Hyperinsulinemia Induces Myocardial Infarctions and Arteriolar Medial Hypertrophy in Spontaneously Hypertensive Rats


To investigate the effects of hyperinsulinemia on the myocardial vessels, long acting insulin (mixtard, a combination of 30% regular human insulin and 70% NPH human insulin) was injected daily for 8 weeks, intraperitoneally, in two strains of rats, normotensive WKY and hypertensive SHR. There were four groups in all, a control group, and an insulin-injected group in each strain. The drinking water contained 10% glucose to prevent hypoglycemia in the insulin-injected rats. At the end of the 8 weeks experimental period, after measuring blood pressure and taking blood for the determination of glucose, urea, creatinine, and insulin, the rats were killed. The organs were fixed in formaldehyde.

The blood glucose levels were higher at the end of the experiment, in both the placebo- (saline-) and the insulin-injected rats. Blood pressure rose significantly only in the insulin-injected SHR. The intramyocardial arterioles in the insulin-injected SHR had a significantly thicker vascular wall than the placebo-injected SHR, as represented by the vessel wall to lumen ratio, because of hypertrophy of the media. When compared with the placebo injected WKY rats, there was a higher wall/lumen ratio of the intramyocardial arterioles in the insulin-injected WKY, but the difference did not reach significance. Heart weights factored by body weights was significantly higher in insulin-injected as compared with placebo-injected SHR.

Myocardial infarctions were observed in four of eight rats in the insulin-injected SHR group despite the fact that there were no signs of atherosclerosis or intimal thickening. It is possible that the increase in heart weight and the probable increase in metabolic activity resulting from hyperinsulinemia, together with the increased oxygen demand of the myocardium and the arteriolar narrowing, may have contributed to the occurrence of myocardial infarctions in the absence of atherosclerotic coronary occlusion. Am J Hypertens 1997;10:646–653 © 1997 American Journal of Hypertension, Ltd.

Key Words: Insulin, myocardial arterioles, blood pressure, vascular smooth muscle cells, atherosclerosis, myocardial infarction.

Hyperinsulinemia has been implicated in the pathogenesis of hypertension, as well as in accelerated atherosclerosis. Insulin-injected chickens given a normal diet developed lipid-containing lesions in the aorta without weight gain and without changes in blood lipids. Furthermore, in chickens, insulin prevented the regression of atherosclerosis when a cholesterol-rich diet...
was withdrawn. Insulin-stimulated cultures of monkey aortic smooth muscle cells proliferate in a dose-dependent manner. Insulin has also been shown to cause hypertrophy of smooth muscle cells and of cardiomyocytes and proliferation of noncardiomyocytes in vitro. The addition of insulin to cultures of smooth muscle cells obtained from aortas of male albino rats resulted in a significantly greater proliferation of these cells. The major effect of insulin on blood vessels was that it resulted in marked thickening of the arteriolar wall and considerable narrowing of the vascular lumen. Previously we have shown that 3 weeks of intraperitoneal insulin injections caused myocardial arteriolar wall hypertrophy with narrowing of the lumen.

Clinically, it has been observed that insulin resistance in non-insulin-dependent diabetes mellitus (NIDDM) was significantly more marked in patients with ischemic heart disease (IHD), although hypertension, cholesterol levels, and body mass index are also, each independently, associated with IHD. Furthermore, hyperinsulinemia is a common disturbance in nondiabetic men with premature coronary artery disease. Morris et al, in their review on insulin and hypertension, drew on three major clinical studies to conclude that insulin may be a direct risk factor for the development of atherosclerosis. These studies were the Helsinki police study, in which coronary events were found to be more common in patients with high fasting and 1 and 2 h postglucose insulin levels; the Paris prospective study, in which fasting plasma insulin independently correlated with events of coronary heart disease; and the Busselton study, in which insulin was associated with all causes of mortality in men.

