Insulin Decreases Circulating Vitamin E Levels in Humans

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Both hyperinsulinemia and free oxygen radicals have been implicated in the pathogenesis of atherosclerosis, but the relationship between insulin levels or insulin action and the oxidant/antioxidant balance has not been explored. We measured the effect of physiologic hyperinsulinemia on plasma concentrations of vitamin E, a major free radical scavenger molecule. Isoglycemic clamps (at an insulin infusion rate of 6 pmol · min^{-1} · kg^{-1}) were performed in four groups of subjects: (1) 12 non–insulin-dependent diabetic (NIDDM) patients, (2) eight patients with essential hypertension, (3) 11 nondiabetic obese individuals, and (4) 12 healthy subjects. In 10 healthy volunteers, a time-control experiment was performed by replacing the insulin infusion with normal saline. Vitamin E and plasma lipid levels were determined at baseline and after 2 hours of insulin/saline infusion. Insulin sensitivity was reduced in diabetic, obese, and hypertensive groups in comparison to healthy controls, but fasting plasma vitamin E concentrations were similar in all groups. A consistent decrement in plasma vitamin E concentrations (averaging 12% of baseline, $P < .0001$) was observed in all subjects receiving insulin regardless of the level of insulin sensitivity, whereas no significant changes in plasma vitamin E were seen in subjects receiving saline infusion ($P < .001$ *v* insulin infusion groups). The insulin-induced decrement persisted in all study groups when plasma vitamin E concentrations were corrected for total serum cholesterol levels (−8.9% ± 1.2% *v* −0.4 ± 2.3% of saline controls, $P = .0004$) or serum low-density lipoprotein (LDL (−10.0% ± 1.2% *v* −0.4% ± 2.2%, $P = .0002$). We conclude that insulin infusion acutely depletes vitamin E in circulating lipids regardless of insulin resistance. This effect may represent a physiologic means of transferring vitamin E into cell membranes; alternatively, it might reflect a pro-oxidant action of insulin in vivo.

LONGITUDINAL epidemiological evidence has shown that hyperinsulinemia is associated with an increased risk of coronary heart disease (CHD).[1-3] Hyperinsulinemia is generally considered a surrogate of insulin resistance.[4] States of insulin resistance such as non–insulin-dependent diabetes mellitus (NIDDM), essential hypertension, and dyslipidemia are known to be associated with a higher frequency of CHD.[5] Moreover, it has been reported that insulin resistance may be found in patients with atherosclerosis even in the absence of other known cardiovascular risk factors.[6] However, the pathophysiologic basis of the association between insulin resistance/hyperinsulinemia, cardiovascular risk factors, and atherosclerosis remains elusive.

A wealth of clinical and experimental data have suggested that free radical reactions (reactive oxygen species) participate in the genesis of atherosclerosis.[7] The oxidation hypothesis of atherosclerosis envisions that the atherosclerotic process can be initiated by damage to the vascular endothelium produced by oxidative modifications of low-density lipoprotein (LDL) cholesterol particles.[8] Oxidation of LDL leads to enhanced LDL cholesterol uptake by arterial wall macrophages.[9,10] Initially, this macrophage scavenger function is able to protect the arterial wall. As the oxidative process is exacerbated and/or antioxidant power declines, an excess of oxidized LDL cholesterol is taken up and the macrophage is converted to a foam cell,[11] the precursor of the atherosclerotic lesion.

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Under normal conditions, a fine balance exists between reactive oxygen species formation and antioxidant defense. It has been suggested that antioxidants such as ascorbic acid, β-carotenes, and vitamin E can protect LDL cholesterol from oxidative stress.[12,13] Recent epidemiologic evidence has indicated that vitamin E can reduce the incidence of CHD,[14,15] thus lending support to the idea that the oxidant/antioxidant balance plays an important role in atherosclerosis.

