Restoration of Prostacyclin/Thromboxane A_2 Balance in the Diabetic Rat
Influence of Dietary Vitamin E

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SUMMARY

The effect of dietary vitamin E on the synthesis of platelet thromboxane A_2 (TxA_2) and vascular prostacyclin (PGI_2) in streptozotocin-induced diabetic rats was investigated. Thrombin-induced platelet TxA_2 synthesis in diabetic rats was significantly elevated, whereas no significant difference was found with collagen-induced synthesis. Arterial PGI_2 production was significantly decreased in diabetic rats. Serum lipid peroxide levels were elevated, and platelet vitamin E levels decreased in diabetic rats. When the diabetic rats were maintained on a high vitamin E diet for 2–3 mo, thrombin-induced platelet TxA_2, vascular PGI_2, and lipid peroxides were restored to levels found in nondiabetic rats. Platelets from diabetic rats maintained on a high vitamin E diet synthesized significantly less collagen-induced TxA_2 than platelets from nondietary vitamin E-supplemented diabetic rats or nondiabetic controls. DIABETES 31:947–951, November 1982.

Several studies have shown that platelets from human diabetic subjects are hypersensitive to aggregating agents[1,2] and produce enhanced prostaglandin E[3] and TxA_2.[2,4] Hyperaggregability has also been demonstrated in platelets from streptozotocin-induced diabetic rats.[5] Decreased arterial PGI_2 release has been reported in diabetic rats[6,7] and swine.[8] We have recently reported an altered balance of PGI_2/TxA_2 in streptozotocin-induced diabetic rats.[9] Since vitamin E influences the modulation of PGI_2/TxA_2 in nondiabetic rats,[10] we have examined in the present study the influence of dietary vitamin E on the disturbed PGI_2/TxA_2 balance in streptozotocin-induced diabetic rats.

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MATERIALS AND METHODS

Diet. All diets were formulated by ICN Pharmaceutical Co. The basal vitamin E-deficient diet followed the formulation of Draper et al.[11] The vitamin E-supplemented diet consisted of the deficient diet supplemented with 200 mg of vitamin E acetate per kg of diet. The control diet consisted of the deficient diet supplemented with 44 mg of vitamin E acetate per kg of diet, which the authors and ICN Pharmaceuticals have determined to be the vitamin E content (40–50 mg/kg) of normal rat chow.

Prostaglandins were generously supplied by Dr. John E. Pike (Upjohn Chemical Company, Kalamazoo, Michigan). Tritiated thromboxane B_2 (150 Ci/mmol), prostaglandin E_2 (100 Ci/mmol), and 6-keto-prostaglandin $F_{1\alpha}$ (100 Ci/mmol) were purchased from New England Nuclear (Boston, Massachusetts). Bovine achilles-tendon collagen, bovine thrombin, bovine albumin (BA), and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, Missouri). Soluble calfskin collagen was purchased from Worthington Biochemical Corp. (Freehold, New Jersey). Alpha-D-tocopherol was purchased from Eastman Kodak Co. (Rochester, New York). Urine test strips (Keto-Diastix) were purchased from Ames Division, Miles Laboratories, Inc. (Elkhart, Indiana).

Thrombin was dissolved in calcium-free Krebs-Henseleit buffer immediately before use. Radioimmunoassay (RIA) buffer (pH 7.6) consisted of TRIS (50 mM)-BA (0.1%). Tritiated prostaglandins were dissolved in ethanol, stored at −20°C, and diluted in RIA buffer before use. Unlabeled prostaglandins were diluted in RIA buffer and stored at −20°C. Bovine achilles-tendon collagen suspension was prepared according to Nakanishi et al.,[12] and standardized using soluble calfskin collagen and the protein determination of Lowry et al.[13]

