PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 251–257 (1982)

6-Keto-$PGF_{1\alpha}$ Synthesis in Diabetic Rat Aorta: Effect of Substrate Concentration and Cholesterol Feeding (41507)

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Abstract. The effect of short-term feeding of a 1% cholesterol diet to normal and streptozotocin-induced diabetic rats on aortic 6-keto-$PGF_{1\alpha}$ synthesis from exogenous and endogenous arachidonic acid (AA) was investigated. Diabetes and cholesterol feeding (by themselves) resulted in a reduction in aortic synthesis of 6-keto-$PGF_{1\alpha}$ from both exogenous and endogenous arachidonic acid. An additive effect of diabetes and cholesterol feeding together was found for synthesis of 6-keto-$PGF_{1\alpha}$ from exogenous but not endogenous AA. Experiments in which the AA concentration was varied suggested that the inhibition of aortic 6-keto-$PGF_{1\alpha}$ synthesis by diabetes was competitive in nature. The diabetic rat was also found to be severely compromised in its ability to handle dietary cholesterol, as evidenced by a dramatic increase in plasma total cholesterol.

Epidemiological studies have suggested that patients with diabetes mellitus have an increased susceptibility to atherosclerosis (1, 2). Abnormalities of platelet function such as increased sensitivity to ADP, epinephrine, collagen, and arachidonic acid-induced aggregation have been noted (3, 4). This is of importance since altered platelet function may contribute to the atherosclerotic process.

Platelets from diabetic patients appear to synthesize greater amounts of the proaggregatory thromboxane A_2 (TXA_2) (5–8). Aortic tissue of human diabetics (9), streptozotocin-induced (10, 11), and spontaneously (12) diabetic rats show a reduced production of the antiaggregatory prostacyclin (PGI_2) as measured by its stable breakdown product 6-keto-$PGF_{1\alpha}$ or bioassay. It has been suggested that the balance between these two prostaglandin-like substances (TXA_2/PGI_2) may be an important determinant in thrombotic disorders of diabetics (11).

Little is known about the mechanism of the decreased aortic PGI_2 synthesis in diabetes. Gerrard *et al.* (11) demonstrated that release of the substrate arachidonic acid from phospholipids was not impaired in the aorta of streptozotocin-induced diabetic rats. Rather, the defect appeared to be in the conversion of free arachidonic acid to 6-keto-$PGF_{1\alpha}$, although the reason for this defect was not determined. Furthermore, how diabetic rat aorta will respond to a stress such as cholesterol feeding, in terms of 6-keto-$PGF_{1\alpha}$ formation, is not known.

In this communication, we have attempted (a) to study the kinetics of 6-keto-$PGF_{1\alpha}$ formation from exogenous arachidonic acid in control and diabetic rat aortas and (b) to assess the sensitivity of normal and diabetic rat aorta to the short-term feeding of a 1% cholesterol diet, in terms of 6-keto-$PGF_{1\alpha}$ formation from both exogenous and endogenous arachidonic acid.

Materials and Methods. *Animals.* Randomly selected male Sprague–Dawley rats (Harlan Animal Supplies, Indianapolis, Ind.), initially weighing between 250 and 300 g, were made diabetic by intravenous (tail vein) injection of 50 mg/kg body weight streptozotocin (Sigma Chemical Co., St. Louis, Mo.) in citrate buffer (0.1 *M* citric acid and 0.145 *M* NaCl, pH 4.5). Controls were injected with an equivalent volume of citrate buffer. The induction of diabetes was confirmed 2 days later by an elevated plasma glucose (greater than 350 mg/dl) in the fed state.

One week after injection of streptozotocin and confirmation of diabetes rats were randomly assigned to each of the following

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groups: (a) control rats on control diet (Purina Rat Chow), (b) control rats on 1% cholesterol diet (Purina Rat Chow base, ICN Nutritional Biochemicals, Cleveland, Ohio), (c) diabetic rat on control diet, (d) diabetic rat on 1% cholesterol diet. The rats were maintained on these diets for 3 weeks and then used to study aortic 6-keto-$PGF_{1\alpha}$ synthesis.

