Effect of High-Dose Vitamin E on Insulin Resistance and Associated Parameters in Overweight Subjects

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OBJECTIVE — Markers of oxidative stress and plasma alanine transferase (ALT) levels are increased and circulating antioxidant concentrations are reduced in individuals with insulin resistance. Vitamin E improves glycemic control in people with diabetes. We tested the hypothesis that vitamin E would decrease markers of oxidative stress and plasma ALT levels and improve insulin sensitivity in overweight individuals.

RESEARCH DESIGN AND METHODS — Eighty overweight individuals (BMI >27 kg/m^2) were randomly allocated to receive either 800 IU vitamin E per day or a matching placebo for 3 months. The dose of vitamin E was increased to 1,200 IU per day for a further 3 months.

RESULTS — Plasma peroxides decreased by 27% at 3 months and by 29% at 6 months in the group that received vitamin E and were positively correlated with plasma vitamin E concentrations at the 6-month time point. At 3 months, fasting plasma glucose and insulin concentrations were significantly reduced and homeostasis model assessment increased. These changes were not apparent at 6 months. Plasma ALT concentrations declined significantly throughout the study period.

CONCLUSIONS — In conclusion, these findings indicate that vitamin E improves oxidative stress and hepatocellular function. Although insulin resistance also improves, this effect appears transient.

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The prevalence of diabetes is increasing dramatically in the western world (1). This increase parallels that of obesity, with insulin resistance explaining the link between these two entities (2). Although the precise mechanism responsible for insulin resistance remains unclear, it would appear that a number of adipocyte-derived factors impair insulin activity and that the secretion of these factors is altered in the obese individual (3). Diabetes results when, in addition to insulin resistance, β-cell dysfunction occurs, leading to relative insulin deficiency. Interventions shown to be effective at preventing the progression to diabetes include lifestyle modification (4,5) and certain pharmacologic agents (4,6).

One of the adipocyte-derived factors implicated in the pathogenesis of insulin resistance is the free fatty acid (FFA) (7). Although the rate of release of FFAs from individual adipocytes may not be raised in obesity, the increased amount of adipose tissue overall leads to an increase in the flux of FFAs to the liver and skeletal muscle, the two tissues most intimately involved in glucose handling (8). Once FFAs enter target tissues, they are either stored as triglycerides or are utilized as a substrate for oxidation by the cell's mitochondria. As a result of the normal process of oxidation, reactive oxygen species (ROS) are produced (9). These ROS are potentially harmful to cellular functions. To prevent these harmful effects, the cell has developed a complex antioxidant system to dispose of ROS. However, antioxidant concentrations are reduced in obese individuals, and the resulting imbalance between the production of ROS and antioxidant defenses results in oxidative stress (10). Although the relationship between oxidative stress and certain diabetes-related complications has been firmly established (11), there is still uncertainty concerning its role in the development of insulin resistance.

The liver has a significant role to play in glucose homeostasis, and hepatic insulin sensitivity is of profound importance in determining fasting glucose concentrations. In longitudinal studies, both plasma alanine transferase (ALT) and γ-glutamyl transferase (GGT) predict future risk of developing diabetes (12). Plasma GGT has important intracellular antioxidant activity. Systemic concentrations of hepatic enzymes reflect hepatocellular health. Raised levels in obese individuals probably reflect nonalcoholic fatty liver disease, which is itself a marker of insulin resistance (13). Whether antioxidant therapy results in improvements in circulating hepatic enzyme concentrations in obese individuals is uncertain.

Antioxidant therapy has been shown to improve insulin signaling in vitro (14) and improves glycemic control in individuals with established type 2 diabetes (15,16). It is unclear, however, whether

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Abbreviations: ALT, alanine transferase; AST, aspartate aminotransferase; CRP, C-reactive protein; FFA, free fatty acid; GGT, γ-glutamyl transferase; HOMA, homeostasis model assessment; IL, interleukin; ROS, reactive oxygen species; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

the use of vitamin E in overweight individuals leads to improvements in insulin sensitivity and/or the other metabolic parameters associated with insulin resistance. This study was designed to investigate these issues.

