Triglyceride-lowering Effect of Dietary Vitamin E in Streptozocin-induced Diabetic Rats

Increased Lipoprotein Lipase Activity in Livers of Diabetic Rats Fed High Dietary Vitamin E

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SUMMARY

High vitamin E supplementation in the diets of streptozocin-induced diabetic rats eliminates accumulation of lipid peroxides in the plasma and the liver, returns the plasma triglycerides toward normal levels, and increases the activity of lipoprotein lipase. Vitamin E has no effect on the levels of insulin or glucose. These findings suggest that vitamin E increases the total hepatic triglyceride lipase activity by increasing the lipoprotein lipase activity possibly by protecting the membrane-bound lipase against peroxidative damage.

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We have previously shown an elevation of lipid peroxides in the plasma of streptozocin-induced diabetic (SID) rats, and that dietary vitamin E supplementation inhibits the accumulation of plasma lipid peroxides in these diabetic rats.[1] Wada et al.[2] have shown that 15-hydroperoxy-arachidonic acid inhibits the activity of lipoprotein lipase bound to the coronary vessels to the rat heart.[2] Nakai et al.[3] reported a decreased hepatic triglyceride lipase activity in SID rats. These findings have prompted us to investigate the effect of high dietary vitamin E on plasma triglycerides, total hepatic triglyceride lipase (THTGL), hepatic triglyceride lipase (HTGL), and lipoprotein lipase (LPL) in diabetic rats. The results of our investigation are reported in the present communication.

MATERIALS AND METHODS

Streptozocin, sodium citrate, egg yolk lecithin, essentially fatty acid-free albumin, and thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, Missouri). Dimethylacetal was purchased from Aldrich (Milwaukee, Wisconsin). Triolein was purchased from Nu-Chek Prep (Elysian, Minnesota). [Carboxyl-^{14}C]triolein (100 mCi/mmol) was purchased from New England Nuclear (Boston, Massachusetts). Vitamin E was purchased from Eastman Kodak (Rochester, New York).

Animals and diets. Male Sprague-Dawley rats (160–200 g, Charles River Laboratories, Wilmington, Massachusetts) were made diabetic by injecting streptozocin (80 mg/kg body wt) or vehicle (0.05 M citrate buffer, pH 4.5) through the tail vein. Rats injected with streptozocin (N = 16) demonstrated glycosuria within 3 days after injection, which persisted throughout the study. After maintaining the diabetic and the nondiabetic control rats (N = 8) on chow diet for 4 wk, the diabetic rats were separated into two weight-matched groups of eight. All three groups of rats were placed on the defined diets described below. The control diet was the vitamin E-deficient diet formulated according to Draper et al.[4] but supplemented with vitamin E-acetate at 28 IU/lb diet. The nondiabetic rats (C-group) and one group of diabetic rats (D-group) were placed on the control diet. The other group of diabetic rats (E-group) was placed on a high vitamin E-supplemented diet, which consisted of the vitamin E-deficient diet (same as described by Draper et al.) supplemented with vitamin E-acetate (90 IU/lb diet). All diets were prepared by ICN Pharmaceutical Co. (Cleveland, Ohio). Rats were maintained for 10 wk on the defined diets, fasted for 6 h, then killed by exsanguination under light ether anesthesia.

Tissue preparation. The liver was removed and quickly rinsed in Tris-saline buffer (50 mM, pH 8.0). The tissue was blotted, weighed, frozen immediately in liquid nitrogen, and stored at −70°C until analysis. The tissue was homogenized in NH_4Cl-NH_4OH buffer (50 mM, pH 8.1) at 4°C with a Polytron homogenizer. The tissue homogenates were extracted at 4°C first with ether and then with acetone by the method of Henson and Schotz.[5] The acetone powder was treated with heparin as described by Tan et al.[6] to release HTGL and LPL.

