Study. In 1989–1990, blood samples were provided by 32 826 participants. Ninety-seven percent of the samples arrived within 24 h after blood drawing. Immediately on arrival, the samples were centrifuged (1200 g, 15 min, room temperature) and divided into aliquots of plasma.
erythrocytes, anduffy coat fractions, which were then stored in liquid nitrogen freezers at \(-130 \, ^\circ C\) or colder until analysis.

Participants who provided a blood sample and who were free of diagnosed cancer and cardiovascular disease at the time of blood drawing were eligible for the current study. Among these participants, 167 cases of nonfatal myocardial infarction (MI) or IHD death were newly diagnosed between the time of blood drawing and June 1996. For each IHD case, 2 control subjects were randomly selected from the participants who were free of IHD at the time of the case’s IHD diagnosis. Cases and controls were matched for age (±1 y), smoking status (never, past, or current), fasting status at blood drawing (fasting for 10 h or not fasting), and the date of the blood drawing. One case and 7 controls had missing data on FA concentrations were missing for 1 case and 7 controls because of contamination, dilution, or loss of blood samples. After exclusion of these participants, 166 cases and 327 controls were available for analysis.

The study protocol was approved by the Institutional Review Board of the Brigham and Women’s Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

Assessment of ischemic heart disease

Nurses who reported a nonfatal MI in the biennial follow-up questionnaires were asked to provide their medical records, which were then reviewed by study physicians who were blinded as to the exposure status of participants. Telephone interviews were conducted if medical records were not provided by the nurses. Nonfatal MI was confirmed if the World Health Organization criteria, which require typical symptoms plus either diagnostic electrocardiographic findings or elevated cardiac enzyme concentrations, were met (13). We identified deaths among the Nurses’ Health Study participants through reports from the next of kin or postal authorities or by searching the National Death Index. At least 98% of deaths were identified (14). IHD deaths were identified if IHD was listed as the cause of death in hospital records, in autopsy reports, or on death certificates. IHD deaths were then confirmed by a previous report of IHD and by a determination that another, more apparent or plausible cause of death was not stated. We included only confirmed IHD cases in the current analysis. Total IHD was defined as nonfatal MI plus fatal IHD.

Assessment of diet

Food and nutrient consumption assessed by semiquantitative FFQs in 1986 and 1990 was primarily used in the present study to represent the usual intake. A detailed description of the validity of the FFQs used in the Nurses’ Health Study and a discussion of the FFQs’ validity were published elsewhere (10, 15). Briefly, these FFQs inquired about the consumption of major dairy foods and other foods in the previous year. For each food item, the frequency of the consumption of that food in a standard portion size was asked. There were 9 possible coding responses, ranging from “never or <1 time/mo” to “≥6 times/d.” The major dairy foods inquired about in these FFQs included skim milk, whole milk, cream, sour cream, sherbet, yogurt, ice cream, cheese, and butter. Dairy fat intake was calculated by multiplying the frequency of consumption of dairy foods by the fat content in the specified amount. Then, the contributions across all foods were summed to represent total dairy fat intake, taking into account the dairy fat intake from other foods in which milk was an ingredient, such as milk chocolate, white bread, home-made bakery products, and other foods. The food composition database was primarily based on US Department of Agriculture publications (16), which were supplemented by data from other publications and individual laboratories (17–19). The intakes of other FAs were also derived by using the same method. Fourteen participants had missing dietary intake. Therefore, when we evaluated biomarkers in relation to dietary intake, we excluded these participants.

Plasma and erythrocyte fatty acid analysis

FA concentrations in plasma and erythrocytes were analyzed by gas-liquid chromatography in 2000 and 2002. A detailed description of the laboratory process has been published elsewhere (12). The concentration of each FA was expressed as a percentage of the total FA content in plasma or erythrocytes. Technicians and laboratory personnel were blinded as to the disease status of the participants. Blood samples from each case-control triplet was shipped in the same batch and analyzed in the same run. Each triplet’s samples were assayed by the same technicians in a random sequence and under identical conditions. Laboratory control samples were tested along with the case-control samples.