6-Keto-$PGF_{1\alpha}$ assays. [^{14}C]Arachidonic acid (55.8 Ci/mole) was obtained from New England Nuclear (Boston, Mass.) and cold arachidonic acid from Nu Chek Prep (Elysian, Minn.). Both were stored in absolute ethanol at −20°. The specific activity of the labeled arachidonic acid was diluted with unlabeled arachidonic acid to obtain the desired concentrations. The ethanol was evaporated under N_2 and the arachidonic acid reconstituted in 10−20 m*M* Na_2CO_3 to the desired concentration. Fresh solutions in Na_2CO_3 were prepared on each day the assays were performed. Approximately 0.25 μCi of [^{14}C]arachidonic acid was used per assay.

The thoracic aorta was quickly dissected following exsanguination, cleared of connective and fatty tissue, and cut into small rings 3−5 mm in length. Individual rings were placed in 0.475 ml of phosphate-buffered saline (NaCl, 8 g/liter; KCl, 0.2 g/liter; $CaCl_2$, 0.1 g/liter; $MgCl_2$ $2H_2O$, 0.1 g/liter; Na_2 HPO_4 $2H_2O$ 1.15 g/liter; KH_2PO_4, 0.2 g/liter; glucose, 1 g/liter, pH 7.2) and preincubated for 2 min at 37° prior to the addition of 25 μl of various concentrations of [^{14}C]arachidonic acid. After a 10-min incubation the reaction was stopped by adding 0.5 ml 2 *M* citric acid. Time course experiments indicated that the aortic production of 6-keto-$PGF_{1\alpha}$ was linear for at least 20 min for both control and diabetic aorta (Fig. 1). The prostaglandins were extracted with 10 ml chloroform/methanol (2:1) and the lower organic layer was collected following the addition of 1 ml 0.9% NaCl. The extract was evaporated to a small volume under N_2 and the various prostaglandins separated by thin-layer chromatography (250 μm silica gel G plates, Analabs, North Haven, Conn.) using a solvent system consisting of ethyl acetate/2,5,5-trimethylpentane/acetic acid/water (90:50:20:100, v/v/v/v, organic layer). Prior to separation, pure standards for PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ (Upjohn Co., Kalamazoo, Mich.) were added to allow visualization following a brief exposure to iodine vapor. The areas corresponding to PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were scraped into vials, 10 ml of Aquasol 2 (New England Nuclear) added, and quantitated by liquid scintillation counting. The remaining areas were also scraped and counted to determine percentage recovery (always greater than 85%). Background counts determined from boiled aortic rings were subtracted prior to the calculation of the molar amount of product formed.

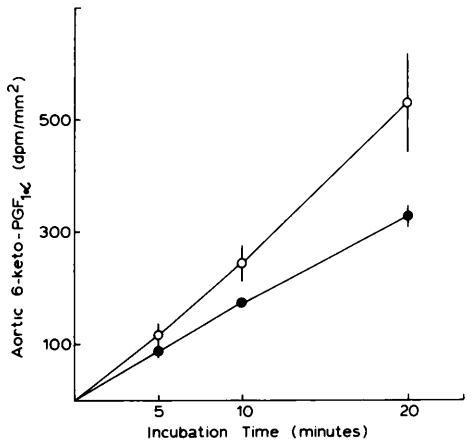

FIG. 1. Aortic 6-keto-$PGF_{1\alpha}$ synthesis (mean ± SD) as a function of incubation time for control (○, n = 4) and diabetic (●, n = 4) rats.

Basal aortic 6-keto-$PGF_{1\alpha}$ synthesis was measured by specific radioimmunoassay (6-keto-[^{3}H]$PGF_{1\alpha}$ kit from New England Nuclear). Aortic rings were suspended in 0.5 ml of phosphate-buffered saline and incubated at 37° for 10 min. At the end of the incubation period the solution was removed with a pipet and frozen at −20°. The concentration of 6-keto-$PGF_{1\alpha}$ was then measured using radioimmunoassay.

