Interaction of Insulin and Prostacyclin Production in the Rat

EUNICE M. LASCHÉ AND RONALD E. LARSON

SUMMARY

The effects of hyperinsulinemia on production of prostacyclin (PGI_2), a potent inhibitor of platelet aggregation, was studied in vitro in the rat. Aortic rings following 1-h preincubation at 37°C and 1 h at 4°C were incubated with and without purified porcine insulin in Krebs' glucose at 37°C for 5 min and immediately percent inhibition of platelet aggregation was determined. PGI_2 production in ng/mg aorta was estimated from a curve using PGI_2 standard. The mean PGI_2 production was significantly decreased in those rings incubated with insulin in concentration of 2500, 500, and 250 μU/ml. Likewise, incubation of rings at 22°C for 30 min resulted in at least ten times less PGI_2 with insulin. Since PGI_2 appears to exert its antiaggregatory effect through cyclic AMP, theophylline was added to the incubation medium resulting in potentiation of inhibition of aggregation which was decreased to control levels when insulin was also added to the medium. Pancreatic slices yielded no significant change in insulin obtained when incubated with and without 5 ng of PGI_2 in 2 ml of 2.8 mM glucose, but in a high glucose medium (28 mM) the PGI_2-treated slices yielded significantly less insulin. Since PGI_2 may play a role in the formation of atherosclerotic plaques, these results suggest a possible deleterious effect of elevated insulin levels in type II and in insulin-treated type I diabetics with regard to macrovascular disease. Suppression of insulin production in presence of PGI_2 in high glucose medium resembles the action of other prostaglandins and suggests it may inhibit insulin secretion after a glucose load. DIABETES 31:454–458, May 1982.

Prostacyclin (PGI_2), a newly discovered prostaglandin generated in vascular endothelial cells, or other cells containing prostacyclin synthetase, from prostaglandin endoperoxides produced from arachidonic acid, is a potent inhibitor of platelet aggregation. A possible homeostatic relationship between PGI_2 and thromboxane A_2, which is formed in the platelets from endoperoxides under the influence of thromboxane synthetase, has been hypothesized, with thromboxane favoring and PGI_2 preventing thrombus formation.[1–5] This has suggested that PGI_2 may have a role in preventing thrombus formation on atherosclerotic plaques and may be an inhibitory factor in the development of atherosclerosis. Reports of low PGI_2 production in vessels of diabetic humans and rats[6–8] and of absence of PGI_2 production by atherosclerotic plaques[9,10] has further suggested that decreased PGI_2 generation might be a factor in the pathogenesis of the earlier and more severe occurrence of macrovascular disease in diabetics. Both human and rodent endothelial cells possess surface-binding sites for insulin; human arterial cells have been reported to bind twice as much insulin[11] and produce less prostaglandin than venous.[2,12]

In this study we have investigated the effect in vitro of high dosage of insulin on prostacyclin production by rat aortic intima and of prostacyclin on insulin production by rat pancreas.

MATERIALS AND METHODS

PGI_2 was determined by a bio-assay previously described and employed by various workers based on its generation by rat aortic rings and assay by its ability to inhibit platelet aggregation.[6–8,13–15] For each determination five Sprague Dawley rats per assay were injected with nembutal, the abdominal aortas rapidly excised and, following removal of adventitia, immersed in Krebs' buffer at 4°C. The tissue was cut into fine rings, weighing 2–3 mg each, and preincubated in Krebs' buffer containing 2.8 mM glucose at 37°C under 95% oxygen and 5% CO_2 for 1 h. The rings were well mixed, removed from the incubation medium, washed, and weighed accurately into samples ranging from 25–30 mg. After being left for at least 1 h at 4°C, each was incubated either with or without purified porcine insulin (Eli Lilly and Co., Indianapolis, Indiana) in amounts of 0.5 mU, 0.1 mU, or

From the University of South Florida, Tampa, Florida.
Address reprint requests to Dr. Eunice M. Lasché, Department of Internal Medicine, Box 19, University of South Florida, College of Medicine, 12901 North 30th Street, Tampa, Florida 33612.
Received for publication 6 July 1981 and in revised form 26 January 1982.