NIDDM, essential hypertension, dyslipidemia, and cigarette-smoking—all insulin-resistant states—are conditions of accelerated atherosclerosis for which enhanced peroxidative mechanisms have been proposed.[9,16-19] The present study was therefore undertaken to test whether there exists a connection between hyperinsulinemia/insulin resistance and the oxidant/antioxidant balance. The approach was chosen to examine the effect of physiologic hyperinsulinemia on plasma levels of vitamin E, the principal physiologic antioxidant, in healthy subjects and in three different groups of patients with insulin resistance.

SUBJECTS AND METHODS

Subjects

We studied four groups of subjects: (1) 12 NIDDM patients, (2) eight patients with essential hypertension, (3) 11 nondiabetic normotensive obese individuals, and (4) 19 healthy subjects. Detailed clinical characteristics of the groups are shown in Table 1. NIDDM was diagnosed using criteria of the National Diabetes Data Group.[20] The diagnosis of hypertension was based on criteria proposed by the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure.[21] Obesity was defined as a body mass index (BMI) greater than 27 kg · m^{-2}. The healthy volunteers had a BMI less than 27 kg · m^{-2}, normal arterial blood pressure, and normal tolerance to oral glucose. All subjects were free of intercurrent acute or chronic illness, and all medications (antidiabetic and antihypertensive drugs) were withdrawn 2 weeks before the study. None of the subjects were taking vitamin supplements.

Table 1. Characteristics of the Study Subjects

Characteristic	Healthy (n = 19)	Obese (n = 11)	Diabetics (n = 12)	Hypertensives (n = 8)	P†
Gender (M/F)	10/9	5/6	12/0	8/0	
Age (yr)	31.5 ± 2.2	39.2 ± 1.6*	51.8 ± 2.5*	41.3 ± 2.3*	<.0001
BMI (kg/m²)	23.2 ± 0.7	31.9 ± 1.1*	27.5 ± 0.9*	25 ± 1.0	<.0001
FPG (mmol/L)	5.0 ± 0.8	5.1 ± 0.1	7.1 ± 0.3*	5.5 ± 0.1	<.0001
FPI (pmol/L)	55 ± 6	76 ± 7*	87 ± 10*	77 ± 9*	<.04
Triglycerides (mmol/L)	1.05 ± 0.14	1.17 ± 0.14	1.88 ± 0.23*	1.32 ± 0.16	<.01
Total cholesterol (mmol/L)	4.83 ± 0.23	4.99 ± 0.23	5.28 ± 0.18	5.26 ± 0.13	NS
LDL cholesterol (mmol/L)	3.52 ± 0.21	3.66 ± 0.22	3.99 ± 0.14	3.94 ± 0.10	NS
HDL cholesterol (mmol/L)	1.11 ± 0.08	1.11 ± 0.05	0.91 ± 0.08	1.06 ± 0.1	NS
SBP (mm Hg)	118 ± 3	119 ± 2	137 ± 4*	145 ± 4*	<.0001
DBP (mm Hg)	81 ± 3	82 ± 2	87 ± 2	98 ± 2*	<.0001
Insulin sensitivity (ml · min^{-1} · kg^{-1} per pmol/mL)	14.9 ± 1.9	9.1 ± 1.9*	10.8 ± 2.9	11.4 ± 1.4	<.04

Abbreviations: FPG, fasting plasma glucose; FPI, fasting plasma insulin; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*$P \leq .05$ *v* healthy control group by unpaired *t* test.
†$P \leq .05$ by ANOVA.

The protocol was reviewed and approved by the Institutional Review Board of the National Research Council Institute of Clinical Physiology. The purpose, nature, and risks involved in the study were explained to all patients before obtaining their consent to participate.