We quantified 37 FAs in plasma and erythrocytes that had meaningful (ie, >0.01% of total) concentrations. In the current study, we reported FAs that are derived exclusively from ruminant sources and that cannot be synthesized by human body (ie, 15:0, 17:0, and trans 16:1n–7) (20, 21) and other FAs that exist in substantial amounts in dairy fat (ie, 14:0, 16:0, and 18:0) (6). The major trans isomer existing in dairy fat is trans 18:1n–7 (vaccenic acid), and it therefore was also included in the analysis. The intraassay CV was 11.1% (plasma) or 19.3% (erythrocytes) for 14:0 (myristic acid); 4.3% (plasma) or 8.8% (erythrocytes) for 15:0; 1.4% (plasma) or 3.3% (erythrocytes) for 16:0 (palmitic acid); 2.3% (plasma) or 7.2% (erythrocytes) for 17:0; 2.1% (plasma) or 3.4% (erythrocytes) for 18:0 (stearic acid); 3.3% (plasma) or 6.8% (erythrocytes) for trans 16:1n–7; and 10.5% (plasma) or 7.0% (erythrocytes) for trans 18:1n–7. Although 12:0 (lauric acid) also mainly comes from dairy foods (6), we did not report this FA because of the high incidence of laboratory measurement errors in relation to it.

Stability of fatty acid contents in plasma and erythrocytes

One study has shown that FAs in serum stored at a low temperature (–80 °C) for 7–12 y showed minimal degradation over time (22). Among the controls of the current study, the proportions of monounsaturated and polyunsaturated FAs were 18.63% (plasma) and 13.25% (erythrocytes) for oleic acid, 30.52% (plasma) and 13.64% (erythrocytes) for linoleic acid, 0.50% (plasma) and 0.18% (erythrocytes) for α-linolenic acid, 7.80% (plasma) and 14.61% (erythrocytes) for arachidonic acid, 0.49% (plasma) and 1.15% for eicosapentaenoic acid, and 1.57% (plasma) and 3.72% (erythrocytes) for docosahexaenoic acid. The contents of these FAs were similar to those observed in previous studies with shorter durations of storage (23, 24), which indicates that the FA contents were stable after long-term storage at a very low temperature (–130 °C).

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Assessment of covariates

Information on the occurrence of disease, medical history, and major lifestyle IHD risk factors of the participants was collected at baseline and updated biennially thereafter with the use of follow-up questionnaires. We primarily used covariates assessed by the 1990 follow-up questionnaire in the current study. To minimize missing data, we carried forward the values obtained in previous questionnaires for values missing in the 1990 questionnaires.

Plasma total and HDL-cholesterol concentrations were measured enzymatically. LDL cholesterol was measured by using a data, we carried forward the values obtained in previous questionnaires. To minimize missing baseline and updated biennially thereafter with the use of follow-up questionnaire in the current study. To minimize missing data, we carried forward the values obtained in previous questionnaires for values missing in the 1990 questionnaires.

Statistical analysis

For continuous variables with normal distribution, means (±SDs) were calculated and Student’s t tests were used to evaluate the significance of difference between cases and controls. Medians and interquartile ranges and Wilcoxon’s rank-sum tests were used for variables with nonnormal distribution. For categorical variables, proportions were calculated, and chi-square tests of significance were used.

Dairy fat intake measured by the FFQs was expressed as a percentage of total fat intake. We determined the biomarkers of dairy fat intake by using data on the controls only, because controls were representative of the source population from which the cases were drawn. Spearman partial correlation coefficients (r) were calculated to determine relations between FA concentrations and dairy fat intake after adjustment for age at blood collection (y), smoking status (never smoker, past smoker, currently smoke 1–14 cigarettes/d, currently smoke 15–24 cigarettes/d, or currently smoke ≥25 cigarettes/d), body mass index (BMI), in kg/m²), current weight (kg), fasting status (yes or no), postmenopausal status (yes or no), postmenopausal hormone use (never, past, or current), and the periods during which the blood samples were assayed. We used both dairy fat intake measured by the 1990 FFQ and the average intake estimated by averaging the 1986 and 1990 FFQ measurements to represent long-term dairy fat intake.

We used multivariate linear regression to detect linear trends of the biomarker concentrations across deciles of average dairy fat intake of controls in 1986–1990. Least-squares means of FA concentrations were calculated for each decile of dairy fat intake after adjustment for total energy intake, age, smoking status, BMI, and other covariates. To allow for deviance from the assumption of normal distribution of dependent variables, we calculated robust estimators of variance for these means (26). Median values of each decile of intake were entered into the models as a continuous variable to calculate P values for linear trend.