0.05 mU in siliconized tubes containing 200 μl of Krebs' glucose at 37°C for 5 min. A few samples were also incubated with insulin dosage of 1–2 mU. After each incubation, aggregation was immediately determined in the Bio-data aggregometer after adding the same aliquot of supernatant as used in the buffer control to rat citrated platelet-rich plasma, 30 s before the addition of ADP 1×10^{-5} M. The quantity of prostacyclin was based on the degree of inhibition of platelet aggregation and was estimated from a standard curve using authentic prostacyclin (kindly furnished by Dr. John Pike of Upjohn Co., Kalamazoo, Michigan), expressed as ng/mg wet weight of aorta and results given as the mean ± 1 SEM. There was only minimal daily variation in the percentage of platelet aggregation by the buffer controls; however, each incubation was compared with the controls obtained on that day, and also with that day's assay of PGI_2 from aortic rings to which insulin had not been added since there was some daily variation in PGI_2 production. Aortic rings from two groups of six rats each were also incubated at 22°C for 30 min with and without insulin, 1 mU, and the amount of PGI_2 determined. Theophylline 1×10^{-3} M was also incubated with and without insulin to observe the effect on platelet aggregation.

The pancreases of the rats were removed, preincubated in 5 ml of Krebs' buffer containing 2.8 mM glucose for 1 h under oxygen and CO_2, washed, cut into four equal sections, sliced, and incubated with and without PGI_2 5 ng in 2 ml of either 2.8 mM glucose or 28 mM glucose for 30 min, and the supernatant was removed for insulin determination (Phadebus, Pharmacia, Piscataway, New Jersey). A similar procedure was done using 6 keto $PGF_1\ \alpha$ instead of PGI_2.

RESULTS

Figure 1 shows a mean PGI_2 production by aortic rings incubated with insulin, 0.5 mU/200 μl, of 0.09 ± 0.02, as compared with a mean production of 0.20 ± 0.02 ng/mg wet weight of aorta by non-insulin-treated controls done during the same assay period ($P < 0.001$). Smaller amounts of insulin, incubated similarly, resulted in PGI_2 production by 0.1 mU insulin concentration of 0.07 ± 0.01 vs. control of 0.14 ± 0.01 ng/mg PGI_2 ($P < 0.001$) and by the 0.05 mU insulin concentration of 0.09 ± 0.01 vs. control of 0.15 ± 0.01 ng/mg PGI_2 ($P < 0.01$). In each group, consisting of insulin concentrations of 2500, 500, and 250 μU/ml, the decreased PGI_2 production as compared with the non-insulin-treated controls was statistically significant. Use of 2 mU per 200 μl completely inhibited PGI_2 production. The control values for PGI_2 production from aortic rings of approximately 0.15–0.20 ng/mg agrees fairly closely with the results of other authors using this method.[6–8,12,13,15]

Although PGI_2 is very unstable with a half-life of 3 min at 37°C, aortic rings continue to produce it so that prolonging the incubation time did appear to result in increasing amounts of prostacyclin being obtained, even though some was continually being degraded. When aortic rings were incubated at 22° rather than 37°C (to decrease the rapidity of degradation of PGI_2) for 30 min, the final PGI_2 obtained was tenfold or higher in controls without insulin as compared with those with insulin (Figure 2). Addition of theophylline to the incubating rings with and without insulin is shown in Figure 3. The results are expressed in this figure as inhibition of