In the present study, we investigated structural changes in the myocardium and myocardial arterioles in SHR and WKY rats after 8 weeks of exogenous hyperinsulinemia induced by the daily intraperitoneal injections of long-acting insulin. To avoid the possibility of an atherogenic effect of the diet, the experiments were done on rats that are known to be resistant to atherogenic diets.

METHODS

Normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), (Tel-Aviv University colony, originally purchased from Harlan Co., Blackthorn, UK), initially weighing 290 ± 30 g, were studied. At the beginning of the experiment, rats of both strains were age matched. Rats of similar weights were chosen from the same litter. They were divided into two groups each. One group received intraperitoneal normal saline as placebo, 0.2 to 0.3 mL per day, and the other received intraperitoneal human insulin retard (Insulin Mixtard, a mixture of 30% regular human insulin and 70% NPH human insulin manufactured by DNA technology, Novo Nordisk A/S, Bagsvaerd, Denmark). The dose used was 0.8 unit/kg body weight/day as reported previously, but in this experiment it was given for 8 consecutive weeks, with the hope of intensifying the expected pathology. The rats were injected daily at about 8 AM. The rats had free access to drinking water. The water contained 10% sugar to prevent hypoglycemia. Eight rats were allotted to each group. Blood pressure was measured under light ether anesthesia, by the tail cuff method. Blood was taken percutaneously from the heart by a 25-G needle between 10 AM and 11 AM for blood glucose and plasma insulin levels. The same manner of taking blood percutaneously through the heart was used in all groups, every time. At the end of 8 weeks, the rats were weighed, lightly anesthetized with ether, blood pressure was measured, and blood was taken from the heart. The abdomen and thorax were opened, all viscera removed and placed in formaldehyde. The heart was removed from the rest of the viscera, weighed after removal of blood clots, embedded in paraffin, cut, and stained with hematoxylin and eosin. Well rounded arterioles within the myocardium, of the size of about 100 μm were examined. Their wall thickness and lumen diameter were measured with a grid mounted microscope eyepiece. Wall thickness was divided by lumen diameter to give the ratio of the vascular wall thickness/lumen diameter, as we have previously described.

Blood was taken for insulin levels at 2 weeks and at 8 weeks of the experiment. Plasma insulin was determined by radioimmunoassay with the Sorin Kit, INSIK-5 (P2796) Sallugia, Italy.

Experimental Procedures Formal approval from the Experimental Animals Committee was obtained. The rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals, as adapted by the US National Institutes of Health. The rats were kept in an air conditioned room with a 12-h dark–light cycle and fed regular chow containing 0.5% sodium chloride and 20% protein.

Blood Pressure Measurement Systolic blood pressure was measured by the tail cuff method using the IITC blood pressure electronic amplifier, model 29-SSP (IITC Life Science, Woodland Hills, CA). Blood was analyzed for glucose, urea, and creatinine by the Hitachi auto analyzer (Hitachi, Mannheim, Germany).

Statistical Analysis Values are presented as mean ± standard error of the mean (SEM). Paired or unpaired t tests, as well as analysis of variance (ANOVA), where applicable, were calculated with the help of the NCSS-61 statistical program (© Dr. Jerry I. Hintze, Kaysville, UT), to compare the different groups. A P of .05 or less was considered significant.
RESULTS

Blood Chemistry  Serum Creatinine  There was no statistical difference between the initial and the final serum creatinine levels of all groups: the initial value in the placebo-injected WKY was 0.63 ± 0.02 and the final value 0.69 ± 0.03 mg%. In the insulin-injected WKY the initial value was 0.66 ± 0.02 and the final value 0.70 ± 0.01 mg%. In the placebo-injected SHR the initial value was 0.70 ± 0.01 and the final value 0.63 ± 0.03 mg%. In the placebo-injected SHR the initial value was 0.63 ± 0.07 and the final value 0.63 ± 0.16 mg%.

