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Thoracic aorta prostacyclin production is not altered during early atherosclerosis development in young swine

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Decreased prostacyclin synthesis by atherosclerotic vascular tissue has been demonstrated in both adult animal models and man. The purpose of this study was to determine whether the decrease in prostacyclin synthesis by the vessel wall was evident during the initial stages of atherosclerosis development and thus a potential contributor to atherogenesis. Thirty-two Yucatan miniature swine entered the study at 3 months of age and were placed on either an atherogenic or regular diet for 10, 30, 90, or 180 days. At that time, the thoracic and abdominal aorta were harvested. Pairs of discs were punched out of the distal thoracic aorta and placed in Krebs Henseleit HEPES buffer. The buffer was then exchanged for either fresh buffer or stimulated with calcium ionophore A23187 (10^{-4} M). Aliquots were collected at 10 min for analysis of prostacyclin production by radioimmunoassay of its stable metabolite 6-keto-prostaglandin $F_{1\alpha}$. Thoracic aorta sections were prepared for atherosclerosis assessment by light microscopy, and the abdominal aorta was stained with Sudan IV. No significant differences were noted between the diet groups at 10 and 30 days. After 90 days, a significant difference was noted ($P < 0.05$) between the diet groups with regard to (1) the early development of atherosclerosis in the thoracic aorta and (2) the percent of abdominal aorta surface area sudanophilically stained. However, no differences ($P > 0.05$) were noted between the diet groups with regard to the basal and stimulated rates of prostacyclin production. Our findings showed that prostacyclin synthesis was not altered during the initial development or early progression of aortic atherosclerosis induced by hypercholesterolemia. (J. Nutr. Biochem. 6: 163–169, 1995.)

Keywords: atherosclerosis; prostacyclin; hypercholesterolemia; calcium ionophore A23187; Yucatan miniature swine

Introduction

Atherosclerosis is one of a number of diseases that has been associated with an imbalance in the prostacyclin-thromboxane A_2 system.[1,2] Prostacyclin (PGI_2) is the main metabolite of the arachidonic acid cascade produced by the vascular tissue and has vasodilatory effects as well as being an inhibitor of platelet adhesion and aggregation. Thromboxane A_2 is the chief product of the arachidonic acid cascade in platelets and has potent effects as a vasoconstrictor as well as an inducer of platelet aggregation.[3] The relationship of these prostanoids to atherosclerosis has been the subject of a great number of investigations since their discovery in the mid-1970s. The specific sequence of events leading to the formation of early atherosclerotic lesions has yet to be fully characterized[4] as does the significance, if

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any, of altered prostanoid levels as related to atherogenesis.[1,2]

PGI_2 is not stored in cells, however, mechanical or chemical irritation of cell membranes is associated with its formation. In the endothelium, pulsatile pressure, shear stress, and a variety of substances including adenine nucleotides, arachidonic acid, bradykinin, calcium ionophore A23187, choline esters, epidermal growth factor, serotonin, substance P, thrombin, and others stimulate PGI_2 formation.[5] Low density lipoproteins, cyclo-oxygenase inhibitors, nicotine, testosterone, and various other compounds known to stimulate the generation of reactive oxygen species within the endothelium inhibit the biosynthesis of PGI_2.[5,6]

The depressed prostacyclin production by the endothelium was believed to allow an increase in adherence and aggregation of platelets on the endothelial surface where lysosomal enzymes, toxic substances, and growth factors could damage the endothelium and stimulate subendothelial cell proliferation and atherosclerotic plaque formation.[7] However, the literature does not demonstrate a clear consensus regarding the biosynthesis of PGI_2 by atherosclerotic arteries or alterations in PGI_2 production relating to atherosclerosis development. Various studies have found PGI_2 production to be increased,[8–11] decreased,[12,13] or unchanged[14] during early atherosclerotic lesion development, while other have reported a cyclic fluctuation in PGI_2 levels.[15,16]

The association of altered PGI_2 production to the evolving atherosclerotic lesion is still not well understood. From the pathological evidence available, it is accepted that the initial development of atherosclerosis originates in childhood and young adulthood long before the appearance of clinical symptoms.[4,17] Thus, developing an understanding of the physiological responses of vessels in the pediatric age group may be more effective in determining early preventive strategies in the future. In assessing if alterations in PGI_2 synthesis may be a potential avenue for developing intervention strategies in the young, it needs to be determined if alterations in prostacyclin production, as noted in vessels with advanced atherosclerosis, actually occurs in the initial atherosclerotic process. The aim of this paper was to address the question Does prostacyclin production decrease prior to the development of atherosclerotic lesions resulting in decreased synthesis which is contributory to the initial atherogenic process, or do changes occur secondary to the formation of atherosclerosis within the vessel wall which, over time, alter prostacyclin production?