We used conditional logistic regressions to estimate the relative risks (RRs) of IHD associated with the biomarkers of dairy fat intake. In nested case-control studies, odds ratios derived from conditional logistic regressions are unbiased estimates of hazard ratios or RRs (27). The distribution among control participants was used to determine the cutoffs for tertiles of these biomarkers. Multivariate RRs were adjusted for established risk factors for IHD: ie, BMI (<25, 25–29, or ≥30), physical activity (≤8.1, 8.2–18.5, or ≥18.6 metabolic equivalent tasks), alcohol intake (0, 1–4, 5–14, or ≥15 g/d), use of aspirin (tablets/ wk), parental history of MI before age 65 y (yes or no), history of hypertension (presence or absence), history of hypercholesterolemia (presence or absence), history of diabetes (presence or absence), postmenopausal status (yes or no), and postmenopausal hormone use (never, past, or current). Matching factors were taken into account as well. We included 18:2n–6 (linoleic acid) and total trans FAs in the multivariate models because their inclusion in plasma changed the RR of ≥1 category of 15:0 tertiles in plasma by >15%.

P values for linear trend were calculated by entering a continuous score based on the median value in each tertile of FA into the models. All P values were 2-sided; 95% CIs were calculated for RRs and least-squares means. Data were analyzed with the use of SAS software (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

We evaluated the validity of plasma and erythrocyte FAs as biomarkers of dairy fat intake in controls only. The comparison between controls and the overall Nurses’ Health Study cohort with respect to baseline characteristic distribution is shown in Table 1. As expected, the controls in the present study did not differ significantly from the overall cohort except in age, which was used to match controls with IHD cases. The FA concentrations in plasma and erythrocytes are described in Table 2.

The Spearman partial correlation coefficients between FA concentrations in plasma and erythrocytes and dairy fat and product intake are shown in Table 3. Uniformly, each FA in both blood fractions had a stronger correlation with average dairy fat intake in 1986–1990 than with 1990 intake alone, probably because of the reduction of random measurement errors by averaging multiple assessments. In plasma, 15:0 had the strongest correlation with dairy fat intake; the Spearman correlation coefficient was 0.36. The second-strongest marker of dairy fat intake in plasma was trans 16:1n−7. In erythrocytes, correlations with dairy fat intake did not differ significantly for 15:0 and trans 16:1n−7; both correlation coefficients were ~0.30. Other FAs in plasma or erythrocytes were weakly associated with dairy fat intake.
Because we observed reasonable correlations for 15:0 and trans 16:1n−7 in both plasma and erythrocytes, we focused on these FAs thereafter. In previous studies (11, 28–31), 17:0 in blood or human tissues was also suggested as a potential biomarker of dairy fat intake. To be comparable to these studies, we also included 17:0 in further analyses. Plasma 15:0 concentrations were correlated with total dairy product intake less than with dairy fat intake; the Spearman correlation coefficient was 0.29 between this FA in plasma and average dairy product intake in 1986–1990. Results are similar for other FAs. Correlations of these FAs with skim milk intake were close to 0 (data not shown). These FAs were not correlated with beef or lamb intake (data not shown).

To be comparable to these studies, we also included participants who were diagnosed with diabetes at baseline characteristics of cases and controls are shown in Table 3. Women diagnosed with IHD events had a significantly higher BMI, drank significantly less alcohol, were significantly lower in cases than in controls (P < 0.01) higher BMI, drank significantly (P = 0.01) less alcohol, were significantly (P < 0.001) more likely to have a parental history of MI, and had significantly (P < 0.001) less favorable plasma lipoprotein values than did controls. For the FAs of interest in both blood fractions, the concentrations among IHD cases and controls did not differ significantly except that 17:0 content in erythrocytes was significantly lower in cases than in controls (P = 0.01).