Blood Glucose  All groups had a similar, statistically significant rise in final blood glucose values when compared with the initial values, as sugar was added to the drinking water in a concentration of 10% to avoid hypoglycemia in the insulin-treated groups (see Table 1). The initial and final blood glucose values in the WKY rats receiving placebo injections were 146 ± 15.9 and 226.3 ± 11.5 mg%, respectively. In the WKY rats receiving insulin they were 152.75 ± 12 and 188.9 ± 8.8 mg%, respectively. In the SHR receiving placebo the initial and final values were 165.4 ± 11.5 and 236.9 ± 12.1 mg%, respectively. In the insulin injected SHR the initial and final glucose values were 160.75 ± 10.2 and 218.3 ± 13.6 mg%, respectively.

Insulin Blood Levels  Insulin blood levels performed at the initial stage of the experiment, after 2 weeks and again after 8 weeks, taken together, were as follows: in control WKY rats it was 37.9 ± 16.2 μIU/mL (mean ± SE, n = 22), in the WKY injected with insulin it was 134 ± 109.8 μIU/mL (n = 12). In the SHR controls it was 54.2 ± 25.6 (n = 28) and in the SHR injected with insulin it was 143.02 ± 109.6 μIU/mL (n = 15), indicating the attainment of hyperinsulinemia. The differences between the controls and the insulin injected rats were significant (P < .001); the difference between the controls WKY and the control SHR was also significant (P < .05).

Body Weight  There was a rise in body weight in all the groups, which was significant. However, no significant difference of weights between different groups was found initially or at the end of the experiment (Table 2). The initial and final body weights in the WKY receiving placebo were 281.4 ± 8.6 g (mean ± SEM) and 322.14 ± 11.5 g, respectively. In the WKY receiving insulin they were 152.75 ± 12 and 236.3 ± 11.5 g, respectively. In the SHR receiving placebo the final body weights were 276.2 ± 9.8 g and 328.8 ± 11.2 g respectively. In the SHR receiving insulin they were 281.3 ± 7.9 g and 313.1 ± 10.0 g respectively.

Blood Pressure  Blood pressure (BP) was normal in the WKY and did not increase at the end of the experiment, either in the placebo controls or in the insulin-injected group. In the SHR, where the BPs were initially higher, there was no significant rise in the placebo-injected group. However, the insulin-injected group showed a statistically significant rise in BP at the end of the experiment (Table 3). The initial and final blood pressures in the WKY receiving placebo averaged 115 ± 3 and 121 ± 5 mm Hg, respectively. In the WKY rats

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<th>TABLE 1. BLOOD GLUCOSE (mg %) TAKEN INITIALLY AND AT THE END OF THE EXPERIMENTAL PERIOD</th>
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P at the end of the experiment; between the groups, was not significant. Values are mean ± SE.

SHR, spontaneously hypertensive rats. SHR insulin, insulin-injected SHR; WKY, Wistar-Kyoto rats; WKY saline controls, saline-injected control WKY; WKY insulin, insulin-injected WKY.

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<th>TABLE 2. INITIAL AND FINAL BODY WEIGHTS IN GRAMS IN THE DIFFERENT GROUPS OF RATS</th>
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Values are mean ± SE. Abbreviations as in Table 1.

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<th>TABLE 3. BLOOD PRESSURE (in mm Hg) AS MEASURED BY THE TAIL CUFF METHOD, INITIALLY AND AT THE END OF THE EXPERIMENT</th>
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<tr>
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Values are mean ± SE. Abbreviations as in Table 1.
receiving insulin, they were 122 ± 3 and 126 ± 6 mm Hg, respectively. The initial and final blood pressures in the SHR receiving placebo were 181 ± 3 and 196 ± 8 mm Hg, respectively. In the SHR receiving insulin they were 179 ± 4 and 232 ± 7 mm Hg, respectively.