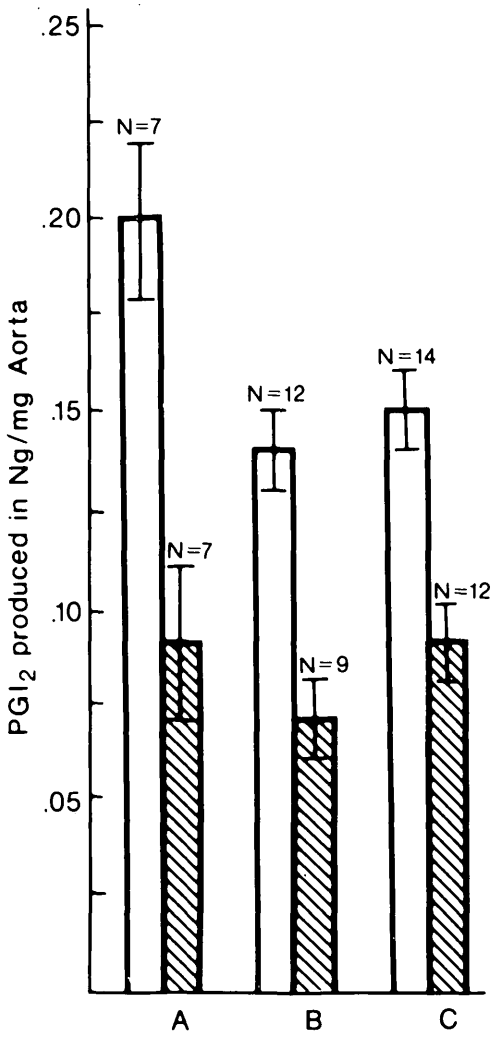

FIGURE 1. Incubation of rat aortic rings (total sample 25–30 mg) with and without insulin in concentration of: A. 0.5 mU/200 μl, B. 0.1 mU/200 μl, C. 0.05 mU/200 μl of Krebs' glucose for 5 min at 37°C. (▭, controls without insulin; ▨, insulin treated) Mean ± SEM. A. $P < 0.001$, B. $P < 0.001$, C. $P < 0.01$. N = number of samples, each from five rats.

platelet aggregation, since it appears most likely that the complete or nearly complete inhibition noted by the open circles, indicating theophylline added to the aortic tissue, represents prolongation of the effect of PGI_2, working through cAMP, by theophylline, based on its effect as a phosphodiesterase inhibitor. It is to be noted that the percent aggregation of supernatant from insulin and theophylline incubations was not significantly different from that obtained by the controls done without addition of either. Addition of insulin or of theophylline to platelet-rich plasma without incubation had no effect on platelet aggregation, indicating theophylline per se did not have an effect on inhibition of aggregation. One would assume that since platelet thromboxane is stated to exert its aggregatory effect through inhibition of cAMP production a phosphodiesterase inhibitor would not be of significance in this situation.

Incubation of pancreatic slices with and without 5 ng of PGI_2 in 2 ml of 2.8 mM glucose resulted in no significant change in insulin obtained, but in the high glucose medium (28 mM), slices with PGI_2 yielded significantly less insulin assayed: 1133 ± 26 vs. 1311 ± 35 mU/ml, P being < 0.01 (Figure 4). No significant difference in insulin production

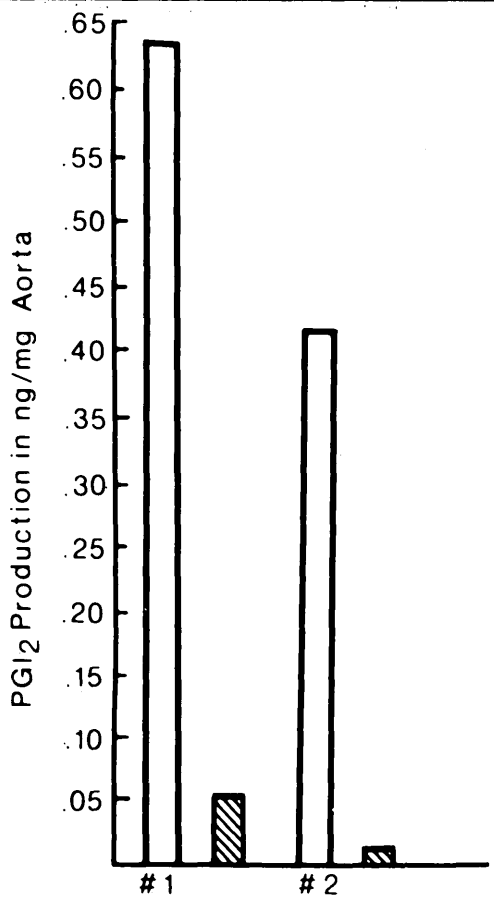

FIGURE 2. Two incubations of rat aortic rings (25 mg) with and without insulin 1 mU/200 μl at 22°C for 30 min. (▭, control; ▨, insulin treated).