Experimental Design and Methods

All subjects were studied at 9 AM after an overnight (10- to 12-hour) fast. A 20-gauge polyethylene catheter was inserted into an antecubital vein for infusion of test substances. A wrist vein was cannulated retrogradely with another catheter, and the hand was placed in a warming box (60°C) for arterialized blood sampling.[22] Insulin sensitivity was assessed by the isoglycemic insulin clamp technique[23] at an insulin infusion rate of 6 pmol · min^{-1} · kg^{-1}. Before the start of the study and at timed intervals during the clamp, multiple arterialized blood samples were obtained for measurement of plasma glucose (glucose oxidase method, Beckman Glucose Analyzer; Beckman Instruments, Fullerton, CA) and insulin (radioimmunoassay) levels. At time 0 and again 2 hours into the clamp study, blood samples were drawn and centrifuged for immediate measurement of plasma vitamin E (α-tocopherol) concentrations with the high-performance liquid chromatography method of Lang et al.[24] Blood samples were also taken at 0 and 2 hours for serum lipid analysis.[25-27] Plasma LDL cholesterol was calculated by subtracting high-density lipoprotein (HDL) cholesterol plus one fifth of triglycerides from total cholesterol concentrations.[28]

In 10 healthy volunteers, a time-control experiment was performed by replacing the insulin infusion with normal saline for a period of 2 hours. Twelve other healthy volunteers received the insulin clamp as described earlier (three subjects were studied with both protocols). In four additional healthy subjects, the insulin clamp was extended to 4 hours and plasma vitamin E and lipids were determined at baseline and 2 and 4 hours into the experiment.

All measurements were made in duplicate. In our laboratory, intraassay coefficients of variation for measurement of plasma concentrations of vitamin E, total cholesterol, and triglycerides are 3.0%, 1.1%, and 2.0%, respectively.

Data Analysis

Since previous studies have demonstrated that hepatic glucose production is fully suppressed during the second hour of an insulin clamp with the insulin dose used in the present studies,[29] whole-body glucose utilization (M value) was calculated from the infusion rate of exogenous glucose after correction for changes in glucose levels in a distribution volume of 250 mL · kg^{-1}.[30] The whole-body glucose clearance rate was calculated as the ratio of M to the steady-state plasma glucose concentration. Insulin sensitivity was expressed as the ratio of glucose clearance to the steady-state plasma insulin concentration.

All data are presented as the mean ± SEM. Glucose disposal rate measurements were averaged over the second hour of the euglycemic clamp study, during which near–steady-state conditions prevailed.

Statistical significance of differences between group means was tested with ANOVA; Fisher's post hoc analysis was used to identify specific intergroup differences. Paired differences were analyzed by paired Student's *t* tests. Simple or multiple linear regression analysis was performed by standard methods.

RESULTS

Clinical characteristics of the study subjects differed between groups, as expected from the selection criteria (Table 1). In addition, serum triglycerides, but not total, HDL, or LDL cholesterol, were significantly higher in the diabetic group as compared with healthy controls ($P < .001$). Fasting plasma insulin concentrations were significantly higher and insulin sensitivity was lower in patients than in controls. Fasting insulin and insulin sensitivity were inversely related ($r = .60, P < .0001$).

Baseline plasma vitamin E concentrations were similar across all groups. This was still the case when plasma vitamin E concentrations were expressed as a ratio to total cholesterol, to LDL cholesterol, or to total lipids (sum of total cholesterol and triglycerides) (Table 2). After 2 hours of isoglycemic hyperinsulinemia, plasma vitamin E levels decreased significantly in all groups ($P < .0001$). No changes were observed in the group receiving saline infusion. The percent decrements in vitamin E levels were comparable in all groups (as were the absolute changes) and all exceeded those observed during the saline infusion experiments ($P < .001$; Fig 1).

