The Influence of Insulin on the Lipids in the Pulmonary Artery and the Lungs of Severely Diabetic Rats

A Histochemical and Chemical Study

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Summary. The effect of insulin on the triglyceride deposits found in the pulmonary artery branches of streptozotocin-diabetic rats was investigated by treating the animals for two, five, nine or 14 days with insulin (3–8 units/day). Histochemical analysis showed that the triglyceride deposits in the pulmonary artery developed within three to four days after the induction of diabetes, but were not present in any animals five days from the initiation of insulin therapy. Plasma triglycerides, non-esterified fatty acids, phospholipid and total cholesterol concentrations were within the normal range within two days of the inception of insulin therapy and random plasma glucose levels were normal within five days. Analysis of lung lipids showed that after 14 days of insulin treatment the decreased content of phospholipids and the increased content of non-esterified fatty acids found in diabetic rats were also normalized. These findings suggest that insulin has an important role in the regulation of lipid metabolism in the pulmonary artery and lung tissue in the diabetic state.

Key words: Diabetes, insulin, non-esterified fatty acids, lipid metabolism, lung, phospholipids, pulmonary artery, rat, streptozotocin, triglycerides.

Lipoprotein lipase (LPL) has been identified in the aortic wall of different species [1, 2]; little is, however, known about the hydrolysis of lipids in the pulmonary artery. The reported effects of insulin and other hormones on lipid metabolism of the arterial wall and on atherogenesis are controversial. Increased lipolytic activity has been observed in the aortic wall of alloxan-diabetic rats, and the adminis-

tration of insulin reduces the activity [3]. On the other hand, a lipolytic effect of insulin in aortic tissue of experimental animals *in vitro* has been described [4].

Recently we reported triglyceride deposits in branches of the pulmonary artery of severely diabetic rats, not treated with insulin [5]. The first aim of the present study was to find out more exactly the time needed for the deposition of triglycerides in the pulmonary artery wall in experimental diabetes, and to establish whether insulin treatment had any effect on the lipid deposits.

A decreased synthesis of phospholipids and neutral lipids of pulmonary tissue has been noted in experimental diabetes, and insulin restores this within a short time [6, 7]. Previously [5] we showed an increased content of pulmonary non-esterified fatty acids (NEFA) and a decreased content of pulmonary phospholipids in severely diabetic rats, not treated with insulin. The second aim of the present study was, therefore, to explore the effect of insulin treatment on the lipid composition of pulmonary tissue in experimental diabetes.

Material and Methods

Induction of Diabetes

Male Sprague-Dawley rats weighing 300–350 g were used. Diabetes was induced with streptozotocin (Upjohn & Co., lot 1613 E, MCM 2), 3 g/100 ml, freshly prepared in 0.1 mol/l citrate buffer, pH 4.5, and injected intracardially in a dose of 65 mg/kg of body weight under pentobarbital anaesthesia [8]. Control rats received an equal volume of intracardiac buffer. Rats were fed ad libitum a commercial chow containing 53.0% carbohydrate, 20.9% protein, 4.5% fat, 3.9% fibre and the usual vitamins and minerals (Hankkija Ltd., Turku, Finland). Only severely diabetic animals were included in the study (weight loss, marked ketonuria: Ketostix® + + +, and glucosuria, > 5 g/100 ml by Clinitest®).