Table 4. Women diagnosed with IHD events had a significantly higher BMI, drank significantly (P = 0.01) less alcohol, were significantly (P < 0.001) more likely to have a parental history of MI, and had significantly (P < 0.001) less favorable plasma lipoprotein values than did controls. For the FAs of interest in both blood fractions, the concentrations among IHD cases and controls did not differ significantly except that 17:0 content in erythrocytes was significantly lower in cases than in controls (P = 0.01). RRs of IHD associated with 15:0, 17:0, and trans 16:1n−7 in plasma and erythrocytes are shown in Table 3. In crude models (model 1), trans 16:1n−7 in plasma and 17:0 in erythrocytes were associated with lower risks of IHD. These inverse associations were attenuated toward the null after further adjustment of established dietary and lifestyle risk factors for IHD and of other FAs in plasma or erythrocytes, whereas the positive associations for 15:0 in plasma were strengthened after such adjustments. These results indicated the presence of negative confounding on these associations caused by the covariates. The RR of IHD between the highest tertile and the lowest tertile of 15:0 in plasma was 2.36 (95% CI: 1.16, 4.78; P for trend = 0.03). Adjustment for other nutrients in which dairy foods are rich—ie, calcium and vitamin D—did not significantly change the associations. Further adjustment for fiber, folate, and fruit and vegetables did not alter these associations. To examine whether these findings may be due to changes in diet subsequent to the diagnosis of diabetes, we excluded participants who were diagnosed with diabetes at baseline. The results did not differ significantly (data not shown).

In a secondary analysis, we found positive but nonsignificant correlations between the biomarker concentrations and the ratio of LDL to HDL cholesterol (LDL:HDL) (data not shown). We observed a correlation coefficient of 0.09 (P = 0.13) between LDL:HDL and 15:0 in plasma. After further adjustment of LDL: HDL in the multivariate models, the associations for 15:0 in plasma were partly attenuated. The RRs across each tertile of 15:0 in plasma were 1.0 (reference), 1.93 (95% CI: 1.0, 3.72), and 2.25 (95% CI: 0.99, 5.15) (P for trend = 0.07). Associations for other biomarkers were attenuated to a similar extent after such adjustment (data not shown).

**DISCUSSION**

In this prospective, nested, case-control study, we explored potential biomarkers of dairy fat intake in plasma and erythrocytes.
and examined their associations with the risk of IHD. We found that
15:0 and trans 16:1n−7 in plasma and erythrocytes could be used as
biomarkers of dairy fat intake. We observed significant associations
between plasma 15:0 concentrations and the risks of IHD after
adjustment for established dietary and lifestyle IHD risk factors and
other FAs in plasma. In comparison with women in the lowest tertile
of 15:0 in plasma, women in the highest tertile had IHD risks more
than twice as great.

FIGURE 1. Relation between cumulative average intake of dairy fat in 1986–1990 and biomarker concentrations in plasma and erythrocytes, the Nurses’
Health Study, 1990. t, trans configuration. n = 306. Least-squares means of fatty acids in plasma (○) and erythrocytes (■) are shown as plotted against median
values by decile of intake (% of total fat) after adjustment for total energy intake, age, smoking, BMI, current weight, postmenopausal hormone use, fasting
status, and period of blood assay. Bars are 95% CIs (t ± SEM: 1.96 × SEM). The range on the y axis in the middle panel differs from that in the top and bottom
panels. Median values of each decile of intake were entered into the models as a continuous variable to estimate the P values for linear trend. All P values for
trend are based on t tests, and P < 0.001.
Cholesterol (mmol/L) MET·h, metabolic equivalent hours; MI, myocardial infarction.

TABLE 4 Baseline characteristics of study participants, the Nurses’ Health Study, 1990