In response to insulin but not to saline infusion, a small but significant decrement of serum total cholesterol was observed at the end of the 2-hour test. This change was largely the result of decreased serum LDL cholesterol concentrations (−0.12 ± 0.02 mmol/L), since HDL choles-

Table 2. Vitamin E and Lipid Concentrations in the Fasting State and Following Insulin Administration*

Parameter	Time Control (n = 10)	Healthy (n = 12)	Obese (n = 11)	Diabetic (n = 9)	Hypertensive (n = 8)	Group*	Treatment†
Vitamin E (μmol/L)							
Fasting	13.9 ± 1.4	17.0 ± 2.1	14.4 ± 0.9	16.2 ± 2.9	16.2 ± 1.7	NS	<.0001
Insulin	13.8 ± 1.4	15.0 ± 1.9	12.8 ± 0.8	13.7 ± 2.3	14.4 ± 1.7		
Total cholesterol (mmol/L)							
Fasting	4.75 ± 0.20	5.01 ± 0.33	4.99 ± 0.23	5.29 ± 0.23	5.26 ± 0.13	NS	<.0001
Insulin	4.67 ± 0.23	4.77 ± 0.31	4.84 ± 0.22	5.10 ± 0.26	5.12 ± 0.13		
LDL cholesterol (mmol/L)							
Fasting	3.47 ± 0.21	3.67 ± 0.31	3.66 ± 0.22	3.99 ± 0.19	3.94 ± 0.10	NS	<.0001
Insulin	3.44 ± 0.22	3.54 ± 0.30	3.54 ± 0.22	3.84 ± 0.19	3.85 ± 0.12		
HDL cholesterol (mmol/L)							
Fasting	1.09 ± 0.10	1.11 ± 0.10	1.11 ± 0.05	0.88 ± 0.08	1.06 ± 0.10	NS	<.001
Insulin	1.06 ± 0.10	1.04 ± 0.07	1.06 ± 0.05	0.85 ± 0.05	1.03 ± 0.10		
Triglycerides (mmol/L)							
Fasting	0.94 ± 0.12	1.19 ± 0.23	1.17 ± 0.11	2.09 ± 0.27	1.32 ± 0.16	<.001	<.001
Insulin	0.81 ± 0.08	0.95 ± 0.18	1.15 ± 0.09	1.98 ± 0.26	1.15 ± 0.17		
Vitamin E/total cholesterol (μmol/mmol)							
Fasting	2.89 ± 0.20	3.29 ± 0.25	2.92 ± 0.10	2.99 ± 0.38	3.09 ± 0.33	NS	<.0002
Insulin	2.92 ± 0.20	3.05 ± 0.23	2.68 ± 0.17	2.64 ± 0.32	2.83 ± 0.35		
Vitamin E/LDL (μmol/mmol)							
Fasting	3.98 ± 0.27	4.51 ± 0.27	4.04 ± 0.29	3.96 ± 0.49	4.15 ± 0.44	NS	<.0001
Insulin	3.99 ± 0.29	4.13 ± 0.26	3.71 ± 0.26	3.50 ± 0.41	3.76 ± 0.46		
Vitamin E/triglycerides (μmol/mmol)							
Fasting	16.3 ± 2.0	17.0 ± 2.3	13.3 ± 1.5	8.8 ± 1.9	13.8 ± 2.5	<.01	NS
Insulin	17.9 ± 2.1	19.2 ± 2.2	11.9 ± 1.3	7.8 ± 1.6	14.8 ± 3.16		
Vitamin E/total lipid (μmol/mmol)							
Fasting	2.42 ± 0.18	2.68 ± 0.19	2.14 ± 0.24	2.36 ± 0.16	2.47 ± 0.25	NS	<.0001
Insulin	2.48 ± 0.17	2.57 ± 0.19	1.91 ± 0.22	2.16 ± 0.14	2.32 ± 0.29		

*Between-group differences.

†Effect of insulin (or saline) by two-way ANOVA with repeated measures over time.

terol levels changed consistently but by a smaller amount (−0.04 ± 0.01 mmol/L). Serum triglyceride levels decreased significantly in response to insulin (but not to saline) in all study groups, except for the obese group (Table 2).

