Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study1–3

Katriina Ylönen, Georg Alfthan, Leif Groop, Carola Saloranta, Antti Aro, Suvi M Virtanen, and the Botnia Research Group

ABSTRACT
Background: The role of antioxidants in the pathogenesis of type 2 diabetes is uncertain.
Objective: We evaluated cross-sectional relations of dietary intakes and plasma concentrations of antioxidants with glucose metabolism in a high-risk population.
Design: The subjects were 81 male and 101 female first- and second-degree, nondiabetic relatives of patients with type 2 diabetes. Antioxidant intake data were based on 3-d food records. Subjects taking supplements containing β-carotene or α-tocopherol were excluded. Plasma antioxidant concentrations were measured by HPLC. By using multiple linear regression analysis and adjusting for demographic, anthropometric, and lifestyle covariates, we studied whether dietary and plasma α- and β-carotene, lycopene, and α- and γ-tocopherol were related to fasting and 2-h concentrations of glucose and nonesterified fatty acids during an oral-glucose-tolerance test, to the homeostasis model assessment index of insulin resistance, and to measures of β cell function (incremental 30-min serum insulin concentration during an oral-glucose-tolerance test and first-phase insulin secretion during an intravenous-glucose-tolerance test).
Results: In men, dietary carotenoids were inversely associated with fasting plasma glucose concentrations (P < 0.05), plasma β-carotene concentrations were inversely associated with insulin resistance (P = 0.003), and dietary lycopene was directly related to baseline serum concentrations of nonesterified fatty acids (P = 0.034). In women, dietary α-tocopherol and plasma β-carotene concentrations were inversely and directly associated, respectively, with fasting plasma glucose concentrations (P < 0.05). In both sexes, cholesterol-adjusted α-tocopherol concentrations were directly associated with 2-h plasma glucose concentrations (P < 0.05).
Conclusion: The data suggest an advantageous association of carotenoids, which are markers of fruit and vegetable intake, with glucose metabolism in men at high risk of type 2 diabetes. Am J Clin Nutr 2003;77:1434–41.

KEY WORDS Glucose concentrations, insulin resistance, insulin secretion, nonesterified fatty acids, dietary intake, plasma concentrations, α-carotene, β-carotene, lycopene, α-tocopherol, γ-tocopherol, relatives of patients with type 2 diabetes

INTRODUCTION
Genetic and environmental factors contribute to the pathogenesis of type 2 (non-insulin-dependent) diabetes, and lifestyle factors act as triggers for the disease among subjects at high risk because of inherited susceptibility (1). The composition of the human diet has changed considerably since ancient times and also during the past few decades (2); these recent changes are thought to be contributing greatly to the increasing incidence of type 2 diabetes (3). Carotenoids and vitamins C and E (tocopherols) are important components of the body’s defense system against oxidative stress (4). Oxidative stress may impair insulin action by changing the physical state of the plasma membranes of target cells for insulin action (5).

Pharmacologic doses of vitamin E were shown to improve insulin-mediated glucose disposal (6–8), and a high dietary intake of vitamin C was associated with a lower incidence of type 2 diabetes (9). In cross-sectional studies, inverse associations of glycated hemoglobin with vitamin C intake (10, 11), plasma vitamin C (12), and vitamin E intake (11) were observed among nondiabetic subjects, whereas no association was seen with β-carotene. A direct relation between vitamin E intake and insulin sensitivity was reported among healthy vitamin E supplement users (13). In cross-sectional studies, serum carotenoid concentrations were inversely associated with insulin resistance (14, 15) and blood glucose concentrations (14). Plasma tocopherol concentrations were inversely correlated with insulin resistance in healthy subjects (15, 16) and in obese children (17), and low plasma vitamin E concentrations were found to increase the risk of type 2 diabetes in a prospective cohort (18).