Diabetes and dietary regimen. Male Sprague-Dawley rats (120–180 g) were administered streptozotocin (80 mg/kg) or vehicle (0.05 M citrate buffer, pH 4.5) through a tail vein. Glucosuria was detected within 5 days and persisted throughout the study. The rats were maintained on Purina Lab Chow and water ad libitum for 5 wk, at which time non-

diabetic controls (C) and part of the diabetic group (D) were placed on the control diet, while the remaining diabetic rats (E) were placed on the vitamin E-supplemented diet. The three groups of rats were maintained on these diets for 8–12 wk. For analysis, nonfasted rats under pentobarbital anesthesia (100 mg/kg i.p.) were exsanguinated using cardiac puncture, and aortas were excised as previously described.[14]

Platelet TxB_2 and PGE_2. Platelet synthesis of TxA_2 was determined by RIA of thromboxane B_2, its stable hydrolysis product. RIA of platelet TxB_2 was performed using a previously described method.[10] Washed platelets (1×10^8/0.5 ml incubation volume) were stirred at 37°C with thrombin, collagen, or arachidonic acid, and assays for TxB_2 and PGE_2 were performed on ether extracts after evaporation and dilution in RIA buffer. Concentrations of thrombin, collagen, and arachidonic acid were selected from linear portions of dose-response plots relating TxB_2 formation to concentrations of these agents. Cross-reactivity of the TxB_2 antiserum is as follows: $PGF_{2\alpha}$, 0.26%; PGE_2, 0.021%; PGD_2, 1.6%; 6-keto-$PGF_{1\alpha}$, 0.023%; arachidonic acid, 0.001%; and α-D-tocopherol, 0.0015%. Cross-reactivity of the PGE_2 antiserum is as follows: $PGF_{2\alpha}$, 0.31%; PGD_2, 0.051%; TxB_2, 0.0078%; 6-keto-$PGF_{1\alpha}$, 0.04%; and arachidonic acid, 0.001%.

Aortic 6-keto-$PGF_{1\alpha}$. Aortic 6-keto-$PGF_{1\alpha}$ was estimated by RIA as previously described.[10] Slices of thoracic aorta (8–12 mg) were incubated in TRIS-NaCl buffer (pH 8.0) at 37°C for 90 min in the presence or absence of 27.4 μM arachidonic acid. When tissue slices are incubated in buffer, PGI_2 is synthesized from endogenous endothelial arachidonic acid and released into the incubation media, where it is converted to 6-keto-$PGF_{1\alpha}$. This condition is referred to as "endogenous" synthesis, whereas when arachidonic acid is added to the aortic incubation buffer, the production of PGI_2 and conversion to 6-keto-$PGF_{1\alpha}$ is referred to as "exogenous" synthesis, even though there is an endogenous contribution to 6-keto-$PGF_{1\alpha}$ synthesis in the latter condition. RIA was performed after appropriate dilution of aqueous aliquots of aortic supernatant with RIA buffer. Cross-reactivity of the 6-keto-$PGF_{1\alpha}$ antiserum is as follows: PGD_2, 0.02%; PGE_2, 0.15%; $PGF_{2\alpha}$, 0.10%; arachidonic acid, 0.005%; and α-D-tocopherol, 0.0015%.

Plasma glucose. Plasma glucose was measured with coupled glucose-oxidase and peroxidase reactions using 5-aminophenazone as the chromophore.[15] The standards and reagents were purchased in kit form from Bio-Dynamics/BMC (Indianapolis, Indiana).

Plasma insulin. RIA of plasma insulin was performed using a guinea pig anti-porcine insulin antiserum that is 100% cross-reactive with rat insulin. Rat insulin standards were used for calibration. Polyethylene glycol precipitation was used to separate bound and free immunoreactive insulin.[16]

Estimation of lipid peroxides. Lipid peroxides in platelet-free plasma (PFP) were estimated as malonyldialdehyde (MDA) by reaction with thiobarbituric acid (TBA) as described by Satoh et al.[17] Fluorescence was measured by excitation at 532 nm and emission at 548 nm on an Aminco-Bowman Spectrophotofluorometer.