Determination of THTGL, HTGL, and LPL. These assays were performed by the method of Nilsson-Ehle and Schotz.[7] To prepare the stock substrate, 500 mg of triolein, 35 mg

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egg lecithin, and 30 μCi of ^{14}C-triolein were placed in a 25-ml counting vial, to which 10 g of glycerol was added. This mixture was homogenized with a Polytron homogenizer at 4°C. The vial was capped under nitrogen and allowed to stand overnight. One volume of stock substrate was added to 4 vol Tris-HCl buffer (0.2 M, pH 8.0) containing 3% (wt/vol) bovine albumin and 1 vol heat-inactivated rat serum, and mixed vigorously. To 0.1 ml of the working substrate, 0.1 ml of the acetone powder extract or an equivalent volume of buffer as control was added. Incubations were carried out at 37°C with gentle shaking. The reaction was stopped by addition of 3.25 ml of methanol-chloroform-heptane (1.41:1.25:1, vol/vol/vol), and the ^{14}C-oleate partitioned by addition of 1.05 ml potassium carbonate-borate buffer (0.1 M, pH 10.5) as described by Schotz et al.[8] NaCl was added to duplicate assay tubes such that the final concentration was 1 M. The activity was defined as HTGL and the difference between the THTGL (without NaCl) and HTGL (with 1 M NaCl) as LPL.

Radioactivity was counted in a Beckman LS8100 liquid scintillation counter (Beckman Instruments, Fullerton, California) with quench correction. Results are expressed as nmol free fatty acid released/mg tissue/h.

Triglyceride analysis. Plasma triglycerides were determined by the method of Sampson et al.[9] using A-Gent triglyceride reagent on a Abbott Bichromatic Analyzer (ABA-100).

Glucose determination. Plasma glucose was determined by the enzymatic glucose-oxidase peroxidase reaction with 5-aminophenonazone as the chromaphore.[10] The standards and reagent were purchased from Bio-Dynamics/BMC (Indianapolis, Indiana).

Insulin determination. Plasma insulin levels were determined by RIA using guinea pig anti-porcine insulin antiserum, which was 100% cross-reactive with rat insulin. Rat insulin standards were used for calibration. Separation of bound from free immunoreactive insulin was achieved by polyethylene glycol precipitation.[11]

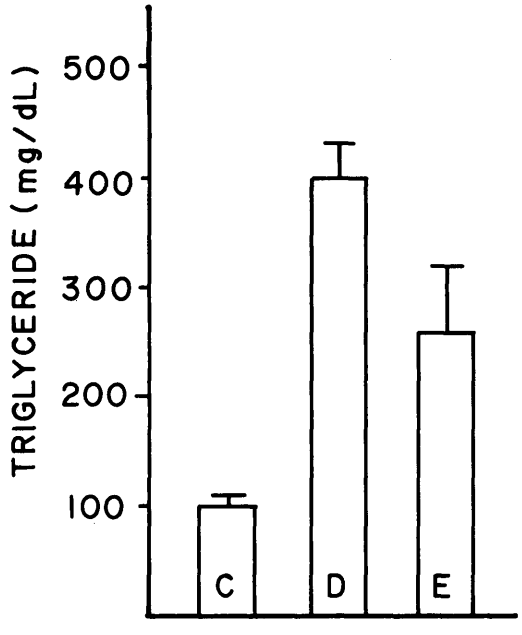

FIGURE 1. Plasma triglyceride for C-, D-, and E-groups of rats (mg/dl). N = 8 animals in each group. (C- vs D-group, P < 0.001; D- vs E-group, P < 0.02; C- vs E-group, P < 0.02).