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The relation between antioxidants and glucose metabolism is, however, not straightforward (19–22). In a longitudinal, nested case-control study, no independent relation was seen between serum α-tocopherol and β-carotene concentrations and the risk of type 2 diabetes (20). Similarly, supplementary β-carotene was not independently related to the development of type 2 diabetes in a randomized, placebo-controlled trial (21).

To address the role of antioxidants in a population at high risk of type 2 diabetes, we studied cross-sectional relations of dietary intakes and plasma concentrations of antioxidants (α- and β-carotene, lycopene, and α- and γ-tocopherol) with insulin sensitivity, insulin secretion, glucose tolerance, and nonesterified fatty acid (NEFA) concentrations in nondiabetic relatives of subjects with type 2 diabetes.

SUBJECTS AND METHODS

Study design

In 1994, the Botnia Dietary Study on relations between diet and glucose metabolism was initiated in relatives of subjects who had type 2 diabetes and were participants in the larger Botnia Study. The Botnia Study is a prospective family study in western Finland aiming at identification of genetic and metabolic defects in type 2 diabetes (23). All patients with known type 2 diabetes from 4 primary health care centers (Jokobstad, Korsholm, Malax-Korsnäs, and Närpes), their relatives, and spouses without a family history of diabetes were invited to participate in the study, which includes baseline and follow-up examinations. The Botnia Study was approved by the ethics committee of the local hospital that participated in the study. As part of the follow-up examination of the Botnia Study, the Botnia Dietary Study was carried out, on average, 5 y after the baseline examination. The inclusion criteria for the dietary study were as follows: 1) a first-degree (including parents, children, and siblings) or second-degree (including grandparents, grandchildren, aunts, uncles, and children of siblings) family history of type 2 diabetes, 2) age 20–70 y at the time of the baseline investigation, and 3) normal or impaired glucose tolerance according to the 1985 World Health Organization criteria (24) at the baseline investigation.

Subjects

Between October 1994 and June 1997, a consecutive sample of 746 subjects who fulfilled the inclusion criteria was invited to participate. The participation rate was 81%. The present study was carried out among a subsample of unrelated subjects who were selected at random from the Botnia Dietary Study population but drawn from only 2 of the participating centers (Malax-Korsnäs and Närpes). Of 308 eligible subjects, 237 (77%) participated in the study and completed the dietary recording acceptably. However, we excluded from subsequent analyses 6 subjects with newly diagnosed diabetes, 31 subjects taking supplementary β-carotene or α-tocopherol, and 18 subjects for whom laboratory or smoking data were missing. Of the remaining 182 subjects (59% of the eligible 308 subjects), 176 (97%) were first-degree relatives of subjects with type 2 diabetes, and 6 (3%) were second-degree relatives. The proportions of men and women did not differ between those who participated and those who did not; nor did the participants and nonparticipants differ in mean age, body mass index (BMI; in kg/m²), waist-to-hip ratio, physical activity, length of education, or fasting plasma glucose concentrations. However, 2-h plasma glucose concentrations and fasting serum insulin concentrations were significantly lower in the participants than in the nonparticipants [5.8 compared with 6.2 mmol/L (P = 0.035) and 49 compared with 57 pmol/L (P = 0.024), respectively].

Dietary data

Food consumption was measured by means of a 3-d estimated food record. Trained study nurses instructed the participants on how to keep a food record. Recording days included mainly 2 weekdays and 1 weekend day. Participants estimated portion sizes by using a food picture booklet. They also recorded the intake of dietary supplements during the study days. Participants returned their food records to the nurse, who reviewed them with the participants and completed missing or incomplete information. Food consumption data were recorded and processed by the NUTNET software developed at the National Public Health Institute, Helsinki (25). Dietary intakes (mean of the 3 recording days) were calculated for the following antioxidants: α- and β-carotene, lycopene, and α- and γ-tocopherol. Intakes of energy and alcohol and energy-adjusted intakes of polyunsaturated fatty acids and dietary fiber were also calculated.