Table 1. Non-fasting levels of plasma glucose, triglycerides and non-esterified fatty acids (NEFA) and the incidence of pulmonary artery triglyceride deposits in diabetic rats two, three and four days after the injection of streptozotocin (65 mg/kg), and in control rats, mean $\pm$ SEM. For details see text

Diabetic rats – Time after induction of diabetes days	n	Plasma glucose mg/100 ml	Plasma triglycerides mg/100 ml	Plasma NEFA mmol/l	Triglyceride deposits in the pulmonary artery, positive cases
2 days	15	280 $\pm$ 7 ^a	660 $\pm$ 69	0.81 $\pm$ 0.05 ^b	0 ^{c, d}
3 days	15	302 $\pm$ 6	758 $\pm$ 81	1.01 $\pm$ 0.10	5 ^c
4 days	15	310 $\pm$ 6 ^a	850 $\pm$ 90	1.10 $\pm$ 0.09 ^b	4 ^d
Control rats	15	102 $\pm$ 2	165 $\pm$ 7	0.40 $\pm$ 0.04	0

^a $p < 0.01$; ^b $p < 0.01$; ^c $p < 0.025$; ^d $p < 0.05$

In order to establish the length of time needed for the development of triglyceride deposits in the pulmonary artery wall, 15 non-treated diabetic rats were killed two, three and four days after the injection of streptozotocin.

Insulin Treatment

Four days after the injection of streptozotocin insulin treatment with lente insulin was initiated (Insulin Novo Lente®). Insulin, 3–8 units, was given once a day sc. The dose of insulin was adjusted according to weight of the rats, plasma glucose concentration, and the amount of glucosuria and ketonuria, these parameters being controlled daily at 9 a. m. Further groups of 15 diabetic rats were killed at two, five and nine days, and 19 rats at 14 days after the initiation of insulin treatment. The 19 non-diabetic control rats were not treated with insulin, and were killed after 14 days.

Handling of Blood and Tissue Specimens: Blood was drawn from the retro-orbital venous plexus of non-fasting rats through a capillary tube under pentobarbital anaesthesia and collected in ice-cold ethylene-diaminetetraacetic acid (EDTA) tubes (final concentration of EDTA, 21.7 μ mol/l). The plasma specimens were stored at -20°C until analysis. Immediately after blood sampling the animals were decapitated, the lungs removed and one lung was perfused through the pulmonary artery with 0.154 mol/l saline until the fluid coming from the pulmonary veins was clear. The perfusion usually lasted 5 minutes. A piece of pulmonary artery was then removed for histochemical analysis. In addition, a piece of pulmonary tissue was taken for chemical analysis from rats treated with insulin for 14 days and from the untreated control rats. Tissue specimens were frozen in liquid nitrogen and kept at -70°C until analysis.

Methods: Histochemical analyses of the pulmonary artery were performed for total hydrophobic lipids (9), NEFA (10), triglycerides (11), total cholesterol (12) and phospholipids (13). The specimens of the pulmonary tissue were freeze-dried and homogenized in chloroform-methanol (2:1; v/v). After the extraction of lipids (14) the total lipids were determined gravimetrically (15). The dried extract was reconstituted to 1 ml with chloroform and analysed chemically for NEFA (16), triglycerides (17), total cholesterol (18) and phospholipids (19). Plasma glucose was measured by the method of Carrol et al. (20), with slight modifications (21), and plasma NEFA, triglycerides, total cholesterol and phospholipids were determined by the methods referred to above.

Values for the lipids of the pulmonary tissue are given as mg/g of total lipids. Non-esterified fatty acids are given in terms of their equivalence in palmitic acid in mmol/l. The results for body weight, plasma glucose, NEFA and triglycerides are expressed as mean $\pm$ SEM. The statistical significance of the differences between these mean values was calculated using Student's t-test. The difference between the proportions of triglyceride-deposit positive and negative cases was tested with the Fisher exact probability test. The differences between plasma glucose and lipid values before and after insulin treatment, and between pulmonary lipids of untreated and treated diabetic rats was tested by analysis of variance.

Results

Two days after the injection of streptozotocin the weight loss of the diabetic rats was 31 ± 2.8 g ($9.9 \pm 0.9\%$), three days after the injection 40 ± 3.1 g ($13.1 \pm 1.0\%$), and four days after the injection 56 ± 3.6 g ($18.9 \pm 1.2\%$). Ketonuria and glucosuria were marked within two days after the induction of diabetes.

