Association of erythrocyte membrane fatty acids with changes in glycermia and risk of type 2 diabetes1–3

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ABSTRACT
Background: The significance of erythrocyte membrane fatty acids (EMFAs) and their ratios to predict hyperglycemia and incident type 2 diabetes is unclear.
Objective: We investigated EMFAs as predictors of the worsening of hyperglycemia and incident type 2 diabetes in a 5-y follow-up of a population-based study.
Design: We measured EMFAs in 1346 Finnish men aged 45–73 y at baseline [mean ± SD age: 55 ± 6 y; body mass index (in kg/m²): 26.5 ± 3.5]. Our prospective follow-up study included only men who were nondiabetic at baseline and who had data available at the 5-y follow-up visit (n = 735).
Results: Our study showed that, after adjustment for confounding factors, palmitoleic acid (16:1n–7; P = 2.8 × 10⁻⁵), dihomo-γ-linolenic acid (20:3n–6; P = 2.3 × 10⁻⁵), the ratio of 16:1n–7 to 16:0 (P = 1.6 × 10⁻⁸) as a marker of stearoyl coenzyme A desaturase 1 activity, and the ratio of 20:3n–6 to 18:2n–6 (P = 9.4 × 10⁻⁷) as a marker of Δ⁵-desaturase activity significantly predicted the worsening of hyperglycemia (glucose area under the curve in an oral-glucose-tolerance test). In contrast, linoleic acid (18:2n–6; P = 0.0015) and the ratio of 18:1n–7 to 16:0 (P = 0.004) nominally predicted incident type 2 diabetes, whereas linoleic acid had an opposite association (P = 0.004), and n–3 polyunsaturated fatty acids did not show any associations.
Conclusion: EMFAs and their ratios are associated longitudinally with changes in glycermia and the risk type 2 diabetes. Am J Clin Nutr 2014;99:79–85.

INTRODUCTION
Fatty acid (FA)4 composition is predominantly determined by dietary intake and endogenous synthesis of FAs (1, 2). Because erythrocyte membranes lack de novo FA synthesis and modification by desaturation or elongation, erythrocyte membrane FAs (EMFAs) mirror especially a long-term dietary intake of n–3 PUFAs and linoleic acid (3–8). EMFAs are interdependent (6–8) and are controlled by several gene variants (9).
Stearoyl coenzyme A desaturases 1 and 2 (SCD1 and SCD2, respectively) catalyze the synthesis of MUFA from SFAs. Δ⁵-Desaturase (D5D) and Δ⁶-desaturase (D6D) are needed for the synthesis of long-chain n–6 (omega-6) and n–3 (omega-3) PUFAs (10). The EMFA product-to-precursor ratios were calculated, which are crude estimates of desaturase activities (10). Elongase activities can be calculated by measuring the ratio of vaccenic acid (18:1n–7) to palmitoleic acid (16:1n–7) (11). The activities of D5D and D6D are known to be genetically controlled by the FADS1 and FADS2 genes (9, 12). A recent Mendelian randomization analysis indicated that genetically determined low D5D activity tends to predict a higher risk of diabetes, whereas a low D6D activity predicts a lower risk of diabetes (13).
Insulin deficiency leads to a decrease in desaturase activity (14–16), whereas insulin resistance increases desaturase enzyme activity (17–19). FA composition, particularly SFAs and PUFAs, in turn modifies membrane properties by altering insulin action, insulin receptor binding, and translocation of glucose transporters (20, 21).
FAs and desaturase activities have been linked with changes in glucose metabolism (9, 22). Most small case-control studies (23–29), but not all (30), have reported that the proportions of individual EMFAs (16:0, 18:0, 24:0, 18:2n–6, 18:1n–9, 20:4n–6, 20:3n–6, and 22:6n–3) and desaturase and elongase activities (ratio of 16:1n–7 to 16:0, ratio of 18:1n–9 to 18:0, ratio of 18:3n–6 to 18:2n–6, and ratio of 20:3n–6 to 18:2 n–6) were higher in individuals with type 2 diabetes than in control subjects. Only a few prospective studies have investigated the proportions of EMFAs as predictors of incident type 2 diabetes, but the findings have not been consistent across studies (13, 31, 32). None of the