Plasma antioxidants

For plasma antioxidant measurements, a fasting sample was drawn and initially stored for 1–3 mo at −20 °C and thereafter at −70 °C until analyzed. Plasma concentrations were analyzed for the following antioxidants: α- and β-carotene, lycopene, and α- and γ-tocopherol. To 0.2 mL plasma, tocotol and echinenon as internal standards for tocopherols and carotenoids, respectively, and a solution [50% (by vol) ethanol] containing 1% (by vol) ascorbic acid were added. After extraction with hexane, the tocopherols (26) and the carotenoids (27) were analyzed separately by HPLC. Ratios of peak height to internal standard were compared with those of a reference plasma for which concentration values were traceable to certified serum standards (standard reference material 968b; National Institute of Standards and Technology, Gaithersburg, MD). The precision between series was 5.7% for the tocopherols and 7.9% for the carotenoids.

Data on glucose metabolism

After an overnight fast, the subjects underwent a 75-g oral-glucose-tolerance test (OGTT). Blood samples for the measurement of glucose and insulin were drawn at −5, 0, 30, and 120 min, and blood samples for the measurement of NEFAs were drawn at 0 and 120 min. On a separate day, the subjects underwent an intravenous-glucose-tolerance test (IVGTT). Briefly, a bolus of glucose [300 mg/kg in a 50% (by vol) solution] was administered intravenously within 2 min. Samples for the measurement of serum insulin were drawn at −10, −5, 0, 2, 4, 6, 8, and 10 min. Plasma glucose concentrations were measured with a glucose oxidase method (Beckman glucose Analyzer II; Beckman Instruments, Fullerton, CA). Serum insulin concentrations were measured by radioimmunoassay (Pharmacia, Uppsala, Sweden), with an interassay CV of < 9%. Serum NEFA concentrations were measured with the use of an enzymatic colorimetric method and a commercially available kit (Wako Chemicals GmbH, Neuss, Germany). Serum total cholesterol concentrations in fasting venous samples were measured with the use of an enzymatic method on a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland).

We used the homeostasis model assessment index of insulin resistance as a surrogate measure of insulin resistance (28). The incremental serum insulin concentration at 30 min during the OGTT was used as a measure of insulin secretion during OGTT, and first-phase insulin secretion during IVGTT was estimated.
from the incremental area under the insulin curve during the first 10 min of the I V G T T (29). Baseline and 2-h serum NEFA concentrations were used as NEFA outcome variables. Glucose tolerance status at the time of this study was determined according to the 1998 World Health Organization criteria (30).

Anthropometric data

Body weight and height were measured while the subjects wore light indoor clothing but no shoes. BMI was used as a measure of relative body weight. Waist and hip circumferences were measured with an inelastic tape while the subjects were standing. Waist circumference was measured midway between the lowest rib and the iliac crest, and hip circumference was measured over the widest part of the gluteal region. The ratio of waist circumference to hip circumference was used as a measure of central obesity.

Physical activity and other data

A structured questionnaire was used to obtain data on physical activity during work, on the way to work, and at leisure time during the past 12 mo (31). Activity levels were changed to meta-

Table 1: 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>55 ± 6</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 2.5</td>
<td>25.9 ± 2.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85 ± 8.5</td>
<td>82 ± 7.5</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>102 ± 9</td>
<td>100 ± 8.5</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.81 ± 0.05</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Moderate</td>
<td>High</td>
</tr>
</tbody>
</table>