Vitamin E analysis. Platelet and plasma vitamin E were assayed by HPLC as described previously by Hatam and Kayden.[18]

TABLE 1
Plasma glucose, insulin, and weights of rats at death

	C	D	E
Glucose (mM)	8.0 ± 0.5	35.9 ± 2.2	32.3 ± 2.1
Insulin (ng/ml)	16.8 ± 3.4	1.1 ± 0.2	1.5 ± 0.3
Weight (g)	472 ± 16	181 ± 9	163 ± 11

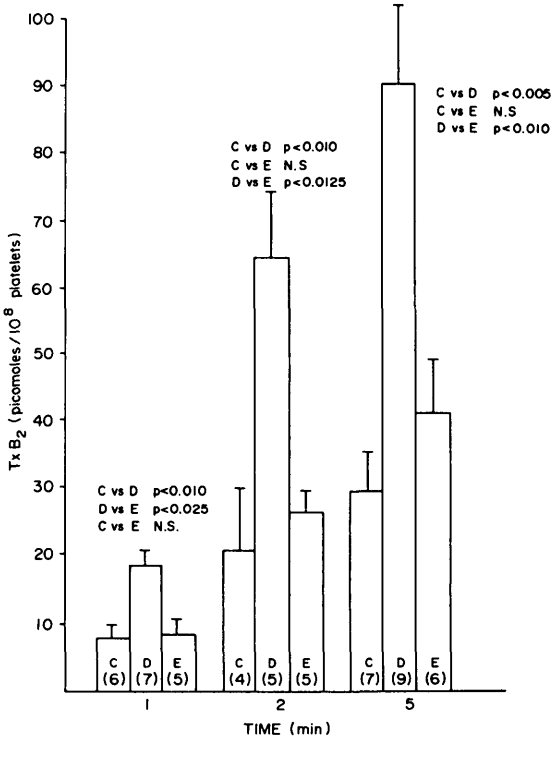
Statistical analysis of data. Differences between means were analyzed for significance using the protected Fisher least significant difference. Data are presented as mean ± SEM and numbers in parentheses represent the number of animals in each group. NS denotes differences as not significant.

RESULTS

Data regarding the metabolic state of the diabetic rats are presented in Table 1. Both groups of diabetic rats displayed similar degrees of hyperglycemia and hypoinsulinemia, and failed to gain weight when compared with the age-matched controls.

Platelet biosynthesis of TxB_2 and PGE_2. Thrombin-induced TxB_2 and PGE_2 production are displayed in Figures 1 and 2, respectively. At all incubation times, both TxB_2 and PGE_2 formation were increased in the D group when compared with the C group. The E group platelets generated amounts of TxB_2 and PGE_2 that were significantly less than the D group and not significantly different from the C group. These data indicate that dietary vitamin E supplementation to diabetic rats restored the increased thrombin-induced platelet TxB_2 and PGE_2 production found in the diabetic group to levels found in age-matched nondiabetic controls.

FIGURE 1. Thrombin (0.5 U/ml)-induced platelet TxB_2 synthesis in C, D, and E group rats. Abscissa denotes time of incubation after thrombin addition.