| Characteristics          | Cases (n = 166) | Controls (n = 327) | P
|--------------------------|-----------------|-------------------|---
| Age (y)                  | 60.6 ± 6.0†     | 60.4 ± 6.1 NS     | NS
| BMI (kg/m²)              | 26.9 ± 5.9      | 25.4 ± 4.5 <0.01  | <0.01
| Physical activity (MET·h) | 10.6            | 13.4              | NS
| Interquartile range      | 4.4–22.4        | 6.3–22.4          | NS
| Diet                     |                 |                   | NS
| Dairy fat intake (g·kcal⁻¹·d⁻¹) | 6.7 ± 3.5      | 6.9 ± 4.0         | NS
| Total                    | 11.2 ± 3.2      | 11.4 ± 3.7        | NS
| Alcohol intake (g/d)     |                 |                   | NS
| Median                   | 0.9             | 1.8               | 0.01
| Interquartile range      | 0.0–3.5         | 0.0–6.7           | NS
| Fasting intake (µg·kcal⁻¹·d⁻¹) | 213.0          | 223.1             | NS
| Median                   | 164.3–352.9     | 160.8–353.8       | NS
| Fruit and vegetable intake (servings/d) | 5        | 6                 | NS
| Medial                   | 4–8            | 4–8               | NS
| Intersquartile range     | 0–6–4          | 0–4               | NS
| Aspirin use (tablets/wk) |                 |                   | NS
| Current smoker           | 32.5            | 31.9              | NS
| Former smoker            | 33.1            | 33.4              | NS
| Never smoker             | 34.3            | 34.7              | NS
| Medical history          |                 |                   | NS
| Diabetes (%)             | 21.1            | 6.7               | <0.001
| Hypertension (%)         | 59.0            | 29.4              | <0.001
| Hypercholesterolemia (%) | 56.0            | 37.0              | <0.001
| Parental MI before age 65 y (%) | 33.7         | 18.7              | <0.001
| Fasting status (%)       | 60.2            | 55.4              | NS
| Postmenopausal (%)       | 92.8            | 90.2              | NS
| Postmenopausal hormone use (%) | 57.8       | 58.6              | NS
| Cholesterol (mmol/L)³    |                 |                   | NS
| HDL                      | 1.34 ± 0.39     | 1.60 ± 0.48       | <0.001
| LDL                      | 3.80 ± 0.87     | 3.34 ± 0.89       | <0.001
| Total                    | 6.25 ± 1.06     | 5.78 ± 1.02       | <0.001
| LDL:HDL                  | 3.1 ± 1.1       | 2.3 ± 1.0         | <0.001
| Fatty acids in plasma    |                 | 16:1n–7          | NS
| 15:0                     | 0.16 ± 0.05     | 0.16 ± 0.04       | NS
| 17:0                     | 0.30 ± 0.05     | 0.31 ± 0.05       | NS
| 16:1n–7                 | 0.14 ± 0.04     | 0.15 ± 0.04       | NS
| Fatty acids in erythrocytes |               |                   | NS
| 15:0                     | 0.10 ± 0.03     | 0.10 ± 0.03       | NS
| 17:0                     | 0.33 ± 0.05     | 0.35 ± 0.05       | 0.01
| 16:1n–7                 | 0.13 ± 0.03     | 0.14 ± 0.03       | NS

1. trans configuration. Percentages are based on nonmissing data.
2. P value estimates are based on Student’s t test for variables expressed as x ± SD, Wilcoxon rank-sum test for variables expressed medians, and Pearson chi-square test for variables expressed as percentages.
4. x ± SD (all such values).
5. Proportions are based on the number of postmenopausal women.
6. Data on HDL cholesterol were missing for 7 women; data on LDL cholesterol were missing for 26 women. To convert values from mmol/L to mg/dL, multiply by 38.6098.

Consumption of whole milk and other high-fat dairy products, such as butter, has been postulated to be associated with the risk of IHD because of the high saturated fat content of those products (1). Although data from ecological studies suggested a strong, positive correlation between dairy food consumption and IHD mortality (2–4), most prospective cohort studies showed mixed results regarding dairy product consumption and the risk of IHD (7, 32–34). These studies varied according to population characteristics, dietary assessment methods, categorization of intake, and assessment and control for confounders, which may explain the inconsistency. Moreover, in addition to the fat content, milk and dairy products contain many nutrients that may play different roles in the development of IHD. For example, intake of calcium, for which dairy products are the main food source, has been associated in some epidemiologic studies with lower risks of IHD (7, 35).

Dairy fat, from which the health concerns arose, has been only indirectly evaluated in a few epidemiologic studies (6, 7). Measuring dairy fat intake can be challenging in epidemiologic studies that use FFQs to quantify it. Apart from the inaccuracies of self-reports or food composition databases (9, 10), measurement errors may also result from the facts that dairy fat exists in many foods, and that the content of dairy fat may vary in the same foods, depending on whether whole milk or low-fat milk is used to produce dairy products (8). Biomarkers of dairy fat intake are not subject to these measurement errors and thus are believed to be more precise than self-reports. Adipose tissue or plasma contents of 15:0 and 17:0 have been found to be valid biomarkers of dairy fat intake (11, 12, 28–31). These 2 odd-numbered FAs are exclusively produced by the bacterial flora in the rumen of ruminants (20). In a Swedish population, Wolk et al (11) documented a correlation coefficient of 0.36 between adipose tissue 15:0 content and FFQ dairy fat measurement, which was similar to our results. In other Swedish studies, the validity of serum 15:0 content as a biomarker of dairy fat intake was examined in relation to diet record measurements, and the authors found somewhat higher correlation coefficients (r > 0.40), which may be due to the fact that fewer measurement errors were associated with the diet records (28, 29, 31). In these studies, 17:0 in human tissues or serum fractions was a weaker marker than 15:0 (11, 28, 29, 31). In a study conducted in Norway, a negative association was documented between dairy fat intake and 17:0 in serum or adipose tissue (30). These findings may be due to different metabolic procedures of these 2 FAs in humans.