Statistical analysis

Statistical analyses were performed separately for the men and the women with the software programs BMDP (version 7.1; BMDP Statistical Software Inc, Los Angeles) and SAS (version 8.1; SAS Institute Inc, Cary, NC). A two-sided P value < 0.05 was considered statistically significant. Before analysis, variables with skewed distributions were logarithmically transformed. Intakes of antioxidants and other nutrients were adjusted for total energy according to the residual adjustment method (32); the residual values were used in the analyses. Intakes of lycopene and alcohol were, however, dichotomized because many subjects had no intake at all. Lycopene intake was considered as 0 when the intake per energy intake was less than the median and was considered as 1 when it was equal to or greater than the median. Alcohol intake was dichotomized as 0 or > 0 g/d. Length of education was categorized into 2 groups (≤ 9 and > 9 y), and smoking was categorized into 3 groups (non-smokers, ex-smokers, and smokers). Plasma tocopherol concentrations were treated in the analyses as cholesterol-adjusted values (divided by the serum cholesterol concentration). The area under the insulin curve during the I V G T T was measured by using the trapezoidal rule.

The significance of differences between the groups in continuous variables was tested by using Student’s unpaired t test, the Mann-Whitney U test, or analysis of covariance, whereas the significance of differences between the groups in categorical variables was analyzed by using the chi-square test. Pearson’s and Spearman’s partial correlation coefficients were used to test relations between dietary and plasma antioxidants after control for the effect of energy intake. Multiple linear regression analysis was used to explore whether dietary and plasma antioxidants were related to glucose metabolism independently of confounding factors. Dietary and plasma antioxidants were included in the models as continuous variables, except for dietary lycopene. In all the analyses, we included age, BMI, waist-to-hip ratio, and physical activity as continuous variables and smoking and length of education as categorical variables. Smoking was included in the models as 2 variables: the first variable was coded as 0 for nonsmokers and 1 for ex-smokers, and the second variable was coded as 0 for non-smokers and 1 for smokers. For each outcome variable, the analyses were performed as follows. First, each dietary and plasma antioxidant was separately entered into the model with the above-mentioned demographic, anthropometric, and lifestyle confounding factors. Second, if > 1 antioxidant variable showed a suggestive association with the outcome variable (P < 0.1) and if the mutual correlation between these variables was lower than 0.6, they were entered simultaneously into a second model. Finally, we also adjusted for dietary covariates, which were the energy-adjusted intakes of polyunsaturated fatty acids, dietary fiber, and alcohol. However, if a dietary covariate turned out to be highly correlated (r > 0.60) with the explanatory variable, that dietary covariate was not included in that analysis.

RESULTS

Most of the men (79%) and most of the women (78%) had normal glucose tolerance (Table 1). The energy-adjusted intake of antioxidants did not differ significantly between the men and the women, but plasma α- and β-carotene concentrations were significantly higher in the women (Table 1). Dietary intakes and plasma concentrations of the antioxidants were directly correlated (r between 0.25 and 0.45, P < 0.05), except for α-tocopherol in both sexes and β-carotene in the men (Table 2). In both sexes, plasma β-carotene concentrations were inversely correlated with BMI, plasma lycopene concentrations were inversely correlated with age, and cholesterol-adjusted γ-tocopherol concentrations were directly correlated with waist-to-hip ratio (Tables 3 and 4). In the analysis of covariance with adjustment for age, there was no difference between nonsmokers, ex-smokers, and smokers in dietary or plasma antioxidants in either the men or the women (data not shown).