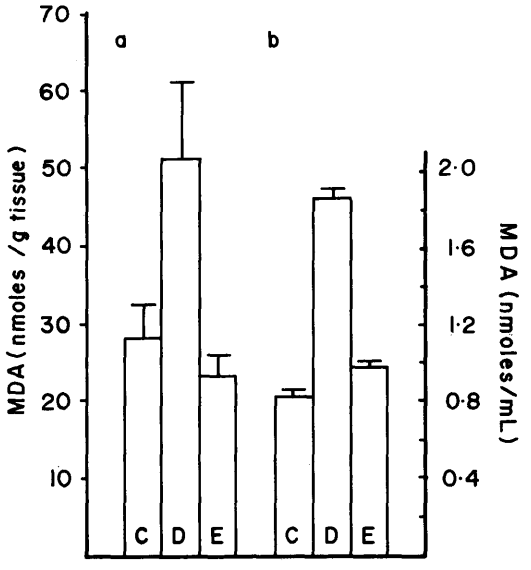

FIGURE 2. (a) Liver lipid peroxides (measured as MDA) for C-, D-, and E-groups of rats. N = 8 animals in each group. (C- vs D-group, P < 0.05; D- vs E-group, P < 0.02; and C- vs E-group, NS). (b) Plasma lipid peroxides (measured as MDA) for C-, D-, and E-groups of rats. N = 8 animals in each group. (C- vs D-group, P < 0.001; D- vs E-group, P < 0.0001; and C- vs E-group, NS).

Vitamin E analysis. Plasma vitamin E levels were determined by the HPLC method of Hatam and Kayden as previously described.[1] Liver vitamin E levels were estimated by a modification of the same procedure. Liver samples were homogenized in NH_4Cl-NH_4OH (50 mM, pH 8.1) and aliquots were removed for the saponification step. Vitamin E content was calculated based on wet tissue weight of the sample expressed as mg vitamin E/g tissue.

Determination of MDA. Lipid peroxides (MDA) in the plasma were measured by a modified method of Satoh[12] and Yagi[13] and the reaction product of thiobarituric acid (TBA) and lipid peroxides was detected fluorometrically.[13] The change from spectrophotometric to fluorophotometric detection increased the sensitivity of the analysis 10-fold. The standard curve for the MDA determinations was linear from 0 to 10 nmol MDA/ml. For the measurement of liver lipid peroxides, the tissue was homogenized in NH_4Cl-NH_4OH buffer (50 mM, pH 8.1) with a Polytron homogenizer. The homogenate was directly used for the TBA reaction. The procedure for estimating MDA in the homogenate is similar to that employed for the plasma.

Data analysis. Differences between means were analyzed for significance with the Fischer least significant difference.[14] Data are presented as mean ± SEM. NS denotes differences are not significant. Significance is defined as a P-value of ≤0.05.

RESULTS

Both D- and E-group rats displayed similar degrees of hyperglycemia and hypoinsulinemia and failed to gain weight when compared with the age-matched control group rats [glucose (mM): C = 8.7 ± 0.67, D = 30.7 ± 7.3, and E = 27.3 ± 6.6; insulin (ng/ml): C = 15.8 ± 1.2, D = 1.1 ± 0.67, and E = 1.5 ± 0.9]. Figure 1 shows plasma triglyceride values in the three groups of rats. The D-group rats exhibited

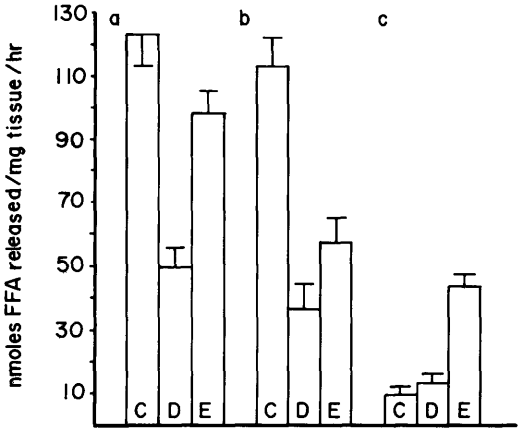

FIGURE 3. Total hepatic triglyceride lipase (a), hepatic triglyceride lipase (b), and lipoprotein lipase (c) for C-, D-, and E-groups of rats. N = 8 animals in each group. (a) C- vs D-group, $P < 0.001$, D- vs E-group, $P < 0.002$, C- vs E-group, NS; (b) C- vs D-group, $P < 0.001$, D- vs E-group, NS, C- vs E-group, $P < 0.01$; (c) C- vs D-group, NS, D- vs E-group, $P < 0.001$, C- vs E-group, $P < 0.001$.