When plasma vitamin E concentrations were expressed as a ratio to total cholesterol, LDL cholesterol, or total lipids, significant decrements from baseline in these ratios were observed in all groups receiving insulin infusion, whereas these ratios were unaltered in time-control experiments. As a ratio to triglycerides alone, plasma vitamin E declined significantly in obese and diabetic patients but not in controls or hypertensives. Figure 2 shows the vitamin E to LDL cholesterol ratio as a percent change from baseline in all study groups, including subjects in whom a 4-hour insulin clamp was performed. In the latter group, the vitamin E to LDL cholesterol ratio decreased by 10% ± 2% after 2 hours, and a further decrement (to −17% ± 5% of baseline) was observed at the end of the experiment ($P = .06$ for the trend).

By simple correlation analysis, plasma vitamin E levels were directly related to total and LDL cholesterol levels both in the fasting state (both $r = .65$, $p < .0001$) and following insulin infusion ($r = .62$ and .61, respectively, $P < .0001$). The corresponding relationships with serum

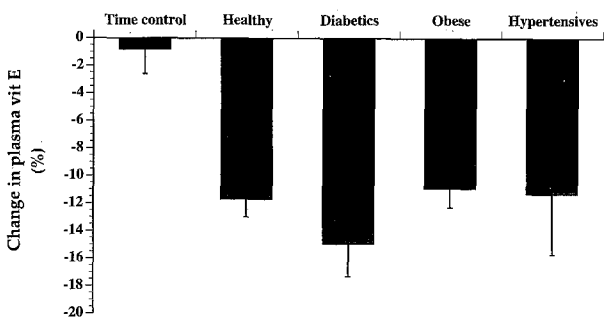

Fig 1. Percent changes from baseline in total plasma vitamin (vit) E concentrations during isoglycemic insulin (or saline for time-control) administration in healthy volunteers and patients with insulin resistance.

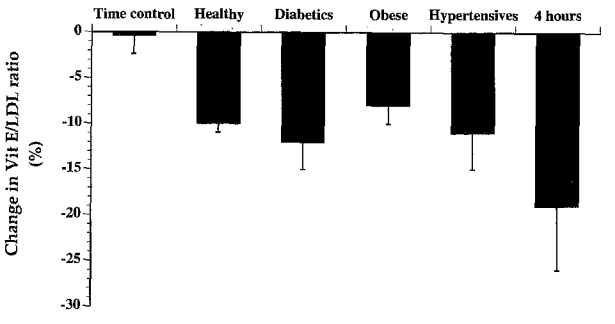

Fig 2. Percent changes from baseline in the ratio of total plasma vitamin E to serum LDL cholesterol during isoglycemic insulin (or saline for time-control) administration in healthy subjects and patients with insulin resistance. 4 hours, the group of healthy subjects in whom insulin infusion was protracted to 4 hours.

triglyceride concentrations were weaker ($r = .47$ and .33 before and after insulin, $P < .01$), whereas there was no association with serum HDL cholesterol. In the pooled data from all studies, changes in plasma vitamin E were directly related to the concomitant changes in LDL cholesterol (Fig 3). None of the vitamin E measurements or changes were related to insulin sensitivity (whether expressed as the M value or the ratio of glucose clearance to steady-state plasma insulin levels).

DISCUSSION

Vitamin E is a potent antioxidant with a protective role against oxidative damage in native LDL cholesterol.[31] Oxidative modification of LDL particles increases their atherogenicity,[9] and is possibly an obligatory step in the pathogenesis of the atherosclerotic lesion.[9] It has been demonstrated that rapid oxidation can only commence when the LDL particle is depleted of vitamin E.[32] Dietary vitamin E supplementation increases the vitamin E content of LDL cholesterol and strengthens its resistance to in vitro oxidation.[33] A high intake of vitamin E is associated with a lower risk of CHD in men[14] and women.[15] Thus, there are sufficient grounds to postulate that any intervention that decreases plasma vitamin E levels has the potential to alter the oxidant/antioxidant ratio, thereby favoring the atherosclerotic process.