Heart Weights and Myocardial Arteriolar Wall Thickness In the WKY, placebo and insulin-injected rats had similar heart weight factored by body weight (heart weight/body weight × 1,000 were 6.41 ± 0.39 v 7.31 ± 1.41, respectively). However, the insulin-injected SHR had a significantly higher heart weight (6.70 ± 0.60 v 8.16 ± 1.90, P < .05) as compared with placebo-injected SHR (Figure 1). Although the arteriolar wall thickness was slightly higher in the insulin-injected WKY rats when compared with the placebo injected group, this difference did not reach statistical significance (wall thickness/lumen ratios were 0.35 ± 0.04 v 0.28 ± 0.03, respectively). In the SHR, however, vascular wall thickness was significantly higher in the insulin-injected group as compared with the placebo group (0.60 ± 0.05 v 0.46 ± 0.03, respectively, P = .038) (Figure 2). Representative arterioles of the different groups are shown in Figure 3.

There were no signs of atherosclerosis, and the intima of all the arterioles was normal. Immunohistochemical staining with α-smooth muscle actin was positive, indicating that the increase in vascular wall thickness was due to medial smooth muscle hyperplasia. This confirmed the Masson stain findings, which showed that the hypertrophied vascular media consisted of vascular smooth muscle (Figure 4).

Blood Glucose mg%

![Blood Glucose mg%](image)

**Figure 1.** Heart weight per body weight × 1000 in the different groups of rats.

Myocardial Histology In the SHR receiving daily insulin injections, myocardial scarring, indicating previous myocardial infarction, was seen in two rats (Figure 5). In two other rats of the same group, acute myocardial infarctions were seen, with areas of inflammatory cell infiltration and necrotic myocytes. In one rat the myocardial infarction was transmural, and in the other it was subendocardial.

**DISCUSSION**

The present study confirmed the findings of our previous experiment in which 3 weeks of hyperinsulinemia induced an increase in BP in SHR but not in WKY rats. The present study was extended to 8 weeks to accentuate the possible structural changes in the myocardium and myocardial vessels. Again we found that hyperinsulinemia caused an increase in BP in the SHR. There were, however, no signs of atherosclerosis. The myocardial arterioles developed medial thickening due to hyperplasia of the smooth muscle cells in the media. This occurred both in the normotensive WKY, where the changes were slight and did not reach significance, as well as in the SHR, where these changes were significantly more marked. Furthermore, in four of eight insulin-injected SHR, myocardial infarctions, both old and new, were found, despite the fact that frank atherosclerotic lesions were not observed, and the intima seemed normal throughout. However, a significant decrease in the internal lumen of the intramyocardial arterioles was found. This decrease in internal diameter was not caused by intraluminal arteriosclerotic obstruction, as seen in human patients with ath-

FIGURE 4. Immunohistochemical staining with α-smooth muscle actin ×160. Intramyocardial arteriole of SHR insulin-injected, showing marked medial thickening due to smooth muscle hypertrophy.
FIGURE 5. Masson trichrome stain ×160. Myocardial section of SHR insulin-injected, showing moderately vascularized collagenous scar, mildly infiltrated by mononuclear cells, consistent with recent myocardial infarction (about 3 weeks old).

Erosclerosis. It was produced by extensive thickening of the media of the arterioles due to hyperplasia of the vascular smooth muscle cells.

These findings complement the earlier findings of Reaven et al that the SHR is a hyperinsulinemic rat as compared with the normotensive WKY rats, and that hypertension is attributable to abnormalities in insulin metabolism. They have also shown that adipocytes isolated from SHR are resistant to insulin-stimulated glucose uptake. Swislocki and Tsuzuki, as well as Hulman et al, similarly found that the SHR is insulin resistant, which was thought to be the cause of the hypertension.