For both assays the amount of 6-keto-$PGF_{1\alpha}$ formed was normalized by the surface area of the aortic ring. Surface area was measured after cutting the ring open and laying it flat. Two measurements of length and width were made using a Bausch

TABLE I. BODY WEIGHT AND PLASMA GLUCOSE, TRIGLYCERIDE, AND CHOLESTEROL IN CHOW OR CHOLESTEROL-FED CONTROL AND DIABETIC RATS

Group	Body weight	Concentration (mg%)		
		Glucose	Triglyceride	Total cholesterol
Control ($n = 8$)	339 ± 11[a]	186 ± 10	27 ± 6	57 ± 1
Diabetic ($n = 8$)	229 ± 12	478 ± 17*	450 ± 91*	99 ± 3*
Control + 1% cholesterol ($n = 8$)	348 ± 6	177 ± 18	66 ± 11**	69 ± 3
Diabetic + 1% cholesterol ($n = 4$)	202 ± 18	481 ± 35*	2009 ± 388*	3175 ± 381*

[a] Results are means ± SEM.
* $P < 0.01$, as compared to the control (range test).
** $P < 0.05$.

and Lomb measuring magnifier equipped with a metric scale (one division = 0.1 mm). Surface area was calculated by taking the product of the average length and width.

Plasma lipid analysis. Plasma lipids were determined by the LRC method (14). Plasma glucose was determined by the glucose oxidase technique, using a Beckman glucose analyzer.

Statistical analysis. Statistical comparisons between means were made using the Newman–Keuls range test after analysis of variance (15). The curves drawn in Figs. 2 and 3 were fit using weighted least squares. Weights were obtained from the inverse of the variance for each arachidonic acid concentration.

Results. The mean body weights and plasma levels of glucose, triglycerides, and cholesterol for the four groups of rats are presented in Table I. Diabetes caused a significant increase in plasma triglycerides and total cholesterol. Diabetic rats fed the 1% cholesterol diet possessed greatly elevated plasma triglycerides and total cholesterol. Control rats fed the 1% cholesterol diet experienced only a small nonsignificant elevation in plasma total cholesterol.

Table II shows the aortic conversion of exogenous [^{14}C]arachidonic acid to 6-keto-PGF$_{1\alpha}$. As previously observed by other investigators, diabetes resulted in a decreased ($P < 0.05$) aortic production of 6-keto-PGF$_{1\alpha}$. Cholesterol feeding alone also resulted in a significant decrease in aortic 6-keto-PGF$_{1\alpha}$ production. The mean aortic 6-keto-PGF$_{1\alpha}$ production for cholesterol-fed diabetic rats was the lowest of all the groups, but only significantly different from the chow-fed control group.

We also studied the effect of exogenous arachidonic acid concentration on the synthesis of 6-keto-PGF$_{1\alpha}$ by aortic rings from the four groups of rats. The data are presented in Figs. 2 and 3 in the form of double reciprocal plots. The difference in the capacity of control and diabetic aorta to form 6-keto-PGF$_{1\alpha}$ was found to be dependent on the substrate concentrations. Specifically,

TABLE II. CONVERSION OF 10 μM [^{14}C]ARACHIDONIC ACID TO 6-KETO-[^{14}C]PGF$_{1\alpha}$ IN RAT AORTA

Group	Aortic 6-keto-PGF$_{1\alpha}$ synthesis (pmole/mm^2/10 min)
Control ($n = 8$)	2.21 ± 0.19[a]
Diabetic ($n = 8$)	1.61 ± 0.17*
Control + 1% cholesterol ($n = 8$)	1.72 ± 0.14*
Diabetic + 1% cholesterol ($n = 4$)	1.23 ± 0.10*

[a] Results are means ± SEM.
* $P < 0.05$, as compared to the control group (range test).