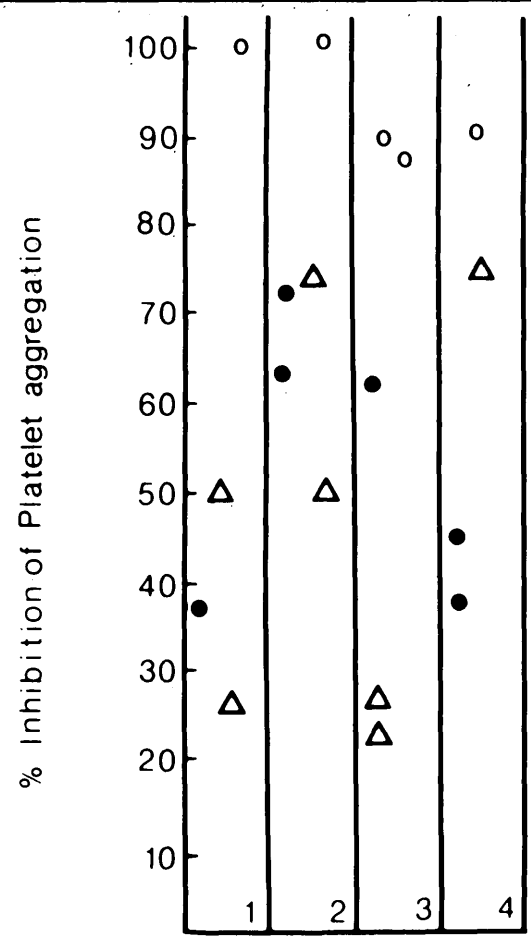

FIGURE 3. Four separate incubations of rat aortic rings with theophylline 1 × 10^{-3} M (○), theophylline and insulin (1 mU) (△), and aortic rings alone (●) in 200 μl Krebs' glucose, showing potentiation of inhibition of platelet aggregation by theophylline.

was obtained in either medium using 6 keto PGF_1 α, indicating that this metabolic product of PGI_2 did not appear to be biologically active.

DISCUSSION

It is generally believed that prostacyclin exerts its antiaggregatory effect through increasing cyclic AMP in the platelets.[16,17] The antithrombotic activity of phosphodiesterase inhibitors, such as dipyridamole and theophylline, appears to depend on the activation of platelet adenylate cyclase by prostacyclin and consequent stimulation of cyclic AMP.[18] Consistent with this theory is our finding of nearly complete inhibition of platelet aggregation after incubation of aortic rings with theophylline. The finding that the addition of insulin to the incubation mixture lessens the inhibitory effect of theophylline, agrees with Stout's work, which revealed that db cyclic AMP and insulin had opposing actions on proliferation of smooth muscle cells in the intima,[19] and would be consistent with decreased PGI_2 production by insulin-treated aorta.

These results suggest that insulin, in high dosage at least, decreases prostacyclin production by vascular endothelium in the rat. The role of hyperinsulinemia as a risk factor for atherosclerosis has been suggested by several authors,[20–22] by recent population studies from Finland[23] and by numerous reports of elevated circulating insulin to glucose levels in diabetics and nondiabetics with atherosclerosis, including ischemic heart disease, peripheral vascular disease, and cerebral vascular disease.[23–33] Experimental work has indicated a stimulatory effect of insulin on proliferation of smooth muscle cells in the intima,[19] which is an important pathologic finding and possibly an initial event in the generation of the atherosclerotic plaque.[34] Proliferative changes and thickening of the media of the arterial wall with significant increase in fatty acid and cholesterol content has been reported in the legs of dogs in which intraarterial injection of insulin was infused.[35] Likewise experimental work revealed that hyperglycemia in the absence of insulin did not result in the development of atheromata as occurred in normoglycemic rabbits in the presence of insulin when both were given a high cholesterol diet.[36] Platelet hyperaggregability has also been noted in atherosclerosis[37] and in insulin-induced hypoglycemia.[38] Although it has been reported that blood vessels of untreated streptozotocin-diabetic rats produced decreased amounts of PGI_2 and that restoration to normal production occurs with control of the diabetes by insulin,[15] it must be emphasized that this type of experimental diabetes, based on beta-cell destruction, is not an appropriate model for type II diabetes. The fact that PGI_2 production could not be restored to normal by acute administration of insulin in two separate studies[6,15] suggests that insulin per se may not have been the factor affecting PGI_2 production in this study. It is possible that other factors associated with the diabetic state that have