RESEARCH DESIGN AND METHODS

— Eighty-six healthy subjects, aged 31–65 years, with a BMI >27 kg/m^2 were recruited from respondents to a newspaper advertisement. Exclusion criteria were cigarette smoking, current treatment with anti-inflammatory or other medications, use of antioxidant supplements, serious illness, and clinical or biochemical evidence of acute or chronic infection. Subjects received a clinical examination, and anthropometric, health, and lifestyle information was collected. Participants gave informed and written consent. The study was approved by the Otago Ethics Committee.

Following a 1-month run-in phase, participants were randomized by block randomization with stratification for sex to receive two 400-IU natural vitamin E capsules (GoodHealth Products, Auckland, New Zealand) per day (800 IU/day) or two matching placebo capsules per day (containing only the vegetable oil included in the vitamin E capsules) for 3 months. At the end of this period, the dose of vitamin E was increased to 1,200 IU/day, with a matching increase in placebo for a further 3 months. Subjects were instructed to take vitamin E and placebo with meals. Stratification occurred for sex to ensure equal proportions in each group. The participants and the research nurses who interacted with the participants remained blinded to allocation throughout the study. Only one researcher (W.H.F.S.), who did not interact with the participants, was responsible for study drug allocation. Randomization was carried out in blocks of six. Participants were instructed not to make lifestyle changes during the study. Assessments were made at baseline and at 3 and 6 months, when blood specimens, repeat anthropometric measurements, and capsule counts to assess compliance were performed.

Laboratory methods

Venous blood was collected in tubes containing EDTA or plasma and into plain tubes, the tubes were then centrifuged at 1,500*g* for 15 min at 4°C, and plasma and serum were harvested. Aliquots of serum and plasma were stored at −80°C. Serum ALT, GGT, aspartate aminotransferase (AST), and plasma glucose, lipids, and HDL cholesterol were measured by routine automated methods in the Otago Diagnostic Laboratories, Dunedin Hospital. Plasma C-reactive protein (CRP) was measured on a Hitachi 911 autoanalyzer by a high-sensitivity immunoturbidimetric method using a commercial kit and calibrator (Roche Diagnostics, Mannheim, Germany). Plasma interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentrations were measured by high-sensitivity enzyme-linked immunosorbent assay methods using commercial kits (R&D Systems, Minneapolis, MN). The concentration of peroxides in plasma was measured as previously described (17), with an incubation time of 45 min. This method is based on the cleavage of peroxides by horseradish peroxidase, leading to oxidation of tetramethylbenzidine to a colored compound that can be measured spectrophotometrically. Plasma insulin was measured on a Hitachi EL-170 autoanalyzer using a commercial kit and calibrator (Roche Diagnostics). As determined in our laboratory, the median (interquartile range) for fasting plasma insulin in nonobese individuals (BMI <25 kg/m^2) is 27.2 (21.7–48.5) pmol/l. Plasma FFAs were measured using a commercial kit (Roche Diagnostics). Plasma α-tocopherol concentration was measured as previously described (18). Samples from an individual were measured in the same assay to reduce interassay variation for serum ALT, GGT, AST, and plasma insulin, CRP, IL-6, TNF-α, peroxides, FFA, and α-tocopherol. The homeostasis model assessment (HOMA) index of insulin sensitivity was calculated as shown (19): HOMA = (22.5 × 18)/(fasting glucose × fasting insulin).

Statistics

Values are given as median (interquartile range) unless stated otherwise. The data were analyzed according to intention to treat. A random-effects regression model that accounted for the underlying covariance structure in the data and adjusted for the baseline measures was used to analyze each outcome measure collected at 3 and 6 months. Log transformations were used for all variables, the results being reported as percentage change and 95% CIs for each time point. The data were analyzed using STATA (2003). Spearman's rank correlation coefficients were used to test for relationships between variables.