Time Course of Deposition of Triglycerides in the Pulmonary Artery

Two days after the induction of diabetes no triglyceride deposits were seen in the walls of the pulmonary artery branches, but after three days deposits were found in five out of 15 animals ($p < 0.025$) (Table 1). In rats killed four days after the induction of diabetes, significant deposition of triglycerides was also observed ($p < 0.05$ vs. the animals investigated at two days) (Table 1). A triglyceride-deposit positive case is seen in Figure 1. No significant differences in plasma glucose, triglyceride, or NEFA values were found between two and three days after the induction of streptozotocin, but between two and four days the difference between plasma glucose levels was significant, as was the difference between plasma NEFA values.

Fig. 1. Triglyceride deposits (←) in the wall of the pulmonary artery of a diabetic rat, not treated with insulin. The deposits are mostly located in the media (lead-lipase sulphide, original magnification $\times 380$)

Influence of Insulin Treatment

Control of Diabetes: Fourteen days after the initiation of insulin treatment all of the diabetic rats had reached their pre-treatment weight (329 ± 4.6 g vs. 322 ± 3.5 g, respectively). The plasma glucose and lipid values, and the glucosuria and ketonuria readings two, five, nine and 14 days after the start of insulin treatment are shown in Table 2.

Insulin treatment restored plasma lipid values to the normal range within two days ($p < 0.001$). Variance analysis showed the plasma lipid values of the diabetic rats, treated with insulin for two days or longer, to be similar to those of the control rats (variance ratios ranging from 0.21 to 2.10).

Normoglycaemia and aglucosuria were achieved within five and elimination of ketonuria within two days.

Deposition of Triglycerides in the Pulmonary Artery: Triglyceride deposits in the pulmonary artery wall were seen in one of the 15 diabetic rats treated with

insulin for two days. Plasma NEFA in this animal were 0.37 mmol/l, triglycerides 141 mg/100 ml, plasma glucose 136 mg/100 ml, and there was no ketonuria. No triglyceride deposits were observed in any of the rats treated with insulin for five, nine or 14 days. A triglyceride deposit-negative case is shown in Figure 2.

Lipid Composition of the Pulmonary Tissue: The elevated content of NEFA and triglycerides, and the decreased concentration of phospholipids of the pulmonary tissue were normalized in the diabetic rats treated with insulin for 14 days (Table 3) when compared with data obtained from rats not treated with insulin and having a similar severity of diabetes as that of the insulin-treated ones before the start of insulin treatment [5].

Discussion

In the present study triglycerides accumulated mostly in the media of the pulmonary artery, and insulin

Table 2. Plasma glucose, triglycerides, non-esterified fatty acids (NEFA), phospholipids, and total cholesterol levels and the average glucosuria and ketonuria readings four days after the induction of diabetes (streptozotocin 65 mg/kg), and after insulin treatment of diabetic rats, and in non-diabetic control rats

	Diabetic rats					Control rats No insulin treatment	Variance ratio F and significance level	
	Before insulin treatment	Duration of insulin treatment						
		two days	five days	nine days	14 days			
Group	I	II	III	IV	V	VI	I-VI	II-VI
n	19	15	15	15	19	19		
Plasma glucose (mg/100 ml)	325	141	94	112	102	97	25.7 0.1%	4.2 1%
Plasma triglycerides (mg/100 ml)	930	121	146	171	180	160	18.5 0.1%	2.1 NS
Plasma NEFA (mmol/l)	1.20	0.46	0.50	0.49	0.55	0.45	7.2 0.1%	0.2 NS
Plasma phospholipids (mg/100 ml)	230	130	120	132	124	125	8.0 0.1%	0.4 NS
Plasma total chole- sterol (mg/100 ml)	120	85	81	75	73	70	11.2 0.1%	0.9 NS
Glucosuria g/100 ml	5	2	0	2	0	0		
Ketonuria	+++	—	—	—	—	—

