SHORT COMMUNICATION

The effects of treatment with insulin on serum high-density-lipoprotein cholesterol in rats with streptozotocin-induced diabetes

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Summary

1. No significant difference in serum high-density-lipoprotein (HDL) cholesterol was observed when control rats were compared with rats with streptozotocin-induced diabetes of 14 days duration. Blood glucose and serum cholesterol and triglyceride concentrations were significantly higher in the diabetic group.

2. In a second experiment rats with streptozotocin-induced diabetes of 8 days duration were given long-acting porcine insulin (1 unit/day or 3 units/day subcutaneously for 14 days) and were compared with a control group. Blood glucose and serum cholesterol and triglyceride concentrations did not differ significantly with the two doses of insulin and there was no significant difference between serum high-density-lipoprotein cholesterol in control rats and either of the two groups of rats given insulin.

3. When the rats given insulin were grouped according to their blood glucose concentrations, those with blood glucose in the range 20–30 mmol/l were found to have significantly higher serum high-density-lipoprotein cholesterol concentrations than those with higher or lower blood glucose levels or than controls. There was thus a statistically significant curvilinear relationship between blood glucose and serum high-density-lipoprotein cholesterol concentrations.

Key words: cholesterol, diabetes mellitus, high-density lipoproteins, streptozotocin, triglycerides.

Abbreviations: HDL, high-density lipoproteins.

Introduction

In man, the serum high-density-lipoprotein (HDL) cholesterol concentrations of patients with diabetes treated with insulin have been variously reported as low (Lopes-Virella, Stone & Colwell, 1977), normal (Calvert, Graham, Mannik, Wise & Yeates, 1978; Kennedy, Lappin, Lavery, Hadden, Weaver & Montgomery, 1978) and high (Nikkilä & Hormila, 1978; Durrington, 1980). These conflicting reports are probably due to difference in the degree to which diabetes was controlled and in factors other than diabetes and insulin that also influence serum HDL concentrations. In the present report serum HDL cholesterol concentrations were studied in rats with streptozotocin-induced diabetes, with or without insulin.

Methods

Animals

Male litter-mates from the University of Manchester colony of Sprague–Dawley rats were used. They were allowed unlimited access to CRM pellets (Labsure Diets, Poole, Dorset, U.K.) (17·45% protein, 53·9% carbohydrate, 2·35% crude oil and 4·26% fibre) and to water. Intravenous injections of 0·5 ml of sodium chloride solution (0·15 mol/l, saline) in control groups or streptozotocin (65 mg/kg body weight) in saline (adjusted to pH 4 with citric acid 0·025...
mol/l) in diabetic groups were given under ether anaesthesia directly into the exposed jugular vein in the superior thorax. Monocomponent porcine insulin (Monotard MC Insulin Zinc Suspension, Novo, Denmark) was given subcutaneously into the abdominal wall in the rats with diabetes. Blood was obtained by cardiac puncture under ether anaesthesia from non-fasting rats.

Determinations

To separate HDL, 1 ml of rat serum was adjusted to a density of 1.063 g/ml (Havel, Eder & Bragdon, 1955; Lasser, Roheim, Edelstein & Eder, 1973) in cellulose nitrate tubes that were filled with a solution of sodium chloride and potassium bromide of density 1.063 g/ml and ultracentrifuged at 100 000 g for 48 h (Super-speed 65 with 18 × 6.5 ml aluminium rotor, MSE Ltd, Crawley, Sussex, U.K.). The infranatant was then obtained by tube-slicing (Spinco tube slicer, California, U.S.A.) and recovered in a volume of 2 ml. Where possible this procedure was carried out in duplicate for each sample. Cholesterol in HDL and whole serum was measured enzymatically by the method of Allain, Poon, Chan, Richmond & Fu (1974) [CHOD-PAP method; Boehringer Corporation (London) Ltd, Lewes, East Sussex, U.K.], unmodified except that the sample volume was increased to 100 μl. The coefficient of variation of the whole procedure for the measurement of serum HDL cholesterol was 8.2%. Serum triglycerides were also measured enzymatically (Bucolo & David, 1973) [fully enzymatic u.v. method; Boehringer Corporation (London) Ltd], no correction being made for free glycerol. Blood glucose concentration was measured by an automated glucose oxidase method in routine use. Groups of rats were compared by Student’s unpaired t-test or by analysis of variance when there were more than two groups, differences at or below the 5% level being considered statistically significant. These tests and linear and multiple regression analyses were performed by the method of Snedecor & Cochran (1967).