RESULTS

— Six subjects dropped out from the study for personal reasons before starting study medication. The baseline characteristics of the remaining 80 participants are shown in Table 1. No significant differences existed in any variables between the two groups at baseline. At baseline, plasma ALT, GGT, and AST concentrations at baseline were >35 IU/l in 10, 7, and 14 subjects, respectively. Self-reported alcohol consumption indicated that 55% of participants did not drink alcohol, 29% consumed 1–3 units/week, 12% consumed 4–10 units/week, and only 4% consumed >10 units/week. One subject stopped taking vitamin E during the initial 3 months but gave blood at all time points in the study. Three subjects dropped out during the final 3 months of the study (one from the treatment group and two from the placebo group). A pill count indicated that 84% of subjects during the initial 3 months and 74% during the final 3 months complied with treatment, which was defined as <20% of capsules unused. More than 90% of subjects used >70% of the capsules they received during the study.

Plasma vitamin E concentrations increased significantly in participants who were randomized to vitamin E treatment and did not change significantly in those receiving placebo (Fig. 1). Despite an increase in vitamin E dosage in the final 3 months of the study, circulating vitamin E concentrations did not increase.

Figure 2 shows the mean percentage of treatment-placebo differences in plasma glucose, insulin, triglyceride, FFA, and peroxide concentrations and HOMA in the participants during the study. At 3 months, mean treatment-placebo differences in fasting plasma glucose, insulin, and peroxide concentrations were significantly less than zero and the corresponding difference in HOMA significantly greater than zero. Plasma triglyceride concentrations were lower during vitamin E treatment, but not significantly. At 6 months, none of the treatment-placebo differences for these variables were significantly different from zero. The mean percentage of treatment-placebo difference in plasma FFA concentration was not significantly different

Table 1—*Characteristics of the subjects at baseline*

	Placebo	Vitamin E
n	39	41
Age (years)	51 (46–56)	47 (38–57)
Sex (M/F)	10/29	10/31
BMI (kg/m^2)	33.2 (29.8–38.1)	32.3 (30.4–37.5)
Waist circumference (cm)	106 (95–113)	102 (93–111)
Systolic blood pressure (mmHg)	122 (112–134)	130 (120–140)
Diastolic blood pressure (mmHg)	84 (78–88)	82 (80–90)
Plasma concentration		
Vitamin E (μmol/l)	37 (31–42)	35 (31–41)
ALT (units/l)	22 (17–28)	22 (16–30)
GGT (units/l)	21 (16–28)	20 (16–25)
AST (units/l)	25 (22–28)	27 (21–34)
Peroxides (μmol/l)	79 (57–126)	85 (61–134)
Glucose (mmol/l)	5.00 (4.80–5.30)	5.10 (4.75–5.35)
Insulin (pmol/l)	63.1 (40.9–100.7)	71.8 (53.7–109.5)
HOMA	1.26 (0.75–1.70)	1.17 (0.78–1.52)
HbA_{1c} (%)	5.4 (5.2–5.6)	5.4 (5.2–5.6)
FFA (mmol/l)	0.32 (0.24–0.45)	0.33 (0.24–0.48)
Triglyceride (mmol/l)	1.30 (1.00–1.80)	1.30 (0.95–2.10)
Cholesterol (mmol/l)	5.70 (5.13–6.38)	5.20 (4.70–6.15)
HDL cholesterol (mmol/l)	1.24 (0.92–1.45)	1.21 (1.03–1.31)
CRP (mg/l)	2.73 (1.58–5.34)	3.30 (1.67–9.16)
IL-6 (ng/l)	1.70 (1.28–2.09)	2.05 (1.14–2.83)
TNF-α (ng/l)	2.2 (1.7–2.8)	1.8 (1.5–2.3)

Data are median (interquartile range).

from zero at 3 and 6 months during the study.

The mean treatment-placebo differences, expressed as a percentage with 95% CIs, for plasma ALT, GGT, and AST concentrations at 3 months and 6 months are shown in Fig. 3. The differences for plasma ALT at 3 and 6 months were significantly different from zero. The corresponding differences for plasma GGT and AST were close to attaining statistical significance from zero.