To our knowledge, this is the first report that trans 16:1n–7 could be used as a biomarker for dairy fat intake. This FA exists exclusively in ruminant fats, especially dairy fat (21). Because trans FAs cannot be synthesized by humans, this FA possesses the characteristics of a potential biomarker. More studies are needed to confirm this novel finding. Although trans 18:1n–7 is the major trans isomer in dairy fat, the fact that this trans isomer also exists in partially hydrogenated oils may explain its weak correlation with dairy fat intake (21).

In the current study, we found that these FAs in plasma or erythrocytes were correlated with dairy product intake less than with dairy fat intake. This finding is consistent with previous observations (28). As expected, these biomarkers were not correlated with low-fat milk intake in our study. Approximately 80% of the participants in the present study reported drinking low-fat milk rather than whole milk. Although 15:0, 17:0, and trans 16:1n–7 also exist in ruminant meats (eg, beef or lamb),
they were not valid biomarkers of ruminant meat intake because these foods are not the major food sources of these FAs (21, 36).

Few epidemiologic studies have examined the coronary effects of dairy fat intake, and the results indicated that high dairy fat intake may increase the risk of heart disease (6, 7). It is interesting that, in 2 small case-control studies that used biomarkers to represent dairy fat intake, adipose tissue or serum 15:0 and 17:0 concentrations were associated with nonsignificantly lower risks of CHD after adjustment for covariates (36, 37). Residual confounding cannot be excluded, because only a few lifestyle risk factors were controlled for in these 2 studies. In the current study, after we adjusted for established IHD risk factors and other FAs in plasma, 15:0 in plasma, the strongest biomarker identified in our study, was associated with a higher risk of IHD, whereas associations for other weaker biomarkers were not significant.

Clinical feeding trials have provided solid evidence regarding the effects of butter fat intake in increasing atherogenic plasma LDL-cholesterol concentrations. At the same time, butter fat intake modestly increases HDL-cholesterol concentrations (38–40). As a result, butter fat intake causes a small increase in ratio of total to HDL cholesterol (41), which is a stronger risk factor than either LDL or HDL cholesterol alone (25). In the present study, the 15:0 in plasma was correlated with a slightly increased LDL:HDL. However, adjustment of this lipid variable did not entirely abolish the association between 15:0 concentration in plasma and IHD risk. These results suggested that other pathways linking dairy fat intake with a higher risk of IHD also may exist. Dairy fat consists of ∼60% saturated FAs, 30% monounsaturated FAs, and 5% trans FAs (8, 21, 42). Saturated fat intake has effects on promoting platelet aggregation (43), activating factor VII (44), and increasing lipoprotein(a) production (45). trans FAs may increase systemic inflammation (46), cause endothelial dysfunction, and impair insulin sensitivity (47).

The present study has two noteworthy strengths. First, we measured a full spectrum of FAs in plasma and erythrocytes, and we thus had the opportunity to identify a greater number of potential biomarkers than did the previous studies. Second, because of the study’s prospective design, the occurrence of disease could not influence biomarker concentrations in erythrocytes and plasma.