Methods and materials

Tritiated 6-keto-$PGF_{1\alpha}$ was obtained from DuPont NEN (Boston, MA USA). The antibody, anti-6-keto-$PGF_{1\alpha}$, was a gift from Gordon Niswender, Department of Physiology, and Dr. Melvin M. Mathias, Food Science and Human Nutrition, Colorado State University, Fort Collins, CO USA. The 6-keto-$PGF_{1\alpha}$ standard was obtained from Cayman Chemical Company (Ann Arbor, MI USA). Cholesterol and calcium ionophore A23187 were purchased from Sigma Chemical Company (St. Louis, MO USA). Sudan IV stain and liquid scintillation fluid was obtained from Fisher Scientific Company (Fair Lawn, NJ USA). Glutaraldehyde and osmium tetroxide were purchased from Electron Microscopy Sciences (Fort Washington, PA USA). All other reagents were analytical grade.

Thirty-two Yucatan miniature swine from the herd at Colorado State University entered the study at 3 months of age and were randomly assigned to either the control group ($n = 16$) or atherogenic diet group ($n = 16$) for 10, 30, 90, or 180 days. Each group was comprised of 8 males and 8 females. The control group received 500 g of standard mini-pig ration daily which comprised by weight 60.4% chopped corn, 20.4% soy bean meal, 10.0% dehydrated alfalfa meal, 5.0% wheat bran, 3.2% vitamin–mineral mix, and 1.0% soy bean oil. The diet met all the National Research Council's nutrient requirements for swine.[18] Vitamin E (all-rac-α-tocopherol) was provided at 55 IU (50 mg of all-rac-α-tocopherol), since mini-pigs require this dietary vitamin E concentration to prevent symptoms of muscular dystrophy and hepatosis (this was established at the CSU Yucatan swine facility and also by Charles River Laboratories, Boston, MA USA). The atherogenic diet group received 500 g of the atherogenic diet daily consisting of 1.5% cholesterol, 15% beef tallow, and 83.5% standard mini-pig ration based on weight.[19]

Serum lipid profiles were obtained on each pig, in the fasted state (20 to 24 hr), at the time of entry into the study and were repeated at intervals throughout the study. Lipid profiles were determined with a Kodak EKTACHEM Model 700XR Analyzer employing standard protocols using a peroxidase colorimetric analysis of cholesterol. HDL cholesterol was determined utilizing a magnesium precipitation method with dextran sulfate. Hematocrits and body weight were measured at each blood draw session.

At the end of the experimental period, the pigs were killed in accordance with the American Veterinary Medical Association's guidelines for euthanasia[20] and approved by the Animal Care and Use Committee at Colorado State University. Euthanasia was by means of gunshot to the head followed immediately by rapid exsanguination. This method was selected to eliminate the possibility of altered vascular responsiveness secondary to chemical euthanizing methods. The ventral chest wall was incised and the ribs spread allowing rapid entry into the thoracic cavity. The thoracic aorta was cut at the level of the diaphragm and the aortic arch, placed immediately in ice cold 0.9% saline, and processed for determination of 6-keto-$PGF_{1\alpha}$ production and light microscopy. The abdominal aorta was removed, from the level of the diaphragm to the bifurcation of the iliac arteries, for staining with Sudan IV dye.

