Relation between dietary vitamin intake and resistance to insulin-mediated glucose disposal in healthy volunteers\textsuperscript{1-3}

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ABSTRACT The relation between the self-reported intake of various dietary constituents and insulin-mediated glucose disposal was evaluated in 52 healthy volunteers. Insulin-mediated glucose uptake was independently associated with degree of obesity (inversely) and estimates of level of physical activity (directly). An independent relation between increased intake of vitamin A and insulin action was shown, i.e., greater the intake of vitamin A, the more effective was insulin in stimulating glucose disposal. However, there was no independent relation noted between insulin-mediated glucose disposal and estimates of the intake of carbohydrate, protein, amount or kind of fat, fiber, or vitamins C and E. Furthermore, the 20 individuals with estimates of vitamin A consumption $> 10,000$ IU/d had significantly lower plasma glucose ($P < 0.01$) and insulin ($P < 0.05$) responses to oral glucose, and insulin-mediated glucose disposal values that were higher ($P < 0.005$) than those of the 20 individuals whose estimated vitamin A intake was $< 8000$ IU/d. These results suggest that vitamin A intake, but not intakes of vitamin C and E, fiber, fat, or carbohydrate is associated with enhanced insulin-mediated glucose disposal. Am J Clin Nutr 1996;63:946-9.

KEY WORDS Insulin sensitivity, vitamin A, vitamin E, glucose response, insulin response

INTRODUCTION Evidence has been published indicating that insulin sensitivity increased when either normal subjects or patients with non-insulin-dependent diabetes mellitus (NIDDM) consumed pharmacologic doses of vitamin E (1). In addition, it was also shown that the greater the dietary intake of vitamin A, the greater the concentration of high-density lipoprotein (HDL) cholesterol (2, 3). Because there is an inverse relation between insulin resistance and the HDL-cholesterol concentration (4), it seemed possible that insulin action may be modulated by dietary intake of vitamin A as well as vitamin E. The current study was initiated to address this issue, and involved quantifying dietary intake of multiple food constituents by questionnaire in 52 healthy volunteers, and defining the relation between these variables and measurements of plasma glucose and insulin responses to oral glucose and of insulin-mediated-glucose disposal.

SUBJECTS AND METHODS The study population consisted of 52 healthy volunteers, 20 males and 32 females, who responded to a newspaper advertisement indicating our interest in studying factors modulating insulin action and glucose tolerance in healthy individuals. The individuals selected for study were defined as healthy on the basis of medical history, physical exam, a body mass index (BMI; in kg/m$^2$) $< 30$, and normal results from routine laboratory tests and an electrocardiogram, and because they were found to be nondiabetic after a 75-g oral glucose load (5). No subject was taking any drugs known to affect glucose or insulin metabolism. The study protocol was approved by the Stanford University Institutional Review Board and written, informed consent was obtained from all subjects. The mean ($\pm$ SD) age of the volunteers was $45 \pm 14$ y and the BMI was $24.0 \pm 3.0$. Eighteen of the subjects had a family history of diabetes, whereas 21 had a family history of hypertension.

Dietary intake was quantified by use of the self-administered, food-frequency questionnaire developed and validated by Block et al (6, 7). Questionnaires were provided to each subject and they were asked to respond on the basis of their usual food consumption and with supplement intake patterns over the past year. Each subject was interviewed by a research diettian and any ambiguities were clarified in a personal interview. Estimates of nutritional intake were quantified with use of the nutrition analysis program DIETSYS (version 3.0, 1994), developed by the National Cancer Institute (Bethesda, MD).

Level of physical activity was assessed by using a previously validated self-reporting questionnaire (8) in which subjects recalled the amount of time spent during the previous week in various physical activities at work, at home, or during leisure time, classified as moderate, hard, or very hard. Multiple examples of exertion were given in each category, and empirical constants were used to convert the level of each activity into a metabolic rate (8). With this approach, final energy expenditure is estimated from the sum of the energy required for each activity. Body surface area was calculated and energy expenditure expressed as kJ $\cdot$ m$^{-2}$ $\cdot$ d$^{-1}$. The mean ($\pm$ SD) estimate of physical activity of the 52 volunteers was $6658 \pm 1180$ kJ $\cdot$ m$^{-2}$ $\cdot$ d$^{-1}$.