Waist circumference ($P = 0.82$), body weight ($P = 0.77$), BMI ($P = 0.87$), blood pressure, and plasma concentrations of cholesterol, HDL cholesterol, HbA_{1c}, CRP, IL-6, and TNF-α did not change significantly in subjects receiving vitamin E compared with placebo during the study.

The decrease in fasting plasma insulin concentration was significantly correlated with the increase in plasma vitamin E concentration during the initial 3 months of the study ($r = -0.235$, $n = 80$, $P = 0.04$). Plasma peroxide concentrations were correlated significantly with plasma vitamin E concentration at baseline ($r = -0.272$, $P = 0.015$, $n = 80$). The decrease in plasma peroxide concentration was significantly correlated with the increase in plasma vitamin E concentration at 6 months ($r = -0.404$, $P = 0.01$, $n = 39$). After lipid standardization, the results remained unchanged, although the correlation between baseline vitamin E and plasma peroxides was no longer statistically significant ($r = -0.120$, $P = 0.29$).

CONCLUSIONS — Oxidative stress has been implicated in the development of insulin resistance (9), and in some studies but not all (20), antioxidant therapies were shown to reduce ROS and to improve glycemic control in people with type 2 diabetes (15,16). In addition, antioxidant concentrations are significantly reduced in individuals at increased risk of diabetes (10). However, it is unknown if antioxidant therapy improves insulin sensitivity in nondiabetic individuals who are obese. Our results suggest that vitamin E improves insulin sensitivity and the associated features of insulin resistance in overweight individuals.

The reduction in fasting plasma insulin and glucose concentrations, together with an increase in HOMA, during vitamin E supplementation suggests improved insulin sensitivity. Furthermore, the magnitude of this improvement in insulin sensitivity, as indicated by fasting insulin levels, depends on the magnitude of the increase in plasma vitamin E. It is well established that fasting insulin and HOMA together are a good marker of insulin sensitivity and correlate well with

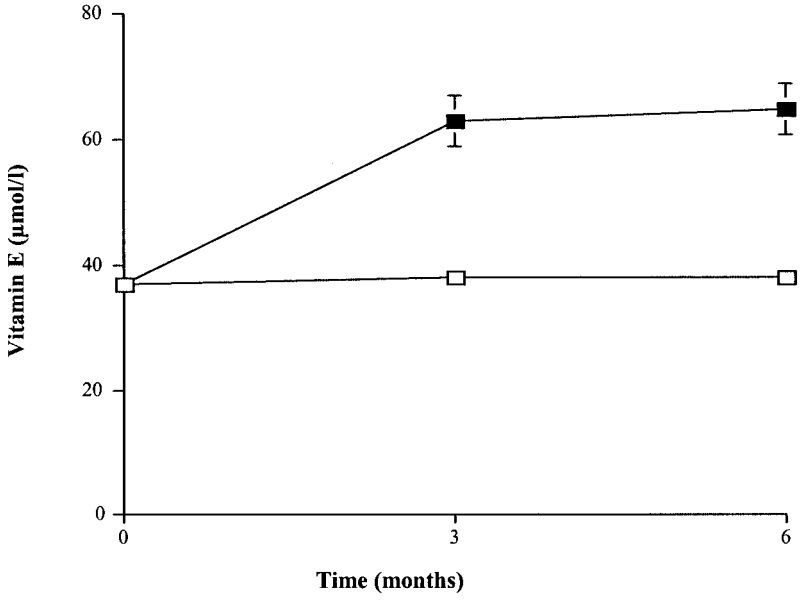

Figure 1—*Plasma concentration of vitamin E in participants receiving vitamin E (■) or placebo (□) during the study. Values are mean ± SE.*