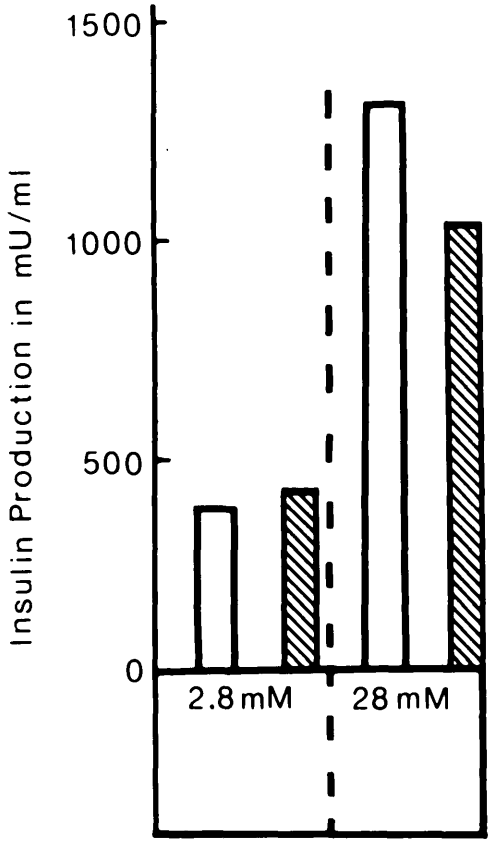

FIGURE 4. Incubation of pancreatic slices in glucose medium, 2.8 mM and 28 mM with and without PGI_2, 5 ng/2 ml of medium. Each bar represents 12 samples (□, control slices; ▨, PGI_2-treated slices). Insulin production is significantly decreased in the high glucose medium ($P < 0.01$) without significant difference in low glucose medium.

been reported to alter PGI_2 production, such as blood lipid levels[39,40] may have been responsible.

In summary, although some have linked atherogenic lesions with insulin deficiency, considerable experimental work has indicated that insulin has effects that may play a role in the pathogenesis of atherosclerosis. The association between type II diabetes and elevated insulin levels is well known, and even though the mild diabetic may release less insulin per unit of secretory stimulus, the absolute output of insulin is greater due to the more intense hyperglycemia. Postprandial immunoreactive insulin (IRI) levels in type II diabetics have been reported to range as high as 300–400 μU/ml,[41,42] thus approximating some of the concentrations used in this study. Type I diabetics, who also may get macrovascular disease, not infrequently receive greater doses than the pancreas ordinarily puts out and such injected insulin first passes through the systemic circulation instead of through the portal circulation and liver as normally occurs. Accurate IRI levels are difficult to assess in this type of diabetes due to circulating insulin antibodies. However, total IRI, obtained by separating insulin from antibodies prior to assay, has been reported to be extremely high in some individuals and it appears that the antibodies may function as a circulating depot which releases insulin irrespective of the metabolic need.[43]

Some have reported absence of generation of prostacyclin from human atherosclerotic plaques even as early as the appearance of the fatty streak.[9,10] Experimental atherosclerosis in rabbits is reported to be associated with suppression of prostacyclin production from exogenous arachidonic acid by the coronary vascular bed and also was associated with decreased spontaneous prostacyclin formation by incubated rings of mesentery artery.[44] Inhibition of prostacyclin production by insulin might therefore be one of the mechanisms favoring atherosclerosis.

Although studies of the effect of prostaglandins other than PGI_2 on beta-cell function have been somewhat contradictory, prostaglandin E has been reported to inhibit the acute insulin response to a glucose load in both humans and experimental animals.[45] This reported finding would be consistent with the occurrence here of decreased insulin obtained after PGI_2 in a high glucose medium, and would indicate that, in this particular action, PGI_2 has an effect similar to other prostaglandins in that it may have an inhibitory effect on insulin secretion in the presence of a high glucose load.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. Roy Behnke, Chairman of the Department of Internal Medicine, for aid from department funds for this project and to the Upjohn Company for kindly supplying the prostacyclin and 6 keto $PGF_1\ \alpha$.

This project was supported in part in BRSG Grant 5 S07 RR05749 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

REFERENCES

[1] Moncada, S., Gryglewski, R., Bunting, S., and Vane, J. R.: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature *263*:663–65, 1976.

[2] Moncada, S., Vane, J. R., and Higgs, E. A.: Human arterial and venous tissues generate prostacyclin (Prostaglandin X), a potent inhibitor of platelet aggregation. Lancet *1*:18–20, 1977.