The thoracic aorta was kept in ice cold saline while it was rapidly cleaned of extraneous connective tissue, cut longitudinally along the ventral aspect and laid open exposing the luminal surface. Care was taken not to touch the endothelial surface. Six 8 mm diameter discs were punched out of the aorta, side-by-side, starting from the distal end of the vessel and progressing proximally. The first two discs were immersion fixed in 2.5% glutaraldehyde (in 0.1 M Sorensen buffer) for future microscopy work. The remaining discs were placed sequentially in tubes containing 10 mL of Krebs Henseleit HEPES (KHH) buffer. KHH buffer comprised (mM): NaCl, 118; KCl, 4.74; $CaCl_2$, 2.56; $MgSO_4$, 1.18; KH_2PO_4, 1.18; HEPES, 10; pH 7.4. It has previously been shown that there is no difference in prostacyclin production by aortic tissue incubation in Krebs Henseleit bicarbonate buffer, a physiological buffer, or KHH buffer.[21] The tubes, with the head space gassed with a mixture of 95% O_2 and 5% CO_2, were capped and placed in a water bath at 37°C for 30 min. Nelson et al.[22] showed it was necessary to stabilize rat aortic ring incubations by equilibrating them for 1 hr in KHH buffer in order to reduce PGI_2 production to a basal rate. From preliminary experiments we determined that pig thoracic aortic disc incubations required only 30

min in KHH buffer to equilibrate to a basal production rate. Following the 30 min rest period, the buffer was carefully drawn from the tubes containing the aortic disc without touching the discs. Ten milliliters of KHH buffer were placed in the first tubes of each of the remaining disc pairs, and 10 mL of 10^{-4} M calcium ionophore A23187 in each of the subsequent tubes of the disc pairs. The tubes were again gassed with a mixture of 95% O_2 and 5% CO_2 and placed in the water bath (37°C) for 10 min. Following incubation of the discs in KHH buffer or 10^{-4} M calcium ionophore A23187 for 10 min, the supernatant was collected into plastic tubes, capped, and frozen at −20°C until analyzed for 6-keto-$PGF_{1\alpha}$ by radioimmunoassay. The aortic discs were also frozen at −20°C until they were analyzed for protein content. The total time from excision of the thoracic aorta from the chest until the beginning of the 30 min rest period in buffer averaged 10 min.

The thoracic aorta discs to be assessed by light microscopy were cut symmetrically in half. One of the thoracic disc halves underwent postfixation in equal volumes of 2% osmium tetroxide and 5% potassium dichromate in 0.1 M Sorensen buffer (pH 7.3). The second mirror image half of the thoracic disc was processed for staining with hematoxylin and eosin, Verhoeff's elastic tissue stain-modified, and Masson's trichrome stain. Standard methods were utilized in all staining procedures employed.[23,24] The sectioning sequence of each specimen for staining was consistent and the sets of slides were randomly sequenced prior to viewing. Each thoracic aorta was evaluated and assigned a lesion classification type, based on the most advanced lesion identified by light microscopy. The lesions were classified as: Type 0 (no lesion), no lipid accumulation in any cells and no foam cells present; Type I (foam cells), macrophage foam cells present usually as isolated cells or sometimes as small clusters of cells; or Type II (fatty streak), multiple layers of cells overloaded with lipid droplet inclusions and extracellular lipid may be present.

The abdominal aorta was carefully dissected out, washed with 0.9% saline, then cut longitudinally and mounted on a cork background with the luminal surface up and exposed. Care was taken to restretch it to its original in vivo length during mounting. The vessel underwent staining with Sudan IV and was then photographed. Tracings were made of the luminal surface of the abdominal aorta, and morphometry was performed using a digitalizer board and the computer program BIOQUANT SYSTEM IV (R&M Biometrics Inc., Nashville, TN USA). The percent luminal surface area stained with sudanophilic lesions to total luminal surface area of the abdominal aorta was calculated.

Radioimmunoassay (RIA) was used to assess 6-keto-$PGF_{1\alpha}$ concentrations, the stable spontaneous (nonenzymatic) degradation product of prostacyclin, in the supernatant collected from the thoracic aortic discs. The RIA utilized had been previously validated,[25] and had been found to be unaffected by hyperlipidemic plasma samples.[26] All assays were performed in triplicate under equilibrium conditions. The thoracic aortic discs were incubated in 5 mL of 3% NaOH in a water bath at 60°C until dissolved. Aliquots of the dissolved protein were analyzed using the Bradford dye binding assay[27] with premixed reagents from Bio-Rad (Richmond, CA USA). Results were calculated and the milligrams of protein content per disc was determined. The 6-keto-$PGF_{1\alpha}$ data were expressed in pmol/mg of protein.