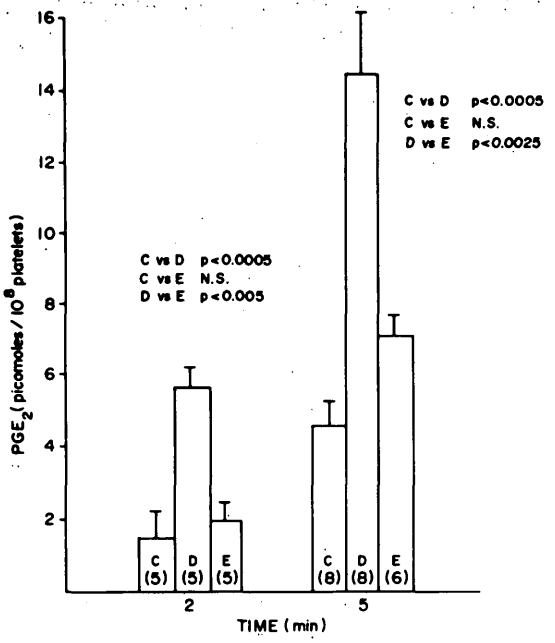
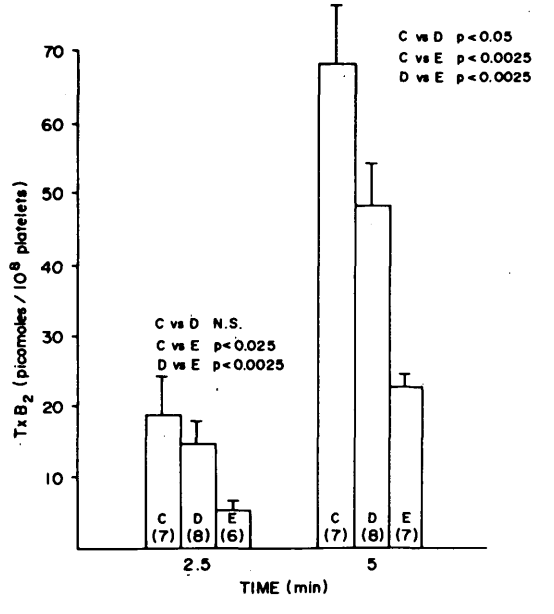


FIGURE 2. Thrombin (0.5 U/ml)-induced platelet PGE_2 synthesis in C, D, and E group rats. Abscissa denotes time of incubation after thrombin addition.

Platelets from diabetic rats responded differently to collagen. Platelets from the D group synthesized marginally less collagen-induced TxB_2 than controls at 5-min incubation ($P < 0.05$), while at 2.5-min incubation, differences were not significant (Figure 3). Collagen-induced PGE_2 production was unchanged between the D and C groups (Figure 4). The E group animals produced significantly less collagen-induced TxB_2 and PGE_2 when compared with the D group animals, indicating that vitamin E supplementation to diabetic rats decreases TxB_2 and PGE_2 synthesis in their platelets.

Synthesis of platelet TxB_2 from exogenous arachidonic acid (4.4 μM) was 288 ± 23, 301 ± 16, and 292 ± 19

FIGURE 3. Collagen (84 μg/ml)-induced platelet TxB_2 synthesis in C, D, and E group rats. Abscissa denotes time of incubation after collagen addition.



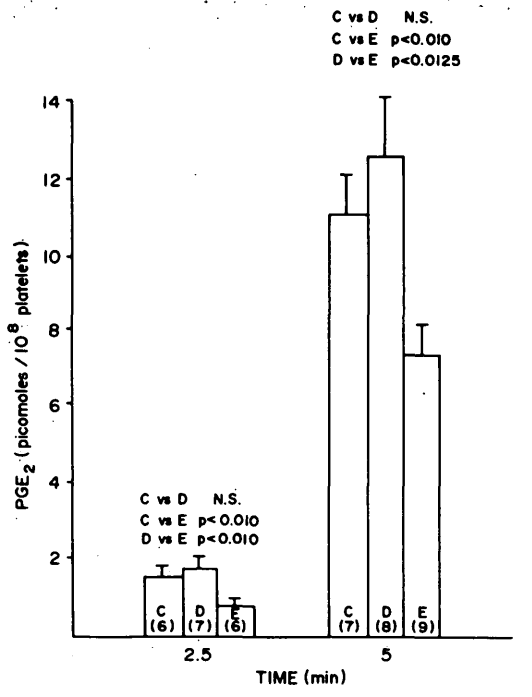


FIGURE 4. Collagen (84 μg/ml)-induced platelet PGE_2 synthesis in C, D, and E group rats. Abscissa denotes time of incubation after collagen addition.

pmol/10^8 platelets for the C, D, and E groups, respectively (N = 10). Differences between the C, D, and E groups were not significant. These data confirm our previous study showing that the cyclooxygenase-thromboxane synthetase system is unaltered in diabetic rat platelets.[9]

Aortic PGI_2 production. Production of aortic PGI_2 from endogenous and exogenous arachidonic acid was measured using an RIA for 6-keto-$PGF_{1\alpha}$. Regardless of whether endogenous or exogenous synthesis of 6-keto-$PGF_{1\alpha}$ is considered, D group aortas produced less PGI_2 than aortas from the C or E groups, while PGI_2 formation from aortas of the latter two groups was not statistically different (Figure 5). Dietary vitamin E supplementation restored the depressed levels of PGI_2 production by diabetic aortas to control group levels.