a fourfold increase in plasma triglyceride content when compared with the C-group rats ($P < 0.001$). The E-group rats exhibited significantly lower (38%) plasma triglycerides compared with the D-group rats ($P < 0.02$), while still being significantly different from the C-group rats ($P < 0.02$).

Lipid peroxides measured as MDA in the liver tissue of the D-group rats were significantly elevated ($P < 0.05$) when compared with those in the liver of the C-group rats (Figure 2A). Lipid peroxides in the liver tissue of E-group rats were significantly decreased compared with those in the liver of D-group rats ($P < 0.02$) but not significantly different from those of C-group rats. Lipid peroxides measured as MDA in platelet-free plasma (PFP) of the D-group rats were significantly elevated compared with MDA of C-group rats ($P < 0.001$, Figure 2B). Lipid peroxides in PFP of the E-group rats were significantly decreased compared with the D-group rats ($P < 0.001$) but not significantly different from the C-group rats.

Plasma vitamin E content of the D-group rats was 3.6 times higher than that of the C-group rats (C = 3.8 ± 0.2 μg/ml and D = 13.5 ± 2.5 μg/ml, $P < 0.01$). Plasma vitamin E content of the E-group rats was also significantly higher than that of the C-group rats (E = 13.8 ± 2.0 μg/ml, $P < 0.01$) but was not significantly different from that of the D-group rats. Even though there was no significant difference in the liver vitamin E content between the C- and D-group rats, liver vitamin E content of the E-group rats was 2.5 times higher than that of the D-group and C-group rats (C = 2.7 ± 0.2 mg/g, D = 3.3 ± 0.5 mg/g, and E = 8.2 ± 0.6 mg/g, C,D versus E, $P < 0.001$). Thus high dietary vitamin E in the E-group rats increased liver vitamin E content and the ratio of plasma vitamin E to plasma triglyceride (μg vitamin E/mg triglyceride) without changing their total plasma vitamin E content (C = 3.8 μg/mg, D = 3.4 μg/mg, and E = 5.3 μg/mg).

The activities of THTGL, HTGL, and LPL for the C-, D-, and E-group rats are shown in Figure 3. The activity of THTGL for the D-group rats was significantly reduced compared with the C-group rats ($P < 0.001$, Figure 3A). The activity of THTGL for the E-group rats was significantly increased when compared with the D-group rats ($P < 0.002$), but not significantly different from that for the C-group rats. In the presence of 1 M NaCl, LPL activity is inhibited and the remaining activity is defined as HTGL (Figure 3B). The activity of HTGL in the D- and E-group rats is significantly decreased when compared with HTGL activity in C-group rats ($P < 0.001$, $P < 0.01$, respectively). Due to variation of results there is no significant difference in HTGL activities between D- and E-group rats even though E-group HTGL activities are approximately 50% increased over D-group HTGL activities. However, with subtraction of HTGL activities from THTGL activities, there is a significant increase (approx. 400%) in the LPL activity of the E-group rats compared with that of C- and D-group rats ($P < 0.001$, Figure 3C).

DISCUSSION

The current study demonstrates the effects of dietary vitamin E on the concentration of plasma triglycerides and the activities of THTGL comprised of HTGL and salt-sensitive LPL in SID rats. Our observation of high plasma triglycerides in the D-group rats is consistent with studies from other laboratories.[15,16] When the diabetic rats were placed on a high vitamin E diet, we observed partial reduction of the concentration of plasma triglycerides.