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4Abbreviations used: D5D, Δ⁵-desaturase; D6D, Δ⁶-desaturase; EMFA, erythrocyte membrane fatty acid; FA, fatty acid; METSIM, Metabolic Syndrome in Men; OGTT, oral-glucose-tolerance test; SCD, stearoyl coenzyme A desaturase.

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longitudinal studies has investigated the association of EMFAs with the worsening of hyperglycaemia and putative mechanisms explaining these associations.

The main aims of our study were to investigate the fasting proportions of EMFAs as predictors of the worsening of hyperglycaemia and incident type 2 diabetes in a 5-y follow-up of the population-based Metabolic Syndrome in Men (METSIM) cohort and the role of insulin sensitivity and insulin secretion in these associations.

**SUBJECTS AND METHODS**

**Subjects**

Our population-based METSIM study was performed in 2005–2010 at the Clinical Research Unit of the University of Eastern Finland, including a total of 10,197 Finnish men. In this cohort, EMFAs were measured in 1346 Finnish men [mean ± SD age: 55 ± 6 y; BMI (in kg/m²): 26.5 ± 3.5], who were selected randomly with equivocal percentages of each glucose category as compared with the original METSIM cohort of 10,197 men. Clinical and laboratory characteristics, including EMFAs, are presented as means ± SDs (Table 1). The glucose tolerance category was classified according to the American Diabetes Association criteria (33) as follows: 456 (33.9%) had normal glucose tolerance, 681 (50.6%) had isolated impaired fasting glucose, 32 (2.4%) had isolated impaired glucose tolerance and isolated impaired fasting glucose, 118 (8.8%) had both impaired fasting glucose and impaired glucose tolerance, and 59 (4.4%) had newly diagnosed type 2 diabetes. Individuals with previously diagnosed type 1 or type 2 diabetes at baseline were excluded from this study.

Our prospective ongoing 5-y follow-up study performed in 2010–2013 included only men who were nondiabetic at the baseline study. Thus, individuals with previously diagnosed type 2 diabetes were excluded. A total of 735 nondiabetic individuals with EMFA measurements at baseline participated in the follow-up study of whom 705 remained nondiabetic and 30 developed newly diagnosed type 2 diabetes (7 received a diagnosis of type 2 diabetes between the baseline and follow-up studies, all of whom were taking antidiabetic medication; 23 had newly detected type 2 diabetes in an oral-glucose-tolerance test (OGTT) performed at the 5-y follow-up visit). The study was approved by the Ethics Committee of the University of Eastern Finland and Kuopio University Hospital and was conducted in accordance with the principles of the Helsinki Declaration. All study participants gave written informed consent.

**Anthropometric measurements**

Height and weight were measured as previously described (34), and BMI was calculated as weight (kg) divided by height (m) squared.

**OGTT**

A 2-h OGTT (75 g glucose) was performed, and samples for plasma glucose and insulin were collected at 0, 30, and 120 min to assess glucose tolerance and insulin response to an oral glucose load.

**Laboratory measurements**

Plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab Systems reagents; Thermo Fischer Scientific). Plasma insulin was measured by immunoassay (ADVIA Centaur Insulin IRI no. 02230141; Siemens Medical Solutions Diagnostics).