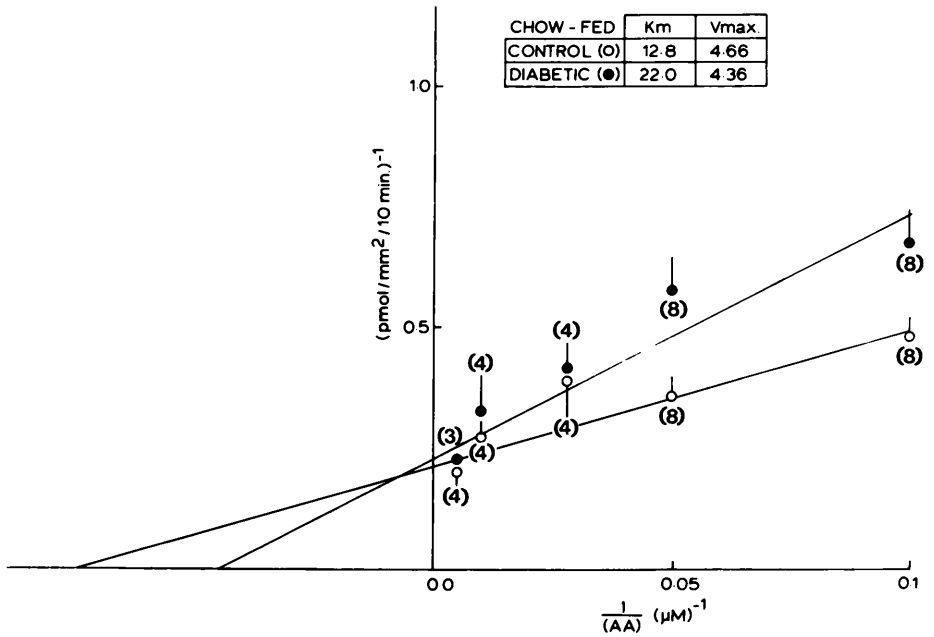

FIG. 2. Aortic 6-keto-$PGF_{1\alpha}$ synthesis (pmole/mm²/10 min) as a function of exogenous arachidonic acid concentration (AA) in control and diabetic rats. The data are presented in the form of a double reciprocal plot. The apparent maximum velocity (pmole/mm²/10 min) and K_m (μM) values were calculated from the regression lines. The number of rats are given in parentheses and the vertical bars represent the SD.

differences were more evident at lower substrate concentrations. The calculated apparent maximum velocity and apparent K_m values are given in Figs. 2 and 3. A useful comparison of the four groups can be obtained from these values. Diabetes resulted in an increased apparent K_m with no change in the apparent maximum velocity, as compared to the chow-fed control group. The apparent K_m was also increased for the cholesterol-fed control group and even more so for the cholesterol-fed diabetic group. Cholesterol-feeding also resulted in a slightly greater apparent maximum velocity for both diabetic and control rats.

Basal aortic synthesis of 6-keto-$PGF_{1\alpha}$ from endogenous arachidonic acid was also measured and found to follow a similar trend. Both cholesterol feeding and diabetes resulted in lower mean values as compared to the chow-fed group (Table III). However, the cholesterol-fed diabetic group did not possess the lowest mean value.

Discussion. The results of the present study confirm previous observations (10–13) demonstrating that the diabetic state decreases the formation of 6-keto-$PGF_{1\alpha}$ in rat aorta. The results further demonstrate that short-term cholesterol feeding (3 weeks) also results in a reduced aortic synthesis of 6-keto-$PGF_{1\alpha}$ in the adult male rat. Our studies using exogenous [^{14}C]-arachidonic acid suggested that feeding cholesterol to diabetic rats may further alter aortic 6-keto-$PGF_{1\alpha}$ synthesis. The magnitude of the change in aortic 6-keto-$PGF_{1\alpha}$ synthesis at 10 μM exogenous arachidonic acid and the apparent K_m for the cholesterol-fed diabetic group was roughly equal to the sum of the separate effects of cholesterol feeding and diabetes. Thus, the reduction in aortic 6-keto-$PGF_{1\alpha}$ synthesis found in the cholesterol-fed diabetic group may simply be an additive effect of cholesterol feeding and diabetes. An interesting finding was that diabetes appeared to result mainly in an increased apparent K_m with