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**VITAMIN A AND INSULIN SENSITIVITY**

Plasma glucose (9) and insulin (10) concentrations were determined before and 30, 60, 90, 120, and 180 min after the oral administration of 75 g glucose. The total integrated area of the plasma concentrations during this 180-min period was used to quantify plasma glucose and insulin responses. The ability of insulin to promote glucose uptake was estimated by a modification (11) of the insulin-suppression test as validated by our laboratory (12). After an overnight fast, an intravenous catheter was placed in each of the patient’s arms. Blood was sampled from one arm for measurement of plasma glucose and insulin concentrations and the contralateral arm was used for administration of test substances. Somatostatin was administered [250 μg/h in a solution containing 2.5% (wt:vol) human serum albumin] to suppress endogenous insulin secretion. Simultaneously, insulin and glucose were infused at rates of 25 and 240 mg · m⁻² · min⁻¹, respectively. Blood was sampled every 0.5 h until 150 min into the study, and then every 10 min until 180 min had elapsed. The four values obtained from 150 to 180 min were averaged and considered to represent the steady-state plasma glucose (SSPG) and insulin (SSI) concentrations achieved during the infusion. Because SSSI concentrations are similar in all individuals, SSPG concentrations provide a direct estimate of insulin-mediated glucose disposal in each individual: the lower the SSPG, the more insulin-sensitive the individual.

Results are expressed as means ± SEs. Correlation coefficients were assessed by Pearson product-moment analysis. To adjust for potential confounders such as age, sex, BMI, physical activity, family history of either diabetes or hypertension, and dietary intake, multiple-regression analysis was used. A nonpaired Student’s t test was used for matched comparisons of the two groups with the lowest and highest intake of vitamin A. All P values are at 95% CIs. All calculations were performed with a commercial statistical software package (STATVIEW 512:).

**RESULTS**

The relations between the dietary variables quantified and the plasma glucose and insulin responses to oral glucose and the estimate of insulin-mediated glucose disposal (SSPG) for all subjects are shown in Table 1. It can be seen that there were no significant correlations between dietary intake of fat, protein, carbohydrate, or kind of fat, and plasma glucose or insulin response or SSPG. However, SSPG was inversely associated with intake of vitamin A and E, ie, the greater the intake of the two vitamins, the more insulin-sensitive the subject. Similarly, the greater the vitamin A and E intakes, the lower the plasma glucose response. The only other significant correlation observed was an inverse relation between fiber intake and plasma glucose response.

In an effort to further evaluate the relation between SSPG and the relative intakes of vitamin A and E, we created two subsets of the study population and compared the differences in the 11 dietary variables between the 20 volunteers with the highest (SSPG = 11.4 ± 0.6 mmol/L) and the lowest (SSPG = 3.4 ± 0.2 mmol/L) SSPG values (Table 2). Those with the highest SSPG values were those who also reported the greatest intake of vitamins A and E.

On the basis of the data in Tables 1 and 2, it appeared that insulin resistance was associated with lower intakes of both vitamin A and E. As a consequence, it was not possible to ascertain whether either, or both, were independent predictors of insulin resistance. The situation was further confounded by the fact that a significant relation (data not displayed) existed between the estimated intakes of vitamin A and E (r = 0.59, P < 0.001). In addition (data not displayed), SSPG correlated significantly with both BMI (r = 0.45, P < 0.001) and level of physical activity (r = −0.30, P < 0.05). Thus, to evaluate the independent nature of these multiple relations, multiple-regression analysis was performed with SSPG as the dependent variable and the following eight independent variables: age, sex, BMI, level of physical activity, family history of either diabetes and/or high blood pressure, and intake of either vitamin A or E. The results of this analysis are shown in Table 3. Only BMI, level of physical activity, and vitamin A intake were independently associated with SSPG, accounting for 40% of the individual variance in SSPG in the 52 subjects. When users of vitamin supplements were considered (n = 27 of 52), both vitamin A (r = −0.32, P < 0.05) and vitamin E (r = −0.39, P < 0.04) were significant predictors of SSPG in multivariate analyses, whereas in nonusers, as in the whole study population, only vitamin A was independently related to SSPG (r = −0.38, P < 0.05).

In an effort to further evaluate the relation between vitamin A intake and SSPG, values in 20 individuals with a vitamin A intake > 10 000 IU/d (x ± SE: 18 723 ± 1729 IU/d) were compared with those of 20 individuals with a vitamin A intake < 8000 IU/d (6018 ± 335 IU/d). The characteristics of these two groups as well as their plasma glucose and insulin responses and SSPG values are shown in Table 4. The two groups were not different in terms of age, sex, BMI, family history of diabetes or hypertension, and level of physical activity. On the other hand, those with the lowest vitamin A