**EMFA analysis**

EMFAs were measured as previously described (8), with modifications (7). Erythrocytes were separated from EDTA-blood by centrifugation at 1000 × g for 10 min (4°C) and then hemolyzed in the tris-HCl buffer (pH 7.6, 10 mmol/L). Erythrocyte membranes were prepared by centrifugation of hemolysate at 30,000 × g for 30 min at 4°C. Membrane sediment was resuspended in 0.5 mL distilled water. FA methyl esters were prepared by direct trans-esterification, which gives more complete recovery, especially of sphingomyelin-derived FAs compared with separate extraction and transesterification (8). Briefly, after 0.1 mL membrane suspension and 2 mL methanol-toluene (4:1, vol:vol) were mixed in a glass tube, 0.2 mL acetyl chloride was slowly added, and this mixture was incubated at 100°C for 1 h. After being cooled in cold water, 5 mL 6% K₂CO₃ was carefully added and then vigorously shaken. Toluene was separated to upper phase by centrifugation at
2000 × g for 5 min and then injected into the gas chromatograph (Agilent Technologies 7890A) with a 25-m free fatty acid phase column (Agilent Technologies). Pure standards (NU Chek Prep Inc) were used to identify FA methyl esters and to prepare calibration curves. Heptadecanoic acid methyl ester (17:0) served as an internal standard. The intra- and interassay precisions varied between 0.3% and 4.1% and between 2.0% and 9.1% (relative SD), respectively. The proportion of each FA is expressed as mole percentage of total FAs in all tables.

Calculations

The trapezoidal method was used to estimate the glucose AUC in an OGTT based on samples collected at 0, 30, and 120 min. Calculation of insulin sensitivity (Matsuda insulin sensitivity index), insulin secretion (insulin AUCp0.30/glucose AUCp0.30), and disposition indexes were previously described (34, 35). We evaluated desaturase and elongase enzyme activities expressed as EMFA product to precursor ratios as follows: ratio of palmitoleic acid (16:1n–7) to palmitic acid (16:0) as a marker of D6D activity, ratio of dihomo-γ-linolenic acid (20:3n–6) to linoleic acid (18:2n–6) as a marker of D6D activity, ratio of arachidonic acid (20:4n–6) to 20:3n–6 as a marker of D5D activity, and ratio of vaccenic acid (18:1n–7) to 16:1n–7 as a marker of elongase activity.

Statistical analysis

Statistical analyses were conducted by using SPSS version 19. All traits, except for age, were log transformed to correct for their skewed distributions. EMFA proportions and ratios were compared across the different glucose-tolerance categories by using the general linear model adjusted for age and BMI. Linear regression was used to evaluate baseline EMFA proportions and ratios measured as predictors of the changes in the glucose AUC in an OGTT, insulin sensitivity, and insulin secretion in the 5-y follow-up study. Unstandardized effect sizes (B and SE) were estimated by linear regression analysis by using untransformed dependent variables. Logistic regression analysis was used to assess the association of EMFA proportions and ratios with incident type 2 diabetes during a 5-y follow-up study. In all models, adjustments were done for age, BMI, current smoking (yes or no), and physical activity (physically active, regular exercise ≤30 min/wk; or physically less active; occasional exercise; or no exercise). After Bonferroni correction for multiple comparisons (for 18 EMFAs and their ratios), \( P < 2.8 \times 10^{-3} \) was considered statistically significant and \( P < 0.05 \) as nominally significant in linear and logistic models. Given a difference >8% in EMFA proportions and their ratios between converters and nonconverters to diabetes at baseline, we had >80% power to show their associations with incident diabetes at the level of statistical significance of \( P < 2.8 \times 10^{-3} \).

RESULTS

Proportions of EMFAs in different glucose-tolerance categories at baseline

Proportions of EMFAs and their ratios in different glucose-tolerance categories in nondiabetic individuals and in individuals with newly diagnosed type 2 diabetes are given in Table 2. In comparison with the normal glucose-tolerance reference category, SFAs did not change significantly across the glucose-