In the multiple linear regression analysis, dietary intakes of α-carotene, β-carotene, and lycopene were inversely associated with fasting plasma glucose concentrations in the men (Table 5). These associations remained virtually unchanged when α-carotene or β-carotene was included in the model simultaneously with lycopene (data not shown). After further adjustment for the dietary covariates (polyunsaturated fatty acids, dietary fiber, and alcohol), the regression coefficients (± SEEs) for α-carotene and lycopene were −0.06 ± 0.02 (P = 0.017) and −0.55 ± 0.14 (P = 0.0001), respectively (goodness of fit in the model: R² = 0.46, P < 0.0001). In the joint model for β-carotene
and lycopene, with adjustment for all covariates, the regression coefficients for β-carotene and lycopene were \(-0.17 \pm 0.08 (P = 0.033)\) and \(-0.54 \pm 0.14 (P = 0.0002)\), respectively (goodness of fit in the model: \(R^2 = 0.45, P < 0.0001\)). In the women, dietary α-tocopherol and plasma β-carotene showed inverse and direct associations, respectively, with fasting plasma glucose concentrations (Table 5). The direct association between plasma α-carotene concentrations and fasting plasma glucose concentrations was nearly significant. The associations of dietary α-tocopherol and plasma β-carotene with fasting plasma glucose concentrations remained unchanged in the joint model and also after further adjustment for dietary fiber and alcohol; the regression coefficients for α-tocopherol and plasma β-carotene were \(-0.54 \pm 0.24 (P = 0.031)\) and \(0.25 \pm 0.11 (P = 0.031)\), respectively (goodness of fit in the model: \(R^2 = 0.17, P = 0.087\)). When dietary α-tocopherol and plasma α-carotene were included simultaneously in the model, their associations with fasting plasma glucose concentrations remained virtually unchanged.

To further delineate the association between dietary lycopene intake and fasting plasma glucose concentrations, we divided both the men and the women into 5 groups according to their energy-adjusted lycopene intake: group zero had no intake at all, and those who received lycopene were divided into quintiles. In the men but not in the women, the analysis of covariance showed a significant difference (\(P = 0.001\)) in fasting plasma glucose concentrations between the lycopene intake groups (Figure 1).

A direct association between cholesterol-adjusted α-tocopherol concentrations and 2-h plasma glucose concentrations was observed in both sexes (data not shown). After adjustment for demographic, anthropometric, lifestyle, and dietary covariates, the regression coefficients in the men and the women were \(0.39 \pm 0.18 (P = 0.038)\) (goodness of fit in the model: \(R^2 = 0.32, P = 0.004\)) and \(0.42 \pm 0.16 (P = 0.013)\) (goodness of fit in the model: \(R^2 = 0.24, P = 0.008\)), respectively. Other dietary and plasma antioxidant variables were unrelated to 2-h plasma glucose concentrations.

Plasma β-carotene and cholesterol-adjusted γ-tocopherol concentrations showed inverse and direct relations, respectively, to insulin resistance in the men (data not shown). When these variables were included simultaneously in the model, the regression coefficients for β-carotene and γ-tocopherol were \(-0.29 \pm 0.09 (P = 0.002)\) and \(0.31 \pm 0.14 (P = 0.031)\), respectively. After further adjustment for dietary covariates, γ-tocopherol was no longer associated with insulin resistance (\(P = 0.15\)), but β-carotene was [regression coefficient = \(-0.28 \pm 0.09; P = 0.003\) (goodness of fit in the model: \(R^2 = 0.57, P < 0.0001\))]. In the women, neither dietary nor plasma antioxidants were associated with insulin resistance.

Dietary β-carotene showed an inverse association with insulin secretion during the OGTT in the women (regression coefficient = \(-0.15 \pm 0.07; P = 0.042\)), but the association was abolished after adjustment for dietary covariates (\(P = 0.17\)). In the men, dietary and plasma antioxidants were unrelated to insulin secretion during the OGTT.

In the men, dietary α-tocopherol intakes showed an inverse relation to first-phase insulin secretion during IVGTT (\(P = 0.028\)), but after adjustment for all covariates, including dietary fiber and alcohol, the association was attenuated [regression coefficient = \(-0.72 \pm 0.38; P = 0.059\) (goodness of fit in the model: \(R^2 = 0.24, P = 0.029\))]. In the women, a suggestive inverse association between cholesterol-adjusted γ-tocopherol concentrations and first-phase insulin secretion during IVGTT (\(P = 0.059\)) was abolished (\(P = 0.12\)) after adjustment for dietary covariates.