Statistical analysis

Gender was found not to be a statistically significant variable in any of the tests performed. The final data analysis utilized two-way analysis of variance (ANOVA) tests, Fisher's Protected least significant difference tests, Chi-square tests and correlation analyses. Significance was at the 0.05 level.

Results

Serum lipid profiles

Two-way analysis of variance (ANOVA) results showed a significant increase in the atherogenic diet group compared with the control diet group with regard to serum total cholesterol, LDL cholesterol, and HDL cholesterol, but not for triglycerides. *Table 1* contains the mean values obtained for each of the groups. The percent of the total cholesterol that was LDL cholesterol increased on average from 39% in the control group to 81% in the atherogenic diet group, while the percent of HDL cholesterol comprising the total cholesterol decreased on average from 50% in the control group to 15% in the diet group. No significant differences between the two groups ($P > 0.05$) were noted with regard to hematocrits or body weights throughout the study.

Sudan IV staining of abdominal aorta

Two-way ANOVA demonstrated a significant difference ($P = 0.032$) between the treatment groups by time in the amount of surface area developing sudanophilic lesions. Correlation analysis demonstrated a positive association in the atherogenic diet group ($r = 0.578$, $p = 0.0038$) between the percent surface area with sudanophilic lesions and the length of time in the study. *Figure 1* illustrates the increasing discrepancy in the amount of abdominal sudanophilia between the groups over time in the study. The percentage of abdominal aorta surface area containing sudano-

Table 1 Comparison of lipid profiles between treatment groups

Time in study	Diet group	Total cholesterol†‡	HDL cholesterol†‡	LDL cholesterol†‡	Triglycerides†
10 days	C	72 ± 4	37 ± 3	28 ± 1	35 ± 1
	A	481 ± 96	89 ± 5	383 ± 90	40 ± 10
30 days	C	79 ± 7	38 ± 2	34 ± 6	37 ± 3
	A	692 ± 188	84 ± 8	582 ± 172	49 ± 5
90 days	C	77 ± 3	42 ± 1	26 ± 2	42 ± 7
	A	688 ± 177	101 ± 8	526 ± 129	44 ± 3
180 days	C	78 ± 2	40 ± 1	30 ± 3	42 ± 2
	A	702 ± 178	106 ± 5	583 ± 177	38 ± 3

*C = control diet group, A = atherogenic diet group.
†Values are mmol/L. Mean ± SEM.
‡Significant difference between control and diet groups ($P < 0.05$).

philic lesions in control subjects was minimal (0% in most subjects to a high of 1% in one of the 180 day subjects). The abdominal aorta surface area containing sudanophilic staining in atherogenic diet subjects ranged from a low of 1.4% in a 10-day pig to a high of 54.9% in a 180-day pig. The pooled mean ± SEM of the percent surface area of the abdominal aorta containing sudanophilic lesions in pigs on the atherogenic diet was 16.2 ± 4%.

In addition a strong positive correlation was noted between the percent of surface area with sudanophilic lesions and the total serum cholesterol ($r = 0.838$, $p = 0.0001$), LDL cholesterol ($r = 0.830$, $p = 0.0001$), and HDL cholesterol ($r = 0.653$, $p = 0.0001$) variables. Thus, as the total cholesterol increased, the fraction (%) of LDL cholesterol increased and the fraction (%) of HDL-cholesterol decreased as sudanophilic lesions increased.

Thoracic aorta atherosclerotic lesions

Chi-square analyses showed a significant difference ($P = 0.033$) in atherosclerotic lesion development between the atherogenic diet and regular diet groups as well as the length of time ($P = 0.045$) on the atherogenic diet but no significant difference between the sexes ($P = 0.513$). *Table 2* illustrates the distribution of atherosclerotic lesion type between the control and atherogenic diet groups. Correlation analysis between the length of time the atherogenic diet group consumed the atherogenic diet and lesion development showed a strong association ($r = 0.615$, $P = 0.0077$). In addition a significant association was demonstrated between thoracic aorta atherosclerotic lesion development and total serum cholesterol ($r = 0.554$, $P = 0.001$), LDL cholesterol ($r = 0.56$, $p = 0.0009$), and HDL cholesterol ($r = 0.455$, $p = 0.0088$) but no significant association with triglycerides ($r = 0.011$, $P = 0.952$).