[3] Nalbandian, R. M. and Henry, R. L.: Platelet-endothelial cell interactions. Semin. Thromb. Haemostas. *5*:2:82–111, 1978.

[4] Moncada, S., and Vane, J. R.: Arachidonic acid metabolites and interactions between platelets and blood-vessel walls. N. Engl. J. Med. *300*:1142–47, 1979.

[5] Gryglewski, R. J., Bunting, S., Moncada, S., Flower, R. J., and Vane, J. R.: Arterial walls are protected against deposition of platelet thrombi by a substance (Prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins *12*:685–713, 1976.

[6] Harrison, H. R., Reece, A. H., and Johnson, M.: Decreased vascular prostacyclin in experimental diabetes. Life Sci. *23*:351–56, 1978.

[7] Johnson, M., Harrison, H. E., Raftery, A. T., and Elder, J. B.: Vascular prostacyclin may be reduced in diabetes in man. Lancet *1*:325–26, 1979.

[8] Silberbauer, K., Schernthaner, G., Sinzinger, H., Pisa-Katser, H., and Winter, M.: Decreased vascular prostacyclin in juvenile onset diabetes. N. Engl. J. Med. *300*:366–67, 1979.

[9] D'Angelo, V., Villa, S., Mysliewiec, M., Donati, M. B., and de Gaetano, G.: Defective fibrinolytic and prostacyclin-like activity in human atheromatous plaques. Thomb. Haemost. *39*:535–36, 1978.

[10] Sinzinger, H., Silberbauer, K., Feigl, W., Wagner, O., Winter, M., and Auerswald, W.: Prostacyclin activity is diminished in different types of morphologically controlled human atherosclerotic lesions. Thromb. Haemost. *42*:803–804, 1979.

[11] Bar, R. S., Peacock, M. L., Spanheimer, R. G., Veenstra, R., and Hoak, J. C.: Differential binding of insulin to human arterial and venous endothelial cells in primary culture. Diabetes *29*:991–95, 1980.

[12] Sinzinger, H., Clopath, P., Silberbauer, K., and Winter, M.: Is the variation in the susceptibility of various species to atherosclerosis due to inborn differences in prostacyclin (PGI_2) formation? Experientia *36*:321–23, 1980.

[13] Silberbauer, K., Sinzinger, H., and Winter, M. Prostacyclin production by vascular smooth muscle cells. Lancet *1*:1356–57, 1978.

[14] Moncada, S., Gryglewski, R. J., Bunting, S., and Vane, J. R.: A lipid

peroxide inhibits the enzyme in blood vessel microsomes that generates from postaglandin endoperoxides the substance (Prostaglandin X) which prevents platelet aggregation. Prostaglandins *12*:715–37, 1976.

[15] Harrison, H. E., Reece, A. H., and Johnson, M.: Effect of insulin treatment on prostacyclin in experimental diabetes. Diabetologia *18*:65–68, 1980.

[16] Tateson, J. E., Moncada, S., and Vane, J. R.: Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. Prostaglandins *13*:381–97, 1977.

[17] Bunting, S., and Miller, O. V.: Modulation of human adenylate cyclase by prostacyclin (PGX). Prostaglandins *13*:377–88, 1977.

[18] Moncada, S., and Korbut, R.: Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. Lancet *1*:1286–89, 1978.

[19] Stout, R. W., Bierman, E. L., and Ross, R.: The effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. Circ. Res. *36*:319–27, 1975.

[20] Ganda, O. M. T.: Pathogenesis of macrovascular disease in the human diabetic. Diabetes *29*:931–42, 1980.

[21] Stout, R. W.: Diabetes and atherosclerosis—the role of insulin. Diabetologia *16*:141–50, 1979.

[22] Ducimetiere, J., and Rosselin, G.: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia *19*:205–10, 1980.

[23] Pyörälä, K.: Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease. Results from two population studies in Finland. Diabetes Care *2*:131–41, 1979.

[24] Kashyap, L., Magill, F., Rojas, L., and Hoffman, M. M.: Insulin and non-esterified fatty acid metabolism in asymptomatic diabetics and atherosclerotic subjects. Can. Med. Assoc. J. *102*:1165–69, 1970.

[25] Gertler, M. M., Leetma, H. E., Saluste, E., Rosenberger, J. L., and Guthrie, R. G.: Ischemic heart disease. Insulin, carbohydrate and lipid interrelationships. Circulation *46*:103–11, 1972.