### Table 1

Characteristics of male and female nondiabetic relatives of persons with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 81)</th>
<th>Women (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>53.0 ± 9.0</td>
<td>53.0 ± 10.4</td>
</tr>
<tr>
<td>Length of education [n (%)]</td>
<td>6–9 (basic)</td>
<td>49 (60) 58 (57)</td>
</tr>
<tr>
<td>Smoking [n (%)]</td>
<td>Non-smoker</td>
<td>50 (49)</td>
</tr>
<tr>
<td>Physical activity (MET/d)</td>
<td>10.3 ± 3.5</td>
<td>8.8 ± 3.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 4.5</td>
<td>26.6 ± 3.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.97 ± 0.06</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.4 ± 0.7</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>Fasting serum insulin (pmol/L)</td>
<td>8.9 ± 1.02</td>
<td>5.81 ± 1.05</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>5.88 ± 1.02</td>
<td>5.81 ± 1.05</td>
</tr>
<tr>
<td>Glucose tolerance status [n (%)]</td>
<td>Normal glucose tolerance</td>
<td>64 (79)</td>
</tr>
<tr>
<td>Energy intake (MJ/d)</td>
<td>9.7 (5.11)</td>
<td>6.6 (5.6–7.5)</td>
</tr>
</tbody>
</table>

### Table 2

Energy-adjusted partial correlation coefficients for correlations between dietary intakes and plasma concentrations of antioxidants in male and female nondiabetic relatives of persons with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 81)</th>
<th>Women (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Carotene</td>
<td>0.31</td>
<td>0.45</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.41</td>
<td>0.23</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.25</td>
<td>0.025</td>
</tr>
</tbody>
</table>

7 All r values are Pearson’s correlation coefficients except those for lycopene, which are Spearman’s correlation coefficients; cholesterol-adjusted values are given for plasma α- and γ-tocopherol.
In the men, a direct association was observed between dietary lycopene and baseline serum NEFA concentrations; the regression coefficient in the model with adjustment for all covariates was 0.19 ± 0.09 (P = 0.034) (goodness of fit in the model: R² = 0.18, P = 0.20). In the women, a nearly significant (P = 0.064) direct association between cholesterol-adjusted α-tocopherol concentrations and baseline serum NEFA concentrations was observed, and this association remained unchanged after further adjustment for dietary covariates (P = 0.075).

Both dietary and plasma antioxidants were unrelated to 2-h serum NEFA concentrations in the men. In the women, an inverse association between plasma β-carotene concentrations and 2-h serum NEFA concentrations was observed (regression coefficient = −0.13 ± 0.06; P = 0.038). After further adjustment for dietary covariates, this association was, however, somewhat attenuated (regression coefficient = −0.11 ± 0.06; P = 0.087) (goodness of fit in the model: R² = 0.30, P = 0.0009).

DISCUSSION

In this study in a population at high risk of type 2 diabetes, dietary intakes of α- and β-carotene and of lycopene, as well as...
plasma β-carotene concentrations, showed beneficial associations with glucose metabolism in the men: an inverse association with fasting plasma glucose concentrations was observed for the former, and an inverse association with insulin resistance was observed for the latter. Previous studies on relations between carotenoid intake and glucose metabolism are scarce. Two cross-sectional studies failed to show a relation between β-carotene intake and glycated hemoglobin (10, 11); the role of dietary lycopene has not been considered in any previous studies of which we are aware. Cross-sectional associations between serum carotenoids (α- and β-carotene, cryptoxanthin, lutein and zeaxanthin, and lycopene) and glucose and insulin concentrations were studied in the population-based US third National Health and Nutrition Examination Survey (14). Inverse relations of serum β-carotene, cryptoxanthin, and lycopene concentrations with glucose concentrations were observed, and an inverse relation was also observed between serum carotenoid concentrations and fasting serum insulin concentrations. In addition, in a small cross-sectional study, plasma concentrations of α- and β-carotene and lutein were inversely correlated with insulin resistance (15). Our finding of an inverse association between plasma β-carotene concentrations and insulin resistance in the men in the present study is consistent with these results, but our finding in the women of a direct relation between plasma β-carotene concentrations and fasting plasma glucose concentrations contrasts with the aforementioned findings. However, an independent role for β-carotene in glucose metabolism was not supported by a longitudinal study of middle-aged Finns (20). Although the incident cases had lower serum β-carotene concentrations than did the controls, the inverse association with the incidence of type 2 diabetes was abolished after adjustment for smoking status, hypertension, serum cholesterol, and BMI. Furthermore, the supplementation of US male physicians with β-carotene for an average of 12 y had no effect on their subsequent risk of type 2 diabetes (21). Thus, the studies that have been performed so far do not consistently support an independent role for β-carotene in the development of diabetes.