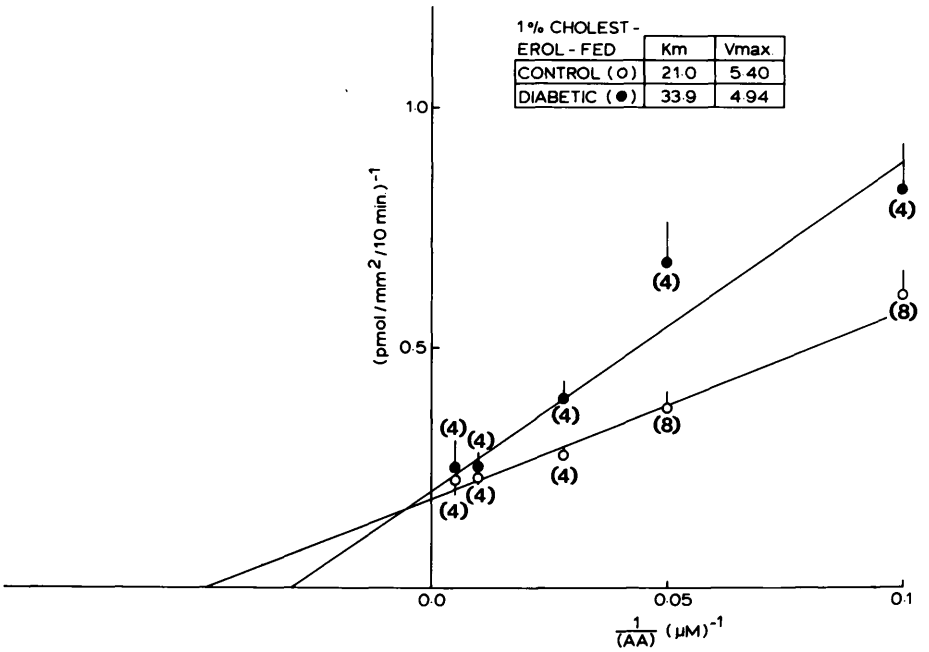

FIG. 3. Aortic 6-keto-$PGF_{1\alpha}$ synthesis (pmole/mm²/10 min) as a function of exogenous arachidonic acid concentration (AA) in control and diabetic rats fed 1% cholesterol. The data are presented in the form of a double reciprocal plot. The apparent maximum velocity (pmole/mm²/10 min) and K_m (μM) values were calculated from the regression lines. The number of rats are given in parentheses and the vertical bars represent the SD.

only minimal effect on the apparent maximum velocity. This resembles a competitive type of inhibition of aortic 6-keto-$PGF_{1\alpha}$ synthesis. Using endothelial cell cultures, Spector, *et al.* (16) found that linoleic and to some degree oleic acid inhibited the conversion of arachidonic acid to 6-keto-$PGF_{1\alpha}$ and that this inhibition appeared to be competitive in nature. Furthermore, increasing the dietary intake of linoleate by feeding corn oil to rabbits has been shown to result in a decreased aortic prostacyclin-producing capacity (17). Thus, it might be speculated that alterations in the levels of certain free or esterified fatty acids may be involved in the diabetes-induced reduction in aortic 6-keto-$PGF_{1\alpha}$ synthesis. Faas and Carter (18) found that liver microsomal Δ6 desaturase activity was depressed in diabetic rats. This depression was presumably the cause of the elevated levels of linoleate found in the liver microsomes. Serum-free fatty acids are also greatly increased in diabetic rats (19). These observations suggest that tissue linoleate would be elevated in diabetic rats.