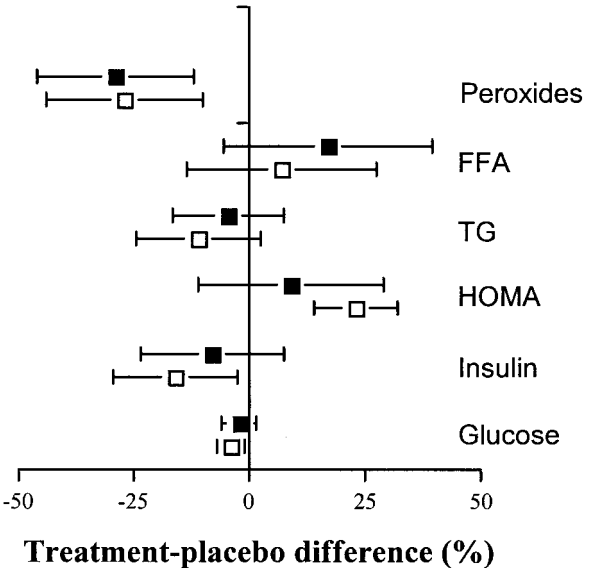

Figure 2—*Treatment-placebo difference and 95% CIs in fasting plasma glucose, insulin, FFA, triglyceride (TG), and peroxide concentrations and in HOMA at 3 (□) and 6 (■) months during the study.*

the gold-standard hyperinsulinemic-euglycemic clamp method (21). Other small studies have shown improvements in insulin sensitivity in elderly subjects (22) and diabetic individuals receiving high-dose vitamin E therapy (15), but there have been no reported studies demonstrating this effect in obese nondiabetic individuals.

Supplementation with high-dose vitamin E may improve insulin action and decrease plasma fasting insulin and glucose levels by decreasing cellular oxidant stress, altering membrane properties, and decreasing inflammatory activity. It has been postulated previously (23) that chronic administration of vitamin E at pharmacological doses (900 IU/day) improves insulin action via an improvement in the chemical-physical state of plasma membranes as a result of a decrease in oxidative stress. Red cell membrane fluidity decreased in association with a decrease in the ratio of serum oxidized to reduced glutathione and concomitantly with an increase in insulin sensitivity during 4 months of vitamin E treatment in elderly subjects (22). In addition, increased vitamin E may enhance the endogenous cellular antioxidant defense system and reduce levels of ROS that are produced by mitochondria. Increased FFA levels may overload the mitochondrial oxidation process, leading to accelerated production of ROS (7) and increased oxidant stress in obese individuals (10). Peroxides are ROS and interfere with insulin signaling (24). In the present study, the decrease in plasma peroxides may therefore contribute to the improvement in insulin sensitivity during 3 months' vitamin E treatment.

Vitamin E has a number of effects at the cellular level that are not dependent on its antioxidant activity and may potentially contribute to improved insulin action. For example, vitamin E inhibits protein kinase C by a nonantioxidant mechanism (25). Vitamin E also accelerates diacylglycerol kinase activity, thereby decreasing levels of diacylglycerol, which is an allosteric activator of protein kinase C (26). Increased protein kinase C activity apparently impairs insulin action by phosphorylating serine or threonine residues on insulin receptor and insulin receptor substrate-1 proteins (27). This decreases insulin-stimulated, phosphatidylinositol 3-kinase–catalyzed phosphorylation of tyrosine residues in these proteins, which is required for effective insulin action. Recent evidence (28) suggests that vitamin E may influence the activity of these enzymes by decreasing the curvature of plasma membranes.

The liver plays an important role in glucose and insulin metabolism. It is the main site of insulin clearance from the blood (29). Animal studies (30) show that genetic knockout of hepatic insulin receptors results in severe insulin resistance, hyperinsulinemia, glucose intolerance, and severe hepatic dysfunction. Nonalcoholic fatty liver disease is common in obese individuals and is associated with hepatic insulin resistance (31) and elevated levels of hepatic enzymes in the blood. Furthermore, in nondiabetic individuals, plasma ALT is associated with percentage of body fat, hepatic insulin resistance, and hepatic glucose output (12). GGT maintains cellular antioxidant protection by regulating intracellular levels of glutathione (32). Hepatic glucose output is the main determinant of fasting blood glucose levels. High-dose vitamin E therapy decreases markedly elevated levels of plasma hepatic enzymes in patients with nonalcoholic fatty liver disease (33). In the present study, supplementation with vitamin E significantly reduced plasma ALT concentrations. Thus, it is possible that the concomitant decrease in fasting insulin and glucose levels may be due to, at least in part, improved hepatocellular function and decreased hepatic insulin resistance and glucose output.