In the present study, dietary α-tocopherol intakes were inversely related to fasting plasma glucose concentrations in the women. Vitamin E intake was inversely associated with glycated hemoglobin in 1 (11) of 2 previous studies (10). In the Insulin Resistance and Atherosclerosis Study, the intake of vitamin E from food and supplements was not associated with insulin sensitivity after adjustment for energy intake and BMI (19). In another study, a direct relation between vitamin E intake and insulin sensitivity was found only in a subsample of supplement users (13). Supplementation with a pharmacologic dose of 900 mg vitamin E/d for

### TABLE 5

Results of multiple linear regression analyses with fasting plasma glucose concentration as the dependent variable and dietary intakes and plasma concentrations of carotenoids and tocopherols as the independent variables in male and female nondiabetic relatives of persons with type 2 diabetes.

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 81)</th>
<th>Women (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient ± SEE</td>
<td>Regression coefficient ± SEE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td><strong>Dietary intake</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Carotene</td>
<td>−0.07 ± 0.02</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>−0.24 ± 0.08</td>
<td>0.02 ± 0.06</td>
</tr>
<tr>
<td>Lycopene</td>
<td>−0.60 ± 0.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>−0.07 ± 0.28</td>
<td>0.81</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>−0.10 ± 0.14</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Plasma concentration</strong></td>
<td></td>
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</tr>
<tr>
<td>α-Carotene</td>
<td>−0.12 ± 0.11</td>
<td>0.25</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>−0.21 ± 0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Lycopene</td>
<td>−0.03 ± 0.11</td>
<td>0.76</td>
</tr>
<tr>
<td>α-Tocopherol (cholesterol-adjusted)</td>
<td>0.09 ± 0.42</td>
<td>0.83</td>
</tr>
<tr>
<td>γ-Tocopherol (cholesterol-adjusted)</td>
<td>−0.01 ± 0.23</td>
<td>0.97</td>
</tr>
</tbody>
</table>

1 Each dietary and plasma antioxidant was entered separately into the model, after adjustment for age, BMI, waist-to-hip ratio, physical activity, smoking status, and length of education.

2 Adjusted for energy according to the residual adjustment method (32) and included in the models as a residual value.

3 Divided by energy intake and dichotomized into 2 groups: 0 = less than the median, and 1 = equal to or greater than the median.
4 mo increased insulin-stimulated glucose disposal, mainly via the nonoxidative route, in healthy normoglycemic subjects, in subjects with type 2 diabetes, and in subjects with coronary artery disease (6–8). Supplementation with 200 mg vitamin E/d for 8 wk potentiated the insulin-lowering effect of fish oil in healthy men (34). The existing data, therefore, do not rule out the possibility that vitamin E, perhaps most clearly at amounts above normal dietary intakes, may be beneficial to glucose metabolism.

Three previous cross-sectional studies found an inverse correlation between plasma tocopherol and fasting plasma insulin concentrations (15–17). In the present study, the direct association between plasma γ-tocopherol concentrations and insulin resistance in the men was abolished after adjustment for dietary covariates. Of 2 previous prospective cohort studies, a low plasma vitamin E concentration was independently associated with an increased risk of diabetes in one of the studies (18) but not in the other (20). Previous studies do not give an explanation for the direct association observed in the present study between plasma α-tocopherol concentrations and 2-h glucose concentrations during OGTT. However, Caye-Vaugien et al (35) found that plasma α-tocopherol concentrations were higher in subjects with type 2 diabetes than in control subjects. The authors suggested that abnormalities in the use and transport of α-tocopherol might be involved.