[26] Gertler, M. M., Leetma, H. E., Saluste, E., Covalt, D. A., and Rosenberger, J. L.: Covert diabetes mellitus in ischemic heart and cerebrovascular disease. Geriatrics *27*:105–20, 1972.

[27] Tzagournis, M., Chiles, R., Ryan, J. M., and Skillman, T. G.: Interrelationship of hyperinsulinism and hypertriglyceridemia in young patients in coronary heart disease. Circulation *38*:1156–63, 1968.

[28] Santen, R. J., Willis, P. W., and Fajans, S. S.: Atherosclerosis in diabetes mellitus. Arch. Intern. Med. *130*:833–43, 1972.

[29] Peters, N., and Hales, C. N.: Plasma insulin concentrations after myocardial infarction. Lancet *1*:1144–45, 1965.

[30] Nikkila, E. A., Miettinen, T. A., Vesenne, M. R., and Pelkonen, R.: Plasma insulin in coronary heart disease. Lancet *2*:508–11, 1965.

[31] Sorge, F., Schwartkopff, W., and Neuhaus, G. U.: Insulin response to oral glucose in patients with a previous myocardial infarction and in patients with peripheral vascular disease. Diabetes *25*:586–94, 1976.

[32] Rubenstein, A. H., Seftel, H. C., Miller, K., Bersohn, I., and Wright, A. D.: Metabolic responses to oral glucose in healthy South African white, Indian, and African subjects. Br. Med. J. *1*:748–51, 1969.

[33] Elkeles, R. S., Lowy, C., Wyllie, A. D. H., Young, J. L., and Fraser, T. R.: Serum insulin, glucose and lipid levels among mild diabetics in relation to incidence of vascular complications. Lancet *1*:880–83, 1971.

[34] Ross, R. and Glomset, J. A.: Atherosclerosis and the arterial smooth muscle cell. Science *180*:1332–39, 1973.

[35] Cruz, A. B., Amatuzio, D. S., Grande, F., and Hay, L. J.: Effect of intra-arterial insulin on tissue cholesterol and fatty acids in alloxan-diabetic dogs. Circ. Res. 9:39–43, 1961.

[36] McGill, H. C., and Holman, R. L.: The influence of alloxan diabetes on cholesterol atheromatosis in the rabbit. Proc. Soc. Exp. Biol. Med. *72*:72–75, 1949.

[37] Gormsen, J., Nielsen, J. D., and Anderson, L. A.: ADP-induced platelet aggregation *in vitro* in patients with ischemic heart disease and peripheral thromboatherosclerosis. Acta Med. Scand. *201*:509–13, 1977.

[38] Hutton, R. A., Mikhailidis, D., Dormandy, K. M., and Ginsburg, J.: Platelet aggregation studies during transient hypoglycemia. J. Clin. Pathol. *32*:434–38, 1979.

[39] Nørdøy, A., Svensson, B., Wiebe, D., and Hoak, J. C.: Lipoproteins and the inhibitory effect of human endothelial cells on platelet function. Circ. Res. *43*:527–34, 1978.

[40] Viinikka, L., and Ylikorkala, O.: An inverse correlation between plasma prostacyclin and serum triglycerides. N. Engl. J. Med. *302*:1424–25, 1980.

[41] Ghu, P. C., Conway, M. J., Krouse, H. A., and Goodner, C. J.: The pattern of response of plasma insulin and glucose to meals and fasting during chlorpropamide therapy. Ann. Intern. Med. *68*:757–69, 1968.

[42] Molnar, G. D., Taylor, W. F., and Langworthy, A. S.: Plasma immunoreactive insulin patterns in insulin-treated diabetics. Mayo Clin. Proc. *47*: 709–19, 1972.

[43] Rasmussen, S. M., Heding, L. G., Parbst, E., and Vøland, Aa.: Serum IRI in insulin-treated diabetics during a 24-hour period. Diabetologia *11*:151–58, 1975.

[44] Dembinska-Kiec, A., Gryglewska, T., Zmuda, A., and Gryglewski, R. J.: The generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rats. Prostaglandins *14*:1025–34, 1977.

[45] Robertson, P. R.: Prostaglandins as modulators of pancreatic islet function. Diabetes *28*:943–48, 1979.