Dietary cholesterol has also been found to alter the composition of rat liver phospholipids (20). The result was an elevation in the percentage composition of linoleate and 8,11,14-eicosatrienoate of rat liver

TABLE III. BASAL AORTIC 6-KETO-$PGF_{1\alpha}$ SYNTHESIS FROM ENDOGENOUS ARACHIDONIC ACID

Group	Aortic 6-keto-$PGF_{1\alpha}$ synthesis (pmole/mm²/10 min)
Control (n = 4)	4.07 ± 0.30[a]
Diabetic (n = 4)	2.42 ± 0.43*
Control + 1% cholesterol (n = 4)	3.31 ± 0.21
Diabetic + 1% cholesterol (n = 4)	2.58 ± 0.44*

[a] Results are means ± SEM.
* $P < 0.05$, as compared to the control group (range test).

phospholipids following the cholesterol supplementation of a control diet containing cottonseed oil. The mechanism of this alteration remains to be determined. It would be of interest to know if cholesterol feeding and diabetes alter aortic 6-keto-$PGF_{1\alpha}$ synthesis by the same mechanism. We found that the apparent K_m value for aortic synthesis of 6-keto-$PGF_{1\alpha}$ was increased for the chow-fed diabetic, cholesterol-fed control, and cholesterol-fed diabetic group. Only cholesterol feeding, however, resulted in an elevation in the apparent maximum velocity. This result suggests the possibility that cholesterol feeding may alter aortic 6-keto-$PGF_{1\alpha}$ synthesis by a mechanism different from diabetes. Further research will be necessary to resolve the mechanisms of dietary cholesterol and diabetes-induced alterations in aortic 6-keto-$PGF_{1\alpha}$ synthesis.

The results of measurements of basal aortic 6-keto-$PGF_{1\alpha}$ synthesis were similar to those using [^{14}C]arachidonic acid except that we found no additional effect of feeding cholesterol to diabetic rats. The reason for this difference is unknown. The work of Needleman *et al.* (21) suggests that the enzymes involved in the synthesis of PGI_2 and PGE_2 by the perfused kidney may exist in multiple compartments within the cell. Thus, to some extent aortic PGI_2 synthesis from endogenous arachidonic acid could involve different enzymes than synthesis from exogenous arachidonic acid. It is possible then that measurements of aortic 6-keto-$PGF_{1\alpha}$ synthesis from exogenous and endogenous arachidonic acid could yield differing results. It should be noted that aortic synthesis of 6-keto-$PGF_{1\alpha}$ from endogenous arachidonic acid (molar amount) was greater than that from 10 μM exogenous arachidonic acid (compare Tables II and III). Karpen *et al.* (22) have also published data demonstrating a greater molar production of 6-keto-$PGF_{1\alpha}$ by rat aortic rings from endogenous arachidonic acid versus 17.6 μM exogenous arachidonic acid after a 90-min incubation at 37°. In contrast the addition of exogenous arachidonic acid to bovine aortic segments and cultured endothelial cells greatly enhances PGI_2 synthesis over the basal level (23). The reason for the difference between these tissues in their 6-keto-$PGF_{1\alpha}$ synthesis from exogenous versus endogenous arachidonic acid is not known, but may be related to differences in the uptake and utilization of exogenous arachidonic acid, the basal capacity for PGI_2 synthesis, or both. Further study will be required to answer the first possibility. In regard to the latter possibility, it has been shown that PGI_2 production by rat aorta was quite high as compared to other species such as rabbit and guinea pig (24). Sinzinger *et al.* (25) have suggested that the susceptability of various species to atherosclerosis may be due to inborn differences in PGI_2 formation. The significance of the difference in our findings when using exogenous versus endogenous arachidonic acid awaits further investigation.

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Received July 1, 1981. P.S.E.B.M. 1982, Vol. 171.