So far, little is known about possible effects of antioxidants on insulin secretion. Our observation of an inverse association between α-tocopherol intake and first-phase insulin secretion in the men needs to be confirmed in further studies. Relations of dietary and plasma antioxidants with serum NEFA concentrations have, to the best of our knowledge, not been studied previously. Our findings suggest that serum NEFAs and antioxidants may be related to each other, and given the importance of serum NEFAs in defects of glucose metabolism, this topic deserves further attention.

Taken together, the available data do not show a consistent effect of carotenoids and tocopherols on glucose metabolism. The reasons for the discrepancies between the studies are unclear but may include uncertainties about dietary recording. The significant correlations between dietary intakes and corresponding plasma concentrations of antioxidants, except α-tocopherol, support the reliability of antioxidant intake data in the present study. A lack of correlation between dietary intakes and plasma concentrations of α-tocopherol has been observed previously (36) and may indicate that plasma α-tocopherol concentrations are regulated more by factors other than intake. Adjusting for potential confounding factors is critical in the analysis of exposure-outcome relations. In the present study, we adjusted for the main demographic, anthropometric, and lifestyle determinants of glucose metabolism. The possibility of residual confounding cannot, however, be ruled out in observational studies. The reasons for the discrepant results between the men and women in the present study are unknown and should be clarified in further studies.

Knowledge about the biological mechanisms linking carotenoids and tocopherols to glucose metabolism is limited. According to one hypothesis (5), oxidative stress impairs insulin action, eg, through a change in the physical state of the plasma membrane of target cells. A significant relation was reported between increased free radical production and reduced glucose disposal (37). Thus, the benefit of carotenoids and tocopherols may occur as a result of protection against oxidative damage. On the other hand, it is unlikely that associations of carotenoids and tocopherols with glucose metabolism are fully explained by their antioxidant effects (38–40). Antioxidants and reactive oxygen species were shown to affect cellular signaling and gene expression (41). Biological activities of carotenoids include induction of cell-cell communication (42). Junctional communication of β cells was shown to contribute to the control of insulin secretion and glucose tolerance (43), and this finding might provide a theoretical explanation for the observed association between dietary lycopene and fasting plasma glucose concentrations. Finally, the intake of carotenoids may simply be an indicator of diets rich in fruit and vegetables, and the intake of tocopherols may simply be an indicator of a high intake of vegetable oils, which may be associated with glucose metabolism by various mechanisms. A dietary pattern including frequent intakes of raw and salad vegetables was negatively correlated with fasting plasma glucose in middle-aged subjects (44). In the present study, dietary, but not plasma, lycopene was associated with reduced fasting plasma glucose concentrations. We have no clear explanation for this discrepancy. However, the fact that physical activity was directly correlated with dietary lycopene intake (Tables 3 and 4) suggests that lycopene intake may have acted only as a marker for an overall lifestyle pattern related to enhanced glucose metabolism. The observed inverse relations between dietary carotenoids and fasting plasma glucose concentrations warrant further studies to define whether a diet high in carotenoid-rich fruit and vegetables has a role in the prevention of diabetes in a high-risk population.

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The study was designed by AA, GA, LG, SMV, and KY. SMV and KY were responsible for dietary data collection, and LG and CS for collection of the other data. GA supervised plasma antioxidant measurements. Statistical data analysis was performed by KY. GA wrote the methods section concerning plasma antioxidant analyses; otherwise, the first draft of the manuscript was written by KY. All authors contributed to the interpretation of the results and the revising of the manuscript. None of the authors had any conflicts of interest.

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