Migration and proliferation of arterial smooth muscle cells from the media to the intima are regarded as the most important initial steps of atherogenesis. Insulin was found to be mitogenic only in conditions where hypoglycemia was not present. Yet these authors caution against extrapolating from rats with short term streptozotocin-induced diabetes mellitus to human diabetics, and against considering proliferation of arterial cells as an initiation of atherosclerosis. Nevertheless, aortic smooth muscle cells possess receptors to insulin, and insulin stimulates the proliferation of aortic smooth muscle cells by almost doubling their number, as evidenced by the incorporation of [3H]thymidine into DNA. Pfeifle et al induced accelerated growth of smooth muscle cells of the aorta of male albino rats by the addition of insulin to the medium. Stout and Vallance-Owen claimed that atherosclerosis occurred in diabetic and in nondiabetic patients who share the common factors of hyperinsulinemia and carbohydrate intolerance. He also showed that hyperinsulinemia induced proliferation of vascular smooth muscles, and suggested that this might be an early process of atherosclerosis. Three important epidemiological studies support these basic observations. The Busselton Study showed that coronary artery disease mortality could be predicted by both systolic and diastolic blood pressures and by 1-h postload plasma insulin levels. The Helsinki Policemen Study showed that 1-h postload plasma insulin, among other factors (age, cholesterol, blood pressure, and smoking), correlated with coronary heart disease. The Paris Prospective Study showed insulinemia to be a strong predictor of coronary heart disease in a population of about 7000 men observed for more than 10 years. These observations indicate that hyperinsulinemia is an independent predictor of coronary heart disease in men.

Hyperinsulinemia was found to be associated with a low HDL cholesterol and a high triglyceride level, which might play an important role in the development of accelerated atherosclerosis seen in hyperinsulinemic states. It was also found to decrease the removal of cholesterol from foam cells in the subintimal region of the vessels. Whether lipid abnormality or insulin per se is the important factor is still not clear. In our experiment, overt atherosclerosis in the arterioles was not observed. On the other hand, proliferation of smooth muscle cells was the dominant finding. This finding seems to be specific to insulin acting as a growth factor.

What are the possible mechanisms of appearance of the myocardial infarctions in hyperinsulinemic rats in the absence of arteriosclerotic lesions in the myocardial vessels? First, it is obvious that myocardial infarctions occurred only in the hyperinsulinemic SHR, even though blood was taken percutaneously from the heart in all the groups in a similar manner. In two SHR there were definite acute changes, namely areas of inflammatory cell
infiltration and necrotic myocytes. These changes take time to develop and therefore could not have occurred due to the needle puncture, which was done immediately before the rats were killed. There were no complete coronary obstructions in or around the infarcted areas. The only morphologic change that was seen in hyperinsulinemic SHRs was a marked decrease in the luminal area of the involved vessels that was caused by hypertrophy and, therefore, thickening of the media. Thus, two pathological processes occurred in the hyperinsulinemic SHR: an increase in myocardial mass, which probably caused an increase in oxygen demand; and a decrease in myocardial blood flow due to the narrowing of the intraluminal spaces of the coronary arterioles.

We cannot exclude the possibility that repeated injections of insulin induced some degree of insulin resistance in our experimental rats. If such a process did indeed occur, it may have enhanced the effects of hyperinsulinemia present in the SHR to produce structural changes.

Other metabolic factors responsible for an increase in metabolic activity and thus an increase in oxygen demand may exist. It has been shown that hyperinsulinemia inhibited protein degradation in animals and in humans with insulin resistance, and that insulin given in supraphysiologic doses stimulated myocardial protein synthesis. This may play a role in the increased metabolic demands of the protein loaded hypertrophic myocardial cells.

It is still debatable whether hyperinsulinemia is capable of inducing myocardial infarctions in genetically predisposed strains. In this study, hyperinsulinemia induced thickening of arteriolar walls, myocardial infarction, and hypertension in a genetically predisposed strain of rats (namely, SHR), without inducing arteriosclerosis. Even though the rat model is a relatively poor model of atherosclerosis, it seems that it is still capable of inducing myocardial infarctions. Sowers advised recently that the treatment of hypertension should incorporate interventions that do not increase insulin resistance and decrease insulin sensitivity, such as pharmacological treatments that include weight loss and physical activity. Thus it seems justified to conclude that it is beneficial to take measures to lower plasma insulin in the hyperinsulinemic patients, for example, by the use of ACE inhibition, which has been shown to improve insulin responsiveness to glucose.

REFERENCES