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Proportions of erythrocyte membrane fatty acids in different categories of glucose tolerance in 1346 subjects without diabetes or with newly diagnosed type 2 diabetes from the METSIM study.</td>
</tr>
<tr>
<td>Category (n)</td>
</tr>
<tr>
<td>NGT (n = 681)</td>
</tr>
<tr>
<td>IGT (n = 32)</td>
</tr>
<tr>
<td>IIFG + IIGT (n = 36)</td>
</tr>
<tr>
<td>Ratio of 20:4n–6 to 20:3n–6 (D5D activity)</td>
</tr>
<tr>
<td>Ratio of 18:1n–7 to 16:1n–7 (elongase)</td>
</tr>
</tbody>
</table>

**All values are means ± SD. P values were calculated over the glucose-tolerance groups (ANOVA) and were adjusted for age and BMI (ANCOVA). The threshold for statistical significance was \( P < 2.8 \times 10^{-3} \), which is considered significant.**
tolerance categories. Of the PUFAs, 18:2n–6 was significantly decreased (P = 3.7 × 10⁻⁸) across the glucose-tolerance categories.

Association of EMFAs with insulin sensitivity and insulin secretion at the 5-y follow-up study

**Insulin sensitivity**

Palmitic acid (P = 4.9 × 10⁻⁴) and vaccenic acid (P = 3.7 × 10⁻⁵) were significantly associated with increased insulin sensitivity, and dihomo-γ-linoleic acid (P = 6.5 × 10⁻⁶) with decreased insulin sensitivity (Table 3). Of the EMFA product-to-precursor ratios, 16:1n–7/16:0 (SCD1 activity; P = 3.1 × 10⁻³) and 20:3n–6/18:2n–6 (D6D activity; P = 1.1 × 10⁻⁵) were significantly associated with increased insulin sensitivity, whereas 20:4n–6/20:3n–6 (D5D activity; P = 3.6 × 10⁻⁴) and 18:1n–7/16:1n–7 (elongase activity; P = 1.2 × 10⁻⁶) were significantly associated with increased insulin secretion.

**Insulin secretion**

Palmitoleic acid (P = 3.9 × 10⁻⁴) and 16:1n–7/16:0 (SCD1 activity; P = 4.3 × 10⁻⁵) were significantly associated with decreased insulin secretion (disposition index), whereas linoleic acid (P = 1.6 × 10⁻⁴) and 18:1n–7/16:1n–7 (elongase activity; P = 4.3 × 10⁻⁵) were significantly associated with increased insulin secretion.

**EMFAs as predictors for hyperglycemia and incident type 2 diabetes**

SFAs did not predict changes in the glucose AUC or in incident type 2 diabetes (Table 4). Palmitoleic acid (P = 2.8 × 10⁻⁷), dihomo-γ-linoleic acid (P = 2.3 × 10⁻⁴), 16:1n–7/16:0 (SCD1 activity; P = 1.6 × 10⁻³), and 20:3n–6/18:2n–6 (D6D activity; P = 9.4 × 10⁻⁷) significantly predicted an increase in the glucose AUC at follow-up after the adjustment for confounding factors, whereas linoleic acid (P = 0.0015) and 18:1n–7/16:1n–7 (elongase activity; P = 1.5 × 10⁻⁹) significantly predicted a decrease in the glucose AUC. Palmitoleic acid (OR: 1.35; 95% CI: 1.07, 1.69; P = 0.010) and 16:1n–7/16:0 (OR: 2.23; 95% CI: 1.29, 3.85; P = 0.004) nominally increased, and linoleic acid (OR: 0.54; 95% CI: 0.35, 0.82; P = 0.004) nominally decreased the risk of incident diabetes after adjustment for confounding factors.

We additionally investigated whether significant associations of different EMFAs and their ratios with the glucose AUC or incident diabetes were dependent on insulin sensitivity or insulin secretion. All statistically significant associations remained, even after further adjustment for insulin sensitivity or insulin secretion. After additional adjustment for fasting plasma glucose, 2-h plasma glucose, or the glucose AUC in separate models, palmitoleic acid, dihomo-γ-linoleic acid (with the exception of adjustment for baseline glucose AUC), SCD1 activity, D6D activity, and elongase activity remained significantly associated with the glucose AUC at the follow-up study.