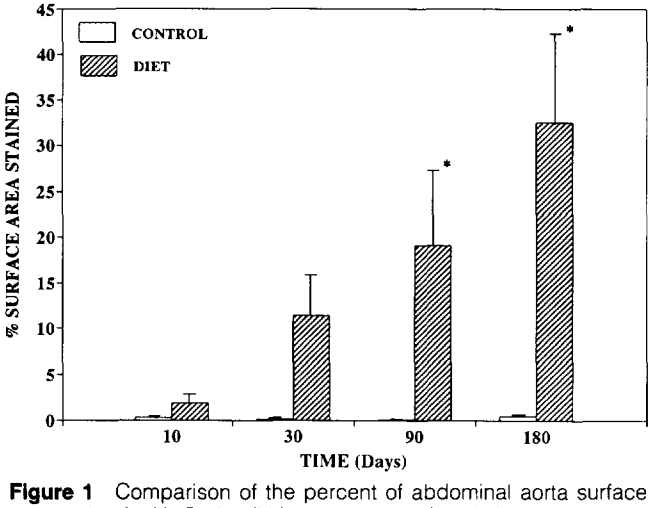

Figure 1 Comparison of the percent of abdominal aorta surface area stained with Sudan-IV between control and diet groups over the time course of the study. Values are expressed as mean ± SEM. *Significant difference ($P \leq 0.05$).

Basal and stimulated production of 6-keto-PGF$_{1\alpha}$ by thoracic aorta

The thoracic aorta basal levels of PGI_2 production measured as 6-keto-PGF$_{1\alpha}$, were evaluated by a two-way ANOVA. No significant difference was observed between the atherogenic diet and control groups with regard to basal production of 6-keto-PGF$_{1\alpha}$. There was a significant decrease ($P < 0.05$) in production in the 90 and 180 day groups as compared with the 10 and 30 day groups for both the atherogenic diet and control subjects. *Table 3* presents the data on basal 6-keto-PGF$_{1\alpha}$ production by the thoracic aorta between the treatment groups over time. A two-way ANOVA on the challenged production of 6-keto-PGF$_{1\alpha}$ by the thoracic aorta revealed no significant differences between treatment groups. Again there was a significant decrease in production levels at 90 and 180 days as compared with 10 and 30 days. *Table 3* shows the data obtained on 6-keto-PGF$_{1\alpha}$ levels under challenged conditions. A pairwise comparison showed a significant difference ($P < 0.05$) between basal and calcium ionophore A23187 stimulated production of 6-keto-PGF$_{1\alpha}$ by the thoracic aorta, indicating that the aorta was responsive to the challenge. The difference between the increase in production of 6-keto-PGF$_{1\alpha}$ under challenged conditions versus basal conditions was calculated to determine the degree of responsiveness of the thoracic aorta. A two-way ANOVA demonstrated no significant differences between the responsiveness of the atherogenic diet group compared with the control group or over time in the study.

The basal production of PGI_2 from the thoracic aorta was lower in the older pigs (90 and 180 day subjects) as compared with the younger pigs (10 and 30 day subjects) independent of diet. However, when challenged with calcium ionophore A23187, the increase in the response of PGI_2 production by the vessel wall was the same regardless of age.