Fig. 2. The wall of the pulmonary artery of a diabetic rat, treated with insulin for nine days. No triglyceride deposits are seen (lead-lipase sulphide, original magnification $\times 230$)

treatment removed the deposits within a few days. No final conclusions can be made about the precise mechanism for the disappearance of triglyceride deposits after insulin treatment. It is possible that triglyceride accumulation is due to hypertriglyceridaemia, resulting from depressed LPL activity in

insulin deficiency [22, 23], the triglycerides being transported in plasma lipoproteins into the arterial wall [24]. However, our present and previous findings [5] do not show any clear differences in plasma triglyceride concentrations between triglyceride deposit-positive and -negative cases. Therefore

Table 3. The level of triglycerides, non-esterified fatty acids (NEFA), phospholipids and total cholesterol in the pulmonary tissue of diabetic rats not treated with insulin, of diabetic rats treated with insulin for 14 days, and of the non-diabetic control rats (mg/g of total lipids)

Lipids in the pulmonary tissue n	Diabetic rats not treated with insulin ^a 43	Diabetic rats treated with insulin 19	Control rats 19	Variance ratio F	Level of significance, %
<i>mg/g of total lipids</i>					
Triglycerides	187	147	151	3.1	5–10
NEFA	159	110	116	16.8	0.1
Phospholipids	536	634	628	13.1	0.1
Total cholesterol	118	109	107	0.9	NS

^a Reinilä, A., Koivisto, V. A., Åkerblom, H. K.: *Diabetologia* **13**, 305–310 (1977)

other mechanisms than hypertriglyceridaemia could also be responsible for triglyceride deposition into the arterial wall during insulin deficiency. The possible role of increased plasma NEFA and glucose levels should not be overlooked.

It is generally assumed that the increased risk of atherosclerosis in diabetes is mediated by high plasma lipid levels, especially those of cholesterol and triglycerides. Some observations, however, suggest that in the diabetic state some factor other than high serum lipid levels might cause atherosclerosis [25, 26]. The tendency to atherosclerosis in diabetes should perhaps also be considered separately depending on whether insulin therapy has been given or not. Further studies are evidently required for the evaluation of the significance of the pulmonary artery triglyceride deposits in the pathogenesis of atherosclerosis, and of the probable particular role of the pulmonary artery in this respect.

Glucose is an important substrate for lung tissue, e. g. as a precursor for lipid synthesis. Physiological concentrations of insulin in the *in situ* rat lung preparation have a clear and significant effect on glucose metabolism [27], suggesting that the lungs may also have an essential non-respiratory metabolic function. It has been proposed that both glucose transport and utilization are reduced during insulin deprivation [28]. Too much insulin is not good for the lungs, since hyperinsulinaemia may impair phospholipid synthesis by reducing dihydroxy-acetone phosphate and glycerol-3-phosphate production, thus causing decreased synthesis of surfactant. The infants of diabetic mothers often have hyperinsulinaemia, and this state may be responsible for the increased frequency of respiratory distress syndrome in these infants [29].

In the present study insulin therapy of diabetic rats restored the decreased concentration of pulmonary phospholipids, the finding corroborating that made previously by others [6, 7]. The increased content of NEFA and triglycerides, and the slightly elevated total cholesterol content in the pulmonary

tissues were also normalized with insulin therapy, confirming that lung tissue of the diabetic rat readily reacts to the correction of insulin deficiency.

In addition to the disturbed lipid metabolism of the lungs in diabetes, some changes of lung elasticity in juvenile diabetics have been reported, suggesting abnormalities in elastin and collagen [30]. On the other hand, another group has reported that lung function tests in juvenile diabetics are similar to those of age- and sex-matched non-diabetic controls [31].

Thus, data from other laboratories and the present findings suggest that many functions of the lung tissue may be adversely affected during insulin deficiency, and that insulin administration corrects the abnormalities.

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