<table>
<thead>
<tr>
<th>Variable</th>
<th>x ± SE</th>
<th>Glucose response</th>
<th>Insulin response</th>
<th>SSPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (% of energy)</td>
<td>34 ± 1</td>
<td>0.25</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>16 ± 1</td>
<td>0.27</td>
<td>0.27</td>
<td>0.12</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>50 ± 1</td>
<td>0.11</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Saturated fatty acids (g/d)</td>
<td>18.6 ± 1.3</td>
<td>0.06</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>9.8 ± 0.6</td>
<td>0.11</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>20.3 ± 1.4</td>
<td>0.13</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>13.9 ± 0.7</td>
<td>−0.30</td>
<td>0.05</td>
<td>0.21</td>
</tr>
<tr>
<td>P:S</td>
<td>0.57 ± 0.02</td>
<td>0.16</td>
<td>0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>Vitamin A (µg retinol/d)</td>
<td>3761 ± 351</td>
<td>11 284 ± 1052</td>
<td>−0.37</td>
<td>0.18</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>569 ± 120</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>90 ± 24</td>
<td>−0.39</td>
<td>0.17</td>
<td>−0.30</td>
</tr>
</tbody>
</table>

1 n = 52. PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; P:S, ratio of PUFA to saturated fatty acids. Saturated fatty acids, PUFA, and MUFA provided 11%, 6%, and 12% of energy, respectively. 2 P < 0.05. 3 P < 0.01.
intake had significantly higher SSPG concentrations and plasma glucose and insulin responses to the glucose challenge \((P < 0.005)\). When a similar comparison was made between the 20 subjects with the highest and lowest intakes of vitamin E, there was no significant difference between the plasma insulin response of the two groups. Furthermore, the increase in the SSPG of those with the lowest vitamin E intake was only 36% compared with the 57% higher SSPG value in those with the lowest reported vitamin A intake.

**DISCUSSION**

The goal of this study was to evaluate the possibility that differences in dietary intake of various types of food modify the ability of insulin to mediate glucose disposal. In particular, we wished to see whether increased consumption of vitamins A, C, or E was associated with enhanced insulin sensitivity. The results indicate that the most robust and independent relation was with regard to estimates of vitamin A consumption. As such, our results are somewhat different from those of Paolisso et al (1), who showed that the intake of pharmacologic doses of vitamin E (900 mg/d) led to an improvement in insulin-mediated glucose disposal in both normal subjects and patients with NIDDM. The most obvious explanation to account for the differences between our results and those of Paolisso et al is that we examined the relation between vitamin intake and measures of glucose and insulin metabolism that reflected the intake of 52 free-living volunteers, not the effect of adding pharmacologic amounts of vitamin E. Thus, Paolisso et al’s conclusion was based on measurement of insulin resistance before and after a large increase in vitamin E intake, and their protocol was more likely to discern relatively subtle changes in the relation between vitamin E intake and insulin resistance than was our evaluation of the cross-sectional relation between dietary estimates of vitamin E intake and measurements of insulin resistance. For example, the average daily intake of vitamin E was 90 mg in the 52 volunteers we studied, only 10% of the daily dose of vitamin E consumed by the subjects in the study by Paolisso et al.

To the best of our knowledge, the relation between increased amounts of vitamin A intake and enhanced insulin sensitivity has not been described previously. However, the fact that this conclusion was derived from both the analysis of the relation between these two variables in the entire 52 volunteers as well as from the fact that insulin resistance was significantly lower in the subset of subjects with the greatest vitamin A intake, lends credence to the basic observation. Furthermore, it is possible that previous (2, 3) descriptions of the direct relation between intake of vitamin A and HDL-cholesterol concentrations were secondary to the enhanced insulin sensitivity and lower plasma insulin concentrations seen in subjects with greater dietary intakes of vitamin A.

In conclusion, vitamin A consumption was the only dietary variable significantly related to insulin resistance in a healthy population of 52 individuals; the greater the estimated intake of vitamin A, the more insulin-sensitive the subject. Although these data do not provide a mechanistic explanation for the relation between vitamin A intake and insulin sensitivity, there are some possibilities worthy of consideration. For example, vitamin A is a physiologic antioxidant, and therefore, may
prevent oxidation of rate-limiting enzymes in the glycolytic and/or glycogen synthetic pathways. Alternatively, vitamin A may enhance endothelium-dependent arterial relaxation (13, 14), with the resultant vasodilation increasing insulin and substrate delivery to muscle capillaries, thereby enhancing insulin-mediated glucose uptake by skeletal muscle (15). Obviously, these possibilities are highly speculative, but should serve to raise hypotheses worthy of pursuit if the relation between vitamin A intake and insulin sensitivity is confirmed by other research groups.

REFERENCES