Discussion

The degree and extent of diet-induced aortic atherosclerosis were assessed by abdominal and distal thoracic aorta sudanophilic staining and histological analysis, respectively. Definitive atherosclerotic lesions were detected in

Table 2 Distribution of thoracic atherosclerosis

	10 Days		30 Days		90 Days		180 Days	
Lesion type	Control	Diet	Control	Diet	Control	Diet	Control	Diet
Type 0	4	4	4	4	4	3	4	1
Type I	—	—	—	—	—	1	—	—
Type II	—	—	—	—	—	—	—	3

Table 3 Thoracic aorta basal and challenged 6-keto-$PGF_{1\alpha}$ production

Time In study	Basal production†		Stimulated production†	
	Control*	Diet*	Control*	Diet*
10 Days	2.22 ± 0.20	2.70 ± 0.47	3.98 ± 0.26	3.45 ± 0.32
30 Days	2.57 ± 0.47	2.07 ± 0.28	3.81 ± 0.96	3.30 ± 0.57
90 Days	0.80 ± 0.11	1.35 ± 0.34	1.64 ± 0.44	2.79 ± 0.69
180 Days	0.80 ± 0.08	0.72 ± 0.12	1.30 ± 0.33	1.08 ± 0.11

*Values are pmol/mg of protein. Mean ± SEM.
†No significant difference between the control and atherogenic diet groups ($P > 0.05$)

the young swine after 3 to 6 months of diet-induced hypercholesterolemia. Prostacyclin production by the thoracic aorta was determined to assess the relationship between prostacyclin production and the initial development of atherosclerosis. This study demonstrated that PGI_2 production by the thoracic aorta was not altered in the prelesion state and that the basal PGI_2 production decreased with age.

In this study on young swine, the atherosclerotic process was accelerated by dietarily inducing hypercholesterolemia. All the serum lipid components except triglycerides were positively correlated with the degree of atherosclerosis induced. No differences in the serum triglyceride level was found between the control and atherogenic diet groups, which was consistent with the findings of Sassen et al.[28] on swine fed an atherogenic diet for 8 months. The abdominal aorta was chosen for Sudan IV staining since it is prone to developing atherosclerosis[29] and is large enough to employ the morphometric method. Sudan IV uptake by the atherosclerotic lesions of the abdominal aorta was quite dramatic with a significant difference noted between the treatment groups with regard to atherosclerosis formation at 90 to 180 days in the study. Early atherosclerotic lesions were also found in the thoracic aorta, histologically, in the pigs after 3 to 6 months of diet-induced hypercholesterolemia. The atherosclerotic lesions we found in the distal thoracic aorta were similar to those described by Gerrity[30] and Kim et al.[31] in the coronary arteries of young swine with diet-induced hypercholesterolemia, and were similar to the Type I and Type II lesions Stary had described in children and young adults.[32] The thoracic aorta was also selected for analysis of prostacyclin synthesis because: (1) it has been shown by Sinzinger et al.[33] that the thoracic aorta is more productive at synthesizing prostacyclin than the abdominal aorta; and (2) the time lag of 10 min from harvest until incubation of the vessel samples could be achieved despite the number of thoracic aortic discs processed.

The research literature relating changes in PGI_2 production to atherosclerosis has been inconclusive in demonstrating a consistent relationship. Some of this may be due to the various models and assay techniques utilized in different studies. Henriksson et al.[14] found no difference in 6-keto-$PGF_{1\alpha}$ production by radioimmunoassay in rabbit aortas between those fed an atherogenic diet for 12 weeks versus a regular diet. All the rabbits in the atherogenic diet group had some degree of atherosclerosis present based on their grading scale estimating the percent surface area with macroscopic evidence of atherosclerosis. Dembinska-Kiec et al.[34] found no significant difference in PGI_2 production, by bioassay, in the aortas of rabbits fed an atherogenic diet for 5 months, with well developed atherosclerotic plaques evident, compared with control rabbits. However, they did note a significant decrease in PGI_2 production of the mesenteric artery in the experimental rabbits.