Platelet vitamin E levels. Vitamin E in the platelets of the D group was undetectable (10^9 platelets were used for assay in each of the three groups). The sensitivity limit for the assay is therefore reported for D group vitamin E values. Mean platelet vitamin E (μg/10^9 platelets) in the C, D, and E groups was 0.41 ± 0.06, ≤ 0.14, and 1.15 ± 0.14 (N = 7), respectively. Dietary vitamin E supplementation increased platelet vitamin E levels (E group) beyond the levels found in the control group platelets.

Plasma MDA levels. MDA in platelet-free plasma (nmol/ml) for the C, D, and E group rats was 0.83 ± 0.07, 1.88 ± 0.17, and 1.00 ± 0.09, respectively (N = 9). MDA was significantly increased in the D group ($P < 0.0005$) when compared with the C or E groups, while MDA in the E group was not significantly different from that of the C group. The mean MDA level in each group was inversely related to the mean aortic PGI_2 production ($r = -0.998$ with PGI_2 production from endogenous arachidonate). Vitamin E supplementation to diabetic rats reduced the elevated plasma MDA to the levels found in nondiabetic rats.

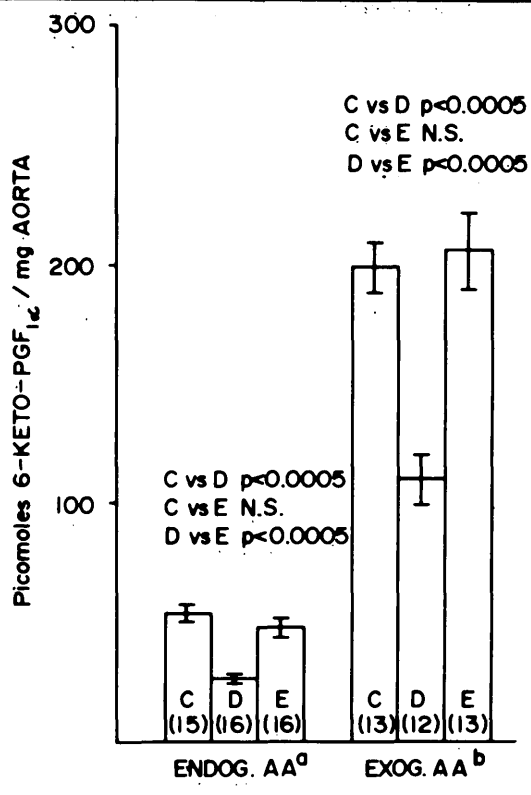


FIGURE 5. Synthesis of 6-keto-$PGF_{1\alpha}$ from aortas of C, D, and E group rats from either endogenous or exogenous (27.4 μM) arachidonic acid (AA). Incubation time = 90 min. [a]Tissue stirred in the absence of added arachidonic acid. [b]Tissue stirred in the presence of added arachidonic acid.

DISCUSSION

The present study demonstrates the effects of dietary vitamin E on the PGI_2/TxA_2 balance in streptozotocin-induced diabetic rats. In a previous study, we reported that in vitamin E-deficient rats, collagen-induced platelet TxA_2 was elevated and arterial PGI_2 was decreased when compared with rats maintained on a vitamin E-supplemented diet.[10] We hypothesized that enhanced platelet TxA_2 synthesis was due to increased deacylation of lipids, and decreased arterial PGI_2 was due to inhibition of PGI_2 synthetase by lipid peroxides formed in the vitamin E-deficient state. In a different study, we demonstrated increased thrombin-induced platelet TxA_2 and decreased vascular PGI_2 production in streptozotocin-induced diabetic rats when compared with nondiabetic control rats.[9]