Using a controlled experimental design, we observed that physiologic hyperinsulinemia (under isoglycemic conditions) causes a consistent decrement in circulating vitamin E levels ($-12\% \pm 1\%$ in the whole study group, $P < .0001$ *v* $-1\% \pm 2\%$ of the saline control experiments). The decrement was similar in healthy insulin-sensitive volunteers and in patients with insulin resistance of different etiologies (obesity, NIDDM, or essential hypertension). Thus, the effect of insulin on vitamin E was independent of the presence of insulin resistance in the glucose metabolic pathways.

Vitamin E is highly lipophilic and circulates bound to lipoproteins.[31,34] In our study, a strong direct relationship existed between total or LDL cholesterol and vitamin E levels both at baseline and following insulin, in accordance with the findings of other investigators.[34] The observed insulin-induced changes in total plasma vitamin E levels could be secondary to changes in the serum concentration of lipid fractions, particularly LDL cholesterol. During insulin infusion, we observed a small but consistent decrease in plasma total cholesterol concentrations in all study groups, which was largely due to the decrease in LDL cholesterol (Table 2). A similar finding has been previously reported in normal individuals[35] and NIDDM patients[36] and has been attributed to augmented LDL cholesterol uptake through insulin stimulation of LDL cholesterol receptor activity.[35] Nevertheless, in our study, the ratio of vitamin E to LDL cholesterol decreased significantly ($P < .0001$) in all insulin infusion studies ($-10 \pm 1\%$, $n = 40$) in comparison to the control infusions ($0.4\% \pm 2.2\%$, $n = 10$, $P = .0007$; Fig 2). Thus, the decrease in circulating LDL cholesterol accounted for only approximately 20% of the change in plasma vitamin E concentrations (as calculated from the regression in Fig 3).

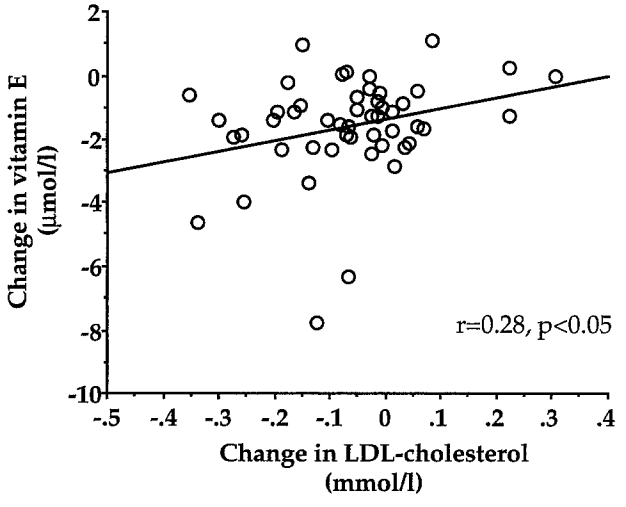

Fig 3. Relationship between insulin-induced changes in plasma vitamin E and LDL cholesterol concentrations in 50 study subjects.

Serum triglycerides also decreased with insulin infusion, the response being blunted in diabetics ($5\% \pm 2\%$ *v* $-19\% \pm 6\%$ of controls, $P = .08$) and absent in obese subjects ($0.3\% \pm 5\%$), presumably as a result of the insulin resistance of lipolysis present in these conditions.[37] It could therefore be argued that vitamin E changes paralleled total lipids rather than LDL cholesterol alone. However, when vitamin E changes were expressed as a ratio to total lipids (total cholesterol plus triglycerides), insulin administration was still associated with a significant decrease in the vitamin E to total lipids ratio in all groups ($-7\% \pm 1\%$ *v* $3\% \pm 3\%$ of saline infusion, $P < .04$). Thus, regardless of the lipid fraction considered, insulin caused a decrement of vitamin E in the circulation.