**DISCUSSION**

A few studies have investigated EMFAs as predictors of type 2 diabetes (9, 13, 31, 32), but no longitudinal studies on the association of EMFAs with the worsening of hyperglycemia have been conducted. We found that palmitoleic acid, dihomo-γ-linoleic acid, 16:1n–7/16:0 (SCD1 activity), and 20:3n–6/18:2n–6 (D6D activity) significantly predicted the worsening of

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**TABLE 3**

Association of baseline proportions of erythrocyte membrane fatty acids with insulin sensitivity and insulin secretion at the 5-y follow-up

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Matsuda ISI</th>
<th>Insulin AUC₀–3₀/glucose</th>
<th>DI30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
<td>P</td>
</tr>
<tr>
<td>16:0 (palmitic acid) (%)</td>
<td>-24.4</td>
<td>11.0</td>
<td>0.008**</td>
</tr>
<tr>
<td>16:1–7 (palmitoleic acid) (%)</td>
<td>-2.1</td>
<td>1.3</td>
<td>0.004**</td>
</tr>
<tr>
<td>18:1–7 (vaccenic acid) (%)</td>
<td>18.2</td>
<td>4.0</td>
<td>3.7 × 10⁻⁶</td>
</tr>
<tr>
<td>18:1–9 (oleic acid) (%)</td>
<td>6.5</td>
<td>6.2</td>
<td>0.548</td>
</tr>
<tr>
<td>Total MUFAs (%)</td>
<td>9.4</td>
<td>7.6</td>
<td>0.377</td>
</tr>
<tr>
<td>18:2–6 (linoleic acid) (%)</td>
<td>3.2</td>
<td>3.1</td>
<td>0.056</td>
</tr>
<tr>
<td>20:3–6 (dihomo-γ-linolenic acid) (%)</td>
<td>-8.6</td>
<td>2.2</td>
<td>6.5 × 10⁻⁶</td>
</tr>
<tr>
<td>20:4–6 (arachidonic acid) (%)</td>
<td>-1.6</td>
<td>4.2</td>
<td>0.408</td>
</tr>
<tr>
<td>20:5–3 (EPA) (%)</td>
<td>1.4</td>
<td>1.0</td>
<td>0.089</td>
</tr>
<tr>
<td>22:4–6 (adrenic acid) (%)</td>
<td>-2.5</td>
<td>1.6</td>
<td>0.029**</td>
</tr>
<tr>
<td>22:6–3 (DHA) (%)</td>
<td>-0.3</td>
<td>2.1</td>
<td>0.807</td>
</tr>
<tr>
<td>Total PUFAs (%)</td>
<td>-1.0</td>
<td>10.6</td>
<td>0.972</td>
</tr>
<tr>
<td>Ratio of 16:1n–7 to 16:0 (SCD1) (%)</td>
<td>-3.1</td>
<td>1.4</td>
<td>3.1 × 10⁻⁴</td>
</tr>
<tr>
<td>Ratio of 20:3n–6 to 18:2n–6 (Δ⁵-desaturase) (%)</td>
<td>-9.8</td>
<td>2.2</td>
<td>1.1 × 10⁻⁴</td>
</tr>
<tr>
<td>Ratio of 20:4n–6 to 20:3n–6 (Δ⁶-desaturase) (%)</td>
<td>6.3</td>
<td>1.9</td>
<td>3.6 × 10⁻⁴</td>
</tr>
<tr>
<td>Ratio of 18:1n–7 to 16:1n–7 (elongase) (%)</td>
<td>4.0</td>
<td>1.3</td>
<td>1.2 × 10⁻⁵</td>
</tr>
</tbody>
</table>