Adipocyte-derived cytokines, includ-

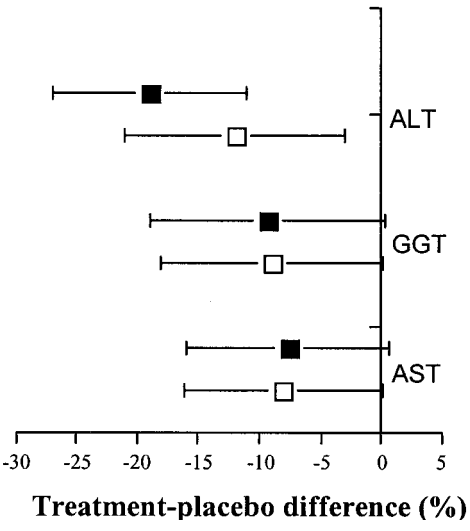

Figure 3—*Treatment-placebo difference and 95% CIs for plasma ALT, GGT, and AST activities at 3 (□) and 6 (■) months during the study.*

ing TNF and IL-6, have been associated (3) with increased inflammation and insulin resistance. Studies from our laboratory (34) and elsewhere (35) have reported decreased systemic inflammation in diabetic patients supplemented with high-dose vitamin E. In the present study, however, we did not demonstrate any significant changes in these inflammatory markers and in plasma CRP, a sensitive marker of systemic inflammation, during vitamin E supplementation. These findings suggest that changes in inflammatory status did not contribute to the observed improvement in insulin sensitivity noted during vitamin E treatment.

The effect of vitamin E on insulin sensitivity and related variables was not maintained during the final 3 months of the study. There is no obvious cause for this observation. Pill count did not suggest that this was due to a reduction in compliance. However, it is possible that intestinal absorption of vitamin E is already saturated at a dose of 800 IU/day. In addition, the antioxidant and other biological effects of vitamin E, indicated by its effect on plasma peroxide and plasma ALT concentrations, did not decline during this period. This finding suggests that mechanisms other than those responsible for these changes may have an important role in the improvement in insulin sensitivity with vitamin E treatment. We noted a trend to an increase in circulating FFAs throughout the study, which could potentially adversely affect insulin action (7). These observations, which are in contrast to the findings of others (15,21), need further investigation. We cannot exclude the possibility that in the present study the increase in the dose of vitamin E from 800 to 1,200 IU/day during the final 3 months may have contributed to the deterioration in insulin sensitivity over this period. We have undertaken a post hoc analysis to investigate the possibility that the study had insufficient power to detect a significant improvement in insulin sensitivity. Samples of the size used in this study, given the variability of the control group, have an 80% chance of detecting reductions of 18% in insulin and 4% in glucose using the 5% level of significance. Although the estimates of the effect of vitamin E were not statistically significant in the last period of the study, the CIs show that effects of a magnitude similar to those in the first follow-up period cannot be excluded. There is therefore insufficient evidence to exclude a clinically important effect of vitamin E, and a larger study is required to clarify this issue.

Numerous other features have been described as being associated with insulin resistance. A number of the more robust associations have given rise to the metabolic syndrome (36). Although the subjects in this study did not necessarily meet the criteria for the metabolic syndrome, the effects of vitamin E on its component features were evaluated. In keeping with an improvement in insulin sensitivity, we showed a significant reduction in plasma triglycerides at 3 months. This effect had disappeared by 6 months, which is consistent with the waning effect of vitamin E therapy over time. While a trend to improvement in other lipid parameters was identified, these were not significant. No change in blood pressure was identified with vitamin E therapy. The lack of weight change between the two groups makes it unlikely that other factors had a significant influence on this finding.

This study shows that vitamin E improves insulin sensitivity and several of its associated parameters in overweight individuals, but the effect of treatment is not sustained. In addition, vitamin E decreased circulating levels of ALT, a risk factor for the development of type 2 diabetes, during entire study period. These results suggest that vitamin E could have a role to play in delaying the onset of diabetes in at-risk individuals.

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