In the present study, we attempted to elevate vitamin E levels in the platelets of diabetic rats with dietary enrichment of vitamin E. When diabetic rats were maintained on a high vitamin E diet for 3 mo, platelet vitamin E stores, otherwise depleted in the D group, were increased to levels above those measured in the C group. Dietary vitamin E had no effect on plasma glucose or insulin levels in diabetic rats. We measured TxA_2 and PGE_2 production in platelets, confirming our earlier observation that thrombin-induced platelet TxA_2 and PGE_2 synthesis was enhanced significantly in D group rats. Thrombin-induced synthesis of TxA_2 and PGE_2 from E group platelets was decreased to the levels of C group platelets. The pattern of collagen-induced TxA_2 and PGE_2 production in diabetic rat platelets was different. Collagen-induced platelet TxA_2 and PGE_2 synthesis from diabetic platelets was not significantly increased over that of nondiabetic controls. Increased vitamin E in platelets of the E group reduced TxB_2 and PGE_2 production to values significantly less than those found in the C and D groups. Our findings support those of Eldor et al.,[5] who reported increased thrombin-induced aggregation and no difference in collagen-induced aggregation in washed platelets of streptozotocin diabetic rats. Because diabetic vitamin E-depleted platelets would be expected to synthesize increased amounts of collagen-induced TxA_2,[10] it can be suspected that other factors related to the diabetic state, in addition to vitamin E levels, govern the platelet sensitivity to collagen in these diabetic rats. The discrepancy between thrombin-induced and collagen-induced TxA_2 and PGE_2 production in D group platelets is to be further explored. Dietary vitamin E supplementation, however, decreased platelet TxA_2 and PGE_2 production induced by collagen in an identical manner to that induced by thrombin.

The data in the present study support the proposal that increased production of TxA_2 and PGE_2 in diabetic rat platelets is due to enhanced deacylation of their platelet phospholipids rather than enhanced cyclooxygenase activity, since arachidonic acid conversion to TxA_2 is unchanged. We hypothesize that increased platelet vitamin E lowers the deacylation of platelet lipids in diabetic rats. This hypothesis is supported by our previous observation that low platelet vitamin E increases TxA_2 production[10] and by the observations of Tagney and Driskell,[19] who showed increased arachidonic acid release in E-deficient rabbit platelets. This idea is further supported by the work of Yasuda and Fugita, who showed that increased mitochondrial lipid peroxidation increases phospholipase-A_2 activity.[20]

We next examined the effect of dietary vitamin E on the release of arterial PGI_2 in diabetic rats. PGI_2 was measured from both endogenous and exogenous arachidonic acid. The depressed PGI_2 release in the D group aortas was increased by dietary vitamin E supplementation in the E group to levels present in the nondiabetic controls. We relate low aortic PGI_2 production in diabetic rats to the high lipid peroxides measured in their circulating plasma. Dietary vitamin E supplementation significantly reduced the elevated lipid peroxides found in D group plasma to values found in the nondiabetic controls.

Decreased vitamin E content in the platelets of the D group implicates vitamin E not only as a potential therapeutic agent that increases aortic PGI_2 and decreases platelet TxA_2, but also as a possible endogenous factor involved in the etiology of the diabetic vasculopathy itself. The factor(s) causing this decrease in platelet vitamin E after induction of streptozotocin diabetes is under further investigation.

Dietary vitamin E supplementation has impressive effects on the altered PGI_2/TxA_2 ratio found in the above diabetic state. If thrombin-induced TxB_2, as measured at 2 min, and 6-keto-$PGF_{1\alpha}$ from exogenous arachidonic acid are considered, the PGI_2/TxA_2 ratios in the C, D, and E groups are 9.4, 1.7, and 8.2, respectively. The greater than fivefold decrease in this ratio in diabetic rats is nearly normalized by dietary vitamin E supplementation. It remains to be established whether this decreased ratio and its reversal by vitamin E supplementation are related to the vascular lesions present in streptozotocin diabetic rats.[21]

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