**TABLE 5**

<table>
<thead>
<tr>
<th>Fatty acid content (%)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P for trend&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
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<td></td>
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<tr>
<td>15:0</td>
<td></td>
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</tr>
<tr>
<td>Mean (range)</td>
<td>0.11 (0.06, 0.13)</td>
<td>0.15 (0.14, 0.16)</td>
<td>0.21 (0.17, 0.30)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>1.36 (0.85, 2.18)</td>
<td>1.39 (0.85, 2.26)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>2.18 (1.20, 3.98)</td>
<td>2.36 (1.16, 4.78)</td>
<td>NS</td>
</tr>
<tr>
<td>17:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>0.25 (0.14, 0.28)</td>
<td>0.30 (0.29, 0.32)</td>
<td>0.36 (0.33, 0.51)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>1.0 (0.63, 1.59)</td>
<td>0.94 (0.59, 1.48)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>1.23 (0.70, 2.17)</td>
<td>1.25 (0.68, 2.30)</td>
<td>NS</td>
</tr>
<tr>
<td>r-16:1n−7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>0.11 (0.05, 0.13)</td>
<td>0.15 (0.14, 0.17)</td>
<td>0.20 (0.17, 0.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>0.77 (0.49, 1.20)</td>
<td>0.61 (0.39, 0.97)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>0.79 (0.46, 1.36)</td>
<td>0.79 (0.44, 1.42)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Erythrocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>0.07 (0.03, 0.08)</td>
<td>0.10 (0.09, 0.11)</td>
<td>0.14 (0.12, 0.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>0.90 (0.55, 1.45)</td>
<td>0.70 (0.39, 1.27)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>1.01 (0.55, 1.83)</td>
<td>0.93 (0.42, 2.05)</td>
<td>NS</td>
</tr>
<tr>
<td>17:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>0.29 (0.19, 0.32)</td>
<td>0.35 (0.33, 0.36)</td>
<td>0.40 (0.37, 0.51)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>0.59 (0.37, 0.94)</td>
<td>0.52 (0.32, 0.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>0.78 (0.44, 1.40)</td>
<td>0.72 (0.38, 1.38)</td>
<td>NS</td>
</tr>
<tr>
<td>r-16:1n−7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>0.10 (0.04, 0.12)</td>
<td>0.14 (0.13–0.15)</td>
<td>0.17 (0.16–0.23)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 1 (matching factors)</td>
<td>1.0</td>
<td>0.97 (0.61, 1.55)</td>
<td>0.92 (0.58, 1.46)</td>
<td>NS</td>
</tr>
<tr>
<td>Model 2 (multivariate)</td>
<td>1.0</td>
<td>0.79 (0.44, 1.43)</td>
<td>0.96 (0.53, 1.83)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup> t, trans configuration. n = 166 cases and 327 controls included for analyses. The lowest tertile is the reference group. The mean and range of each tertile and interquartile range are based on the distributions among controls. Model 1 is conditioned on the matching factors: age at blood draw, smoking status (never, past, or current), fasting status (yes or no), and time of blood drawing. Multivariate model 2 is as model 1 and further controlled for BMI (in kg/m<sup>2</sup>); <25, 25–29, or ≥30); postmenopausal status (yes or no), postmenopausal hormone use (never, past, or current), physical activity (≥8.1, 8.2–18.5, or ≥18.6 metabolic equivalent hours), alcohol intake (0, 1–4, 5–14, or ≥15 g/d), aspirin intake (tablets/wk), parental history of MI before age 65 y (yes or no), history of hypertension (presence or absence), history of hypercholesterolemia (presence or absence), history of diabetes (presence or absence), and linoleic acid and total trans fatty acids in plasma or erythrocytes.

<sup>2</sup> Linear scores derived from the medians of tertiles of fatty acid concentration among controls were used to estimate P values for trend. Estimates of P value for linear trend are all based on t tests.
The present study was also subject to several limitations. First, because we did not have complete data on 15:0 and 17:0 content in foods, we cannot assess whether these FAs reflect their own intakes more than dairy fat intake. Second, single baseline measurements of plasma and erythrocyte FA concentrations will not perfectly reflect long-term dietary intake, which is the variable of interest. Third, we observed higher laboratory measurement errors for erythrocyte FAs than for plasma FAs. This may partly explain why plasma 15:0 content was a better marker and a better predictor than the erythrocyte one. Fourth, in observational studies, residual confounding cannot be entirely ruled out.

In conclusion, 15:0 and trans 16:1n−7 content in plasma and erythrocyte can be used as biomarkers of dairy fat intake as assessed by FFQs. These biomarker data suggest that a high intake of dairy fat may be associated with a moderately greater risk of IHD.

The authors’ responsibilities were as follows—QS: analyzed the data and drafted the manuscript; JM: designed the study and directed the blood sample assays; HC: designed the study, assayed the biomarkers, and prepared the data; FBH: designed the study analytic strategy and supervised the data analysis; and all authors: contributed to the revision of the manuscript. None of the authors had any personal or financial conflict of interest.

REFERENCES

6. Hu FB, Stampfer MJ, Manson JE, et al. Dietary saturated fats and their intake of dairy fat may be associated with a moderately greater risk of IHD. 
41. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol.