The International Collaborative Study on the Fatty Acid–Antioxidant Hypothesis of Atherosclerosis and the Optional Study on Antioxidant Vitamins and Polyunsaturated Fatty Acids by the World Health Organization/MONICA constitutes the largest study (1,176 individuals from 12 European countries) in which plasma levels of vitamin E and lipids were related to the incidence of CHD mortality.[38] A significant inverse association ($r = .49$) between the lipid-standardized α-tocopherol concentrations and CHD mortality was found. From the regression line, one can calculate that every 18% decrement in the plasma lipid-standardized vitamin E level corresponds to an excess of 200 CHD deaths. In the current study, the mean change in vitamin E enrichment of total lipids (-7%) would translate into a detectable increase ($n = 78$) in incident CHD deaths. Moreover, when euglycemic hyperinsulinemia was maintained for a further 2 hours, an additional decrease in the ratio was observed (Fig 2), suggesting that this effect of insulin is time-dependent and in all likelihood biologically relevant.

Total plasma vitamin E content only depends on the relative rates of entry and removal of plasma lipid moieties, since vitamin E consumption does not seem to occur in the

circulation.[39] In fact, the ratio of plasma vitamin E to LDL cholesterol is reported to be a reliable index of tissue vitamin E status.[34,40,41] Under the influence of experimental manipulations (eg, insulin), lipids leaving the plasma compartment (LDL cholesterol) will carry away a proportional amount of vitamin E. Likewise, a reduction in the rate of lipid influx into the circulation (triglycerides) will proportionally decrease the amount of vitamin E reaching the bloodstream. Acute insulin administration, by forcing LDL cholesterol efflux and restraining very–low-density lipoprotein triglyceride influx, effectively favors the transfer of vitamin E from plasma to tissues. However, with either change in lipid traffic, the relative content of vitamin E in plasma lipids will not be altered. Therefore, in the absence of dietary input, an insulin-induced decrease in the ratio of vitamin E to lipids in the plasma must reflect an increased net loss of the vitamin to the body tissues. Such loss could result from accelerated incorporation of vitamin E into cell membranes or reduced incorporation into recycling or de novo secreted lipid particles. In this case, insulin would be a physiologic regulator of vitamin E turnover and cell membrane function. In this regard, it is pertinent to recall that insulin action is a function of the fatty acid composition of membranes, and that an alteration of the latter is associated with insulin resistance.[42] Alternatively, insulin might decrease plasma vitamin E enrichment by enhancing vitamin E consumption. In this case, insulin would behave as a physiologic pro-oxidant.

Is insulin a pro-oxidant? There is some indication that this might be the case. For example, in diabetic rats the production of free radicals is stimulated by intraperitoneal administration of insulin.[43] More direct evidence comes from studies by Krieger-Brauer and Kather.[44] They have shown that exposure of intact human fat cells to physiologic concentrations of insulin leads to a time- and dose-dependent accumulation of hydrogen peroxide in the suspension medium. This effect, which was related to the presence of a membrane-bound NADPH oxidase, persisted after cell disruption and did not require adenosine triphosphate, indicating that the receptor kinase step was bypassed.[44] These findings have been interpreted as evidence that hydrogen peroxide contributes to the intracellular propagation of the insulin signal through a tyrosine kinase–independent pathway. The implication is that in response to insulin, antioxidant activity within or close to the plasma membrane is consumed in an attempt to maintain the oxidant/antioxidant balance. In this case, vitamin E is a likely candidate to buffer insulin-induced production of hydrogen peroxide.

In the current study, insulin-induced consumption of vitamin E was independent of the level of insulin resistance. Dose-response studies are needed to establish the sensitivity of this effect, since differences between patient groups and healthy subjects may emerge at lower insulin doses. However, baseline circulating vitamin E concentrations were not different in our patients versus the controls, and plasma vitamin E has generally been found to be within or above the normal range in NIDDM.[45] On the other hand, Paolisso et al[46] have reported higher plasma free radical levels in NIDDM patients than in nondiabetic volunteers and an inverse relationship between plasma free radicals and insulin sensitivity. Furthermore, the same group has reported that high-dose, long-term vitamin E supplementation improves insulin sensitivity in NIDDM patients.[47] Clearly, more study is needed to elucidate the mechanism and significance of insulin action on vitamin E metabolism.

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