*p = 721 for the Matsuda ISI, insulin secretion index, and DI30 (Matsuda ISI × insulin AUC₀–3₀/glucose AUC₀–3₀); excluding 7 participants who had a diagnosis of diabetes and started receiving antidiabetic medication between baseline and follow-up.* Values were adjusted for age, BMI, smoking, and physical activity in multiple linear regression analyses. P < 2.8 × 10⁻³ was considered statistically significant given the 18 traits tested. #Statistically significant result. **Nominally significant result. DI30, disposition index; ISI, insulin sensitivity index; SCD1, stearoyl coenzyme A desaturase 1.
| Fatty acid | Glucose AUC at follow-up (n = 724) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B² | SE² | p² | p¹ | p⁴ | p⁵ | OR (95% CI)¹ | p⁶ | p⁷ | p⁸ | p⁹ |
| 16:0 (palmitic acid) (%) | -316.2 | 308.6 | 0.272 | 0.874 | 0.128 | 0.790 | 0.76 (0.50, 1.14) | 0.187 | 0.74 (0.48, 1.12) | 0.152 | 0.239 | 0.098 | 0.649 |
| 18:0 (stearic acid) (%) | 458.1 | 345.9 | 0.164 | 0.426 | 0.103 | 0.294 | 1.41 (0.73, 2.72) | 0.307 | 1.25 (0.63, 2.50) | 0.520 | 0.638 | 0.394 | 0.650 |
| Total SFAs (%) | 922.4 | 558.0 | 0.087 | 0.097 | 0.098 | 0.078 | 0.98 (0.68, 1.42) | 0.926 | 0.96 (0.66, 1.41) | 0.836 | 0.834 | 0.859 | 0.804 |
| 16:1n–7 (palmitoleic acid) (%) | 216.1 | 41.4 | 2.8 × 10⁻⁷* | 3.6 × 10⁻⁷* | 4.7 × 10⁻⁷* | 1.7 × 10⁻⁷* | 1.42 (1.14, 1.75) | 0.001* | 1.35 (1.07, 1.69) | 0.010** | 0.010** | 0.015** | 0.028** |
| 18:1n–9 (oleic acid) (%) | -348.5 | 126.9 | 0.004** | 0.054 | 0.001* | 0.011** | 1.00 (0.72, 1.41) | 0.980 | 0.99 (0.71, 1.40) | 0.966 | 0.809 | 0.810 | 0.525 |
| Total MUFAs (%) | -12.2 | 238.9 | 0.814 | 0.876 | 0.774 | 0.350 | 1.14 (0.79, 1.65) | 0.477 | 1.26 (0.86, 1.85) | 0.232 | 0.189 | 0.259 | 0.099 |
| 20:3n–6 (dihomo-γ-linolenic acid) (%) | -284.0 | 97.2 | 1.5 × 10⁻⁷* | 0.008** | 0.001* | 0.036** | 0.48 (0.32, 0.72) | 0.34 × 10⁻⁴* | 0.54 (0.35, 0.82) | 0.004** | 0.005** | 0.013** | 0.020** |
| 20:4n–6 (arachidonic acid) (%) | 88.9 | 130.6 | 0.436 | 0.848 | 0.407 | 0.529 | 1.26 (0.90, 1.75) | 0.172 | 1.24 (0.87, 1.77) | 0.235 | 0.293 | 0.195 | 0.706 |
| 20:5n–3 (EPA) (%) | -39.3 | 32.3 | 0.267 | 0.563 | 0.189 | 0.419 | 1.15 (0.66, 2.01) | 0.612 | 1.04 (0.58, 1.87) | 0.898 | 0.811 | 0.919 | 0.903 |
| 22:6n–3 (DHA) (%) | 125.9 | 50.7 | 0.011** | 0.070 | 0.004** | 0.085 | 1.08 (1.00, 1.18) | 0.062 | 1.09 (0.99, 1.19) | 0.073 | 0.112 | 0.045** | 0.183 |
| Total PUFAs (%) | -436.0 | 330.1 | 0.222 | 0.133 | 0.326 | 0.028** | 0.93 (0.70, 1.24) | 0.624 | 0.89 (0.66, 1.20) | 0.441 | 0.394 | 0.448 | 0.136 |
| Ratio of 16:1n–7 to 16:0 (SCD1) | 25.0 | 44.1 | 1.6 × 10⁻⁸ | 4.5 × 10⁻⁸ | 2.0 × 10⁻⁸ | 2.4 × 10⁻⁷ | 2.53 (1.51, 4.23) | 4.1 × 10⁻⁸ | 2.23 (1.29, 3.85) | 0.004** | 0.005** | 0.006** | 0.020** |
| Ratio of 20:3n–6 to 18:2n–6 (Δ⁶-desaturase) | 324.1 | 69.3 | 9.4 × 10⁻⁶ | 3.4 × 10⁻⁶ | 9.9 × 10⁻⁶ | 1.0 × 10⁻⁵ | 1.12 (1.01, 1.24) | 0.032** | 1.11 (0.99, 1.23) | 0.070 | 0.119 | 0.042** | 0.191 |
| Ratio of 20:4n–6 to 20:3n–6 (Δ⁶-desaturase) | -165.2 | 59.5 | 0.004** | 0.009** | 0.001* | 0.001* | 0.96 (0.76, 1.21) | 0.734 | 0.95 (0.75, 1.21) | 0.685 | 0.791 | 0.603 | 0.735 |
| Ratio of 18:1n–7 to 16:1n–7 (Δ⁴-desaturase) | -248.1 | 40.7 | 1.5 × 10⁻⁸ | 1.3 × 10⁻⁸ | 8.9 × 10⁻¹⁰ | 2.2 × 10⁻⁸ | 0.57 (0.36, 0.92) | 0.022** | 0.63 (0.39, 1.03) | 0.065 | 0.080 | 0.067 | 0.198 |