Even though several studies have demonstrated a correlation between plasma triglycerides and the activities of extrahepatic lipoprotein lipase[15–18] and of HTGL[3,19,20] in diabetic subjects, the precise cause for the accumulation of triglyceride-rich lipoproteins from plasma of SID rats is not clear. HTGL and LPL play important roles in postheparin lipolytic activity[21] and in the metabolism of VLDL and remnants.[22,23] Recently, Nomura et al.[17] demonstrated that low HTGL activity in SID rats is in part responsible for hypertriglyceridemia. We have chosen in this investigation to measure the activities of THTGL, HTGL, and LPL by salt inhibition in the C-, D-, and E-groups of rats in order to explain the decrease of plasma triglycerides by high dietary vitamin E. High vitamin E supplementation in the diet of diabetic rats has restored the hepatic lipase activity through an increase in LPL.

In a different study,[1] we have demonstrated a reduction of lipid peroxides in the plasma of diabetic rats when diets were supplemented with high vitamin E. In the present study, liver lipid peroxides in the D-group were elevated without any significant decrease in their vitamin E content when compared with those in the C-group. However, liver lipid peroxides of the vitamin E-supplemented group were significantly decreased with a concomitant increase in liver vitamin E content when compared with those of the D-group. At the present time it is paradoxical why liver vitamin E content of the D-group rats was not decreased while their lipid peroxides were increased.

The source of lipid peroxides in the D-group rats is not known. However, it is a well-known fact that lipid peroxides result from free radical reactions in the lipids of biomembranes. Possible sources of these free radicals could be the NADPH oxidase system, lipoxygenase and cyclooxygenase. The NADPH oxidase system generates O_2^- [24] and H_2O_2,[25] potent lipid peroxidizing agents. Lipoxygenase activity, which produces hydroperoxy-fatty acids from arachidonic acid, is increased in vitamin E deficiency[26,27] and diabetes.[28] Cyclooxygenase activity, which produces endoperoxides of arachi-

donic acid, is also increased in platelets from diabetic rats[1] and humans.[29] Another consequence of increased lipid peroxides is an increase in phospholipase activity,[30] which will increase the release of arachidonic acid for the lipoxygenase and cyclooxygenase systems.

Koster and Slee demonstrated that some enzyme activities, but not others, are lost when lipid peroxidation takes place in microsomal lipid membranes.[31] Lipid peroxidation occurs in mitochondria and microsomes of liver and can be inhibited by exogenous vitamin E.[32] In this study, D-group livers had significant loss in THTGL activity (HTGL fraction), which was restored with high dietary vitamin E supplementation (salt-sensitive LPL fraction). The decrease in HTGL in SID rats might be explained in part if it was known what fraction of lipoproteins carried the lipid peroxides. Nishigaki et al.[33] reported that HDL contained significantly more lipid peroxides in diabetic subjects than in normal controls. HTGL was reported to primarily metabolize HDL,[34] which in diabetes could be lost due to the peroxidative damage by HDL particles carrying lipid peroxides. The inhibitory effect of lipid peroxides on lipase activity observed in this study is supported by a recent study[2] that demonstrated inhibition of rat coronary vessel membrane-bound lipoprotein lipase by 15-hydroperoxy-arachidonic acid.

We hypothesize that high lipid peroxides accumulate in the liver of diabetic rats and cause damage to the membrane-bound HTGL, which results in low THTGL activity and high plasma triglycerides. The loss in HTGL activity caused by peroxidative damage can be partially compensated for by high dietary vitamin E due to its antioxidatant properties and ability to increase LPL activity in the liver. In view of this observation, it would be of interest to investigate the activities of lipoprotein lipase in other tissues in relation to their vitamin E content and lipid peroxides in the diabetic rat.

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