Voss fed rabbits an atherogenic diet for 4 months and assessed PGI_2 production by bioassay at various sites along the thoracic aorta.[8] The degree of atherosclerosis was determined by Sudan III staining, and significantly higher levels of PGI_2 were found to be produced by tissue samples from atherosclerotic sites versus sites with no evident atherosclerosis. Kanemaru et al.[9] also found PGI_2 production to be greater in aortic strips obtained from rabbits fed an atherogenic diet for 4 months versus a regular diet, with all rabbits in the atherosclerotic diet group having well developed atherosclerotic lesions based on histological examination. Mehta et al.[10] induced atherosclerosis in rabbits by balloon injury of the aorta followed by feeding an atherogenic diet for 3 months. Aortic tissue rings from atherogenic diet–fed rabbits and control rabbits were harvested at 6-keto-$PGF_{1\alpha}$ measured by radioimmunoassay. They found that 6-keto-$PGF_{1\alpha}$ production was greater in tissue samples obtained from the rabbits fed an atherogenic diet as compared with controls. In miniature swine, Sinzinger et al.[11] found the PGI_2 production by the abdominal and thoracic aorta to be enhanced in those swine with a greater degree of hypercholesterolemia, subsequent to balloon injury of the abdominal aorta and feeding an atherogenic diet for 4 weeks, as compared with those swine exhibiting a lesser degree of hypercholesterolemia. FitzGerald et al.[35] found enhanced PGI_2 production in patients with severe atherosclerosis of the lower limbs as measured by urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ by gas chromatography-mass spectrometry.

Conversely, Gryglewski et al.[12] demonstrated by bioassay that PGI_2 production was suppressed in aortic strips from rabbits fed an atherogenic diet for 1 or 3 months with a partial recovery of PGI_2 production noted in the experimental rabbits at 5 months. Wang et al.[13] fed an atherogenic diet to rabbits for 2 months followed by a regular chow diet for 35 days. They showed that 6-keto-$PGF_{1\alpha}$ synthesis by aortic wall tissue, measured by radioimmunoassay, was significantly less in the experimental rabbits than control rabbits and that there was a difference in production between tissue samples obtained from atherosclerotic lesion sites as opposed to nonatherogenic lesion sites in the aortas of the atherogenic diet–fed rabbits. A biphasic response in 6-keto-$PGF_{1\alpha}$ production was noted by Beetens et al.[15] in their study in rabbits fed an atherogenic diet for 10 weeks. A transient increase in 6-keto-$PGF_{1\alpha}$ production, as measured by RIA, was noted after 2 weeks of feeding an atherogenic diet as compared with controls. Following the transient increase in PGI_2 production, a steady decline in PGI_2 production was noted thereafter. More recently, Myers et al.[16] described a triphasic response in PGI_2 production in the thoracic aorta of rabbits. A transient decrease in 6-keto-$PGF_{1\alpha}$ was noted after 2 weeks of feeding an atherogenic diet to rabbits followed by an increase back to normal levels of synthesis by week 5 and a subsequent decline in produc-

tion after week 7. Atherosclerotic lesions were not detected until 7 weeks of feeding the atherogenic diet. The conflicting findings of Beetens[15] and Myers[16] after 2 weeks of feeding an atherogenic diet may be more related to their assay techniques as Beetens assessed intimal PGI_2 production using a well technique whereas Myers assessed PGI_2 production via a microsomal membrane fraction of the whole aortic wall.

Most of the above studies have investigated alterations in PGI_2 production in vessels with atherosclerotic lesions already established, with the exceptions of the studies by Beetens[15] and Myers[16] which showed contradictory findings regarding PGI_2 production prior to atherosclerotic lesion formation in the rabbit. Sinzinger et al.[36] have found that the arteries of humans and swine, which are species more susceptible to atherosclerosis, produce significantly lower amounts of PGI_2 than rats or rabbits. Thus our findings in young swine may be more applicable to the study of human atherogenesis as both species produce similar amounts of PGI_2. The thoracic aorta at the time points of 10, 30, 90, and 180 days provided us with a systematic means of assessing PGI_2 production at potentially critical time periods preceding and during the initial development of atherosclerotic lesions. Our findings clearly demonstrate that despite lesion development in 75% of the 180-day atherogenic diet swine there was no significant difference in PGI_2 production in the control versus atherogenic diet groups. Hence, with no differences in PGI_2 production found before lesion development (despite the age-related decline) and no differences at the fatty streak stage (Type II lesions) we can confidently state that PGI_2 has no role in the initial development of atherosclerosis, at least not up to the fatty streak stage. As such, the effect of decreased PGI_2 synthesis noted in vessels with advanced atherosclerosis would appear to be secondary to the influences of the advanced atherosclerotic lesions in the vascular tissue as opposed to the decreased PGI_2 production being directly etiologic for atherogenesis.

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