¹ n = 724 for glucose AUC at follow-up (excluding 7 participants who had a diagnosis of diabetes and started receiving antidiabetic medication between baseline and follow-up), n = 30 individuals with incident type 2 diabetes diagnosed at follow-up, and n = 705 individuals who remained nondiabetic. B and SE were obtained from a multiple linear regression analysis. ORs and their 95% CIs were obtained from a logistic regression analysis. For logistic regression, EMFAs for the ratio of 16:1n–7 to 16:0 were multiplied by 100, and EMFAs for 16:1n–7, 18:1n–9, 20:3n–6, 22:4n–6, and the ratio of 20:3n–6 to 18:2n–6 were multiplied by 10. P < 2.8 × 10⁻³ was considered statistically significant given the 18 traits tested. *Statistically significant result. **Nominally significant result. EMF, erythrocyte membrane fatty acid; ISI, insulin sensitivity index; SCD1, stearoyl coenzyme A desaturase 1.

² Adjusted for age, BMI, smoking, and physical activity.
³ Adjusted for age, BMI, smoking, physical activity, and the Matsuda ISI.
⁴ Adjusted for age, BMI, smoking, physical activity, and insulin AUC₀–30/glucose AUC₀–30.
⁵ Adjusted for age, BMI, smoking, physical activity, and the disposition index (Matsuda ISI × insulin AUC₀–30/glucose AUC₀–30).
⁶ Unadjusted.
hyperglycemia, and linoleic acid and 18:1n–7/16:1n–7 (elongase activity) predicted the improvement of hyperglycemia. Palmitoleic acid and 16:1n–7/16:0 nominally predicted incident diabetes, whereas linoleic acid prevented diabetes. In contrast, n-3 PUFAs were not associated with glucose concentrations or the risk of diabetes, in line with a recent meta-analysis (36).

Three previously published longitudinal studies that investigated the association of EMFAs with incident diabetes were case-control studies, and they measured somewhat different EMFAs compared with our study (13, 31, 32). Similar to our study, high proportions of palmitoleic acid (16:1n–7) and 16:1n–7/16:0 (SCD1 activity) predicted incident diabetes, and high proportions of linoleic acid (18:2n–6) prevented incident diabetes in the European Prospective Investigation of Cancer–Potsdam Study (13). In the European Prospective Investigation of Cancer–Norfolk cohort study (32), high proportions of erythrocyte membrane palmitic and palmitoleic acids predicted diabetes, whereas plasma linoleic acid and calculated D5D activity had opposite effects on the risk of diabetes. Krachler et al (31) also reported that a high proportion of palmitoleic acid in EMFAs increased the risk of diabetes, whereas linoleic acid had a preventive effect. These consistent findings are, however, likely to underestimate the significance of EMFAs and their ratios for the development of hyperglycemia, because in our study the associations with the glucose AUC—reflecting the glucose burden over time—were substantially more statistically significant than the associations with incident diabetes.

The mechanisms by which EMFAs and their ratios predict hyperglycemia or conversion to diabetes have remained largely unknown. Cross-sectional studies have reported that altered D5D and D6D activities are related to insulin resistance, but the direction of causality is not possible to conclude because dietary factors can also influence insulin sensitivity (37, 38). Our study was the first to measure insulin sensitivity and insulin secretion as possible mechanisms for the worsening of hyperglycemia and incident diabetes related to different EMFA proportions or their ratios. The adjustment for insulin sensitivity and insulin secretion somewhat weakened $P$ values but did not abolish statistical significance. These results differ from those of our previous study, in which we investigated the ability of fasting serum FAs, measured by proton nuclear magnetic resonance, to predict the worsening of hyperglycemia and incident diabetes in a 5-y follow-up study (39). The adjustment for baseline insulin sensitivity or glucose AUC abolished statistical significances for almost all associations. Therefore, the mechanisms by which plasma FAs and EMFAs increase the risk of type 2 diabetes are likely to differ.

EMFAs that reflect both dietary FAs and their complex metabolism in the body may have effects on the risk of type 2 diabetes, largely independent of insulin sensitivity and insulin secretion. The role of gene variants could be important in these associations, but testing this hypothesis needs further study.

The strength of our study was that it was population-based and had a relatively long follow-up period. Furthermore, the diagnosis of diabetes was based on an OGTT, which was performed twice at baseline, and on follow-up studies, which allowed us to determine not only incident diabetes but also the changes in glucose burden over time. The limitations of our study were that the METSIM cohort included only Finnish men; therefore, it is not possible to generalize our findings to women or to any other ethnic group. Although our longitudinal follow-up included 735 men, we had only a limited numbers of participants who developed diabetes during the follow-up. Finally, EMFAs were expressed as the proportions of total fat and thus are interdependent.

In conclusion, our study showed that EMFAs, palmitoleic acid, dihomo-$\gamma$-linolenic acid, 16:1n–7/16:0, and 20:3n–6/18:2n–6 were significantly associated with the worsening of hyperglycemia, whereas linoleic acid and 18:1n–7/16:1n–7 were significantly associated with a decrease in hyperglycemia. Palmitoleic acid and 16:1n–7/16:0 nominally predicted incident type 2 diabetes, whereas linoleic acid prevented type 2 diabetes. These associations were largely independent of insulin sensitivity, insulin secretion, and glucose concentrations. Thus, EMFAs may serve as biomarkers for the risk of disturbances of glucose metabolism in a longitudinal setting.

The authors’ responsibilities were as follows—ML, MU, and JK: designed the research and were involved in the data collection, data analyses, and manuscript preparation; YM, HC, JV, and AS: were responsible for the statistical analyses; JA, MU, and US: were responsible for erythrocyte membrane fatty acid measurements; YM and ML: wrote the manuscript; JA, MU, HC, JV, AS, US, and JK: were involved in the manuscript review and commenting; and ML: had primary responsibility for the final content. The funding bodies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript. No conflicts of interest were reported by the authors.

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