Experiments

First experiment. Rats aged 8 weeks (weight 200–300 g) were randomly allocated to a control group (n = 13) and to a diabetic group (n = 30). Eight days after the injection of saline or streptozotocin the diabetic group received either insulin 1 unit/day (n = 17) or 3 units/day (n = 13). Blood was sampled 14 days after commencing insulin, 6 h after the last dose of insulin had been given.

Results

First experiment: diabetes without insulin

Serum HDL cholesterol was 1.10 ± 0.03 mmol/l (mean ± SEM) in control rats and was not significantly different in the rats with diabetes (1.17 ± 0.04 mmol/l). In the control rats concentration of blood glucose was 7.3 ± 0.2 mmol/l, of serum cholesterol was 1.58 ± 0.05 mmol/l and of serum triglycerides was 0.83 ± 0.07 mmol/l and in rats with diabetes blood glucose was 34.1 ± 1.5 mmol/l, serum cholesterol 1.89 ± 0.07 mmol/l and serum triglycerides 2.33 ± 0.09 mmol/l. These differences in blood glucose, serum cholesterol and serum triglycerides were statistically significant.

Second experiment: diabetes with insulin

No differences were observed in the concentration of serum HDL cholesterol between controls (1.15 ± 0.06 mmol/l; mean ± SEM), rats with diabetes receiving 1 unit of insulin/day (1.21 ± 0.04 mmol/l) and those receiving 3 units/day (1.31 ± 0.09 mmol/l). Serum cholesterol concentrations were similar, being 1.54 ± 0.09 mmol/l in controls, 1.43 ± 0.05 mmol/l in rats with diabetes receiving 1 unit of insulin/day and 1.69 ± 0.10 in those receiving 3 units/day. The serum triglyceride concentration, which was 2.25 ± 0.11 mmol/l in the controls, was significantly elevated in both the rats with diabetes given 1 unit of insulin/day (3.70 ± 0.31 mmol/l) and those given 3 units/day (3.78 ± 0.29 mmol/l), but there was no difference between the two diabetic groups. The blood glucose concentration did not differ significantly between rats with diabetes receiving 1 unit of insulin/day (21.0 ± 3.0 mmol/l) and those receiving 3 units/day (17.6 ± 2.5 mmol/l), although in both these groups it was higher than that of the control group (7.5 ± 0.5 mmol/l).

When the rats given insulin were grouped according to their blood glucose concentrations those with blood glucose in the range 20–30 mmol/l had significantly higher serum HDL concentrations than those with the higher or lower blood glucose and than controls
HDL in diabetic rats

\[ \text{HDL} \text{ in diabetic rats} \]

![Graph](image)

Fig. 1. Concentration of serum high-density-lipoprotein (HDL) cholesterol and triglycerides (mean ± SEM) of 13 control rats and 30 rats with streptozotocin-induced diabetes given insulin grouped according to their blood glucose concentration. There were significant differences: between serum HDL cholesterol of those with a blood glucose concentration in the range 20-30 mmol/l and the other diabetic and control groups, between serum triglycerides in control rats and all groups of rats with diabetes and between diabetic rats with a blood glucose concentration of <10-20 mmol/l and those with 20->30 mmol/l. □ Diabetic group given insulin; □, control group.

(P < 0.005) (Fig. 1). Thus, although there was no significant linear correlation between serum HDL cholesterol and blood glucose concentrations \((R = 0.34)\) in the rats given insulin, there was a significant curvilinear relationship (multiple \(R = 0.50, F = 4.40, P = 0.022\)) given by the quadratic equation: [HDL cholesterol] = 0.794 + (0.044[blood glucose] - 0.00082[blood glucose]).

In the rats given insulin, there was a progressive increase in serum triglyceride concentrations with increasing blood glucose to which these were linearly related \((r = 0.55, P < 0.005)\). In the same group of rats the serum triglyceride concentrations were unrelated to the serum HDL cholesterol concentration \((r = 0.06)\).

Discussion

No significant effect on the serum HDL cholesterol concentration was observed in rats with streptozotocin-induced diabetes without insulin. Raised concentrations of serum HDL cholesterol in rats with streptozotocin-induced diabetes have been reported before (Bar-On, Roheim & Eder, 1976). However, in that study the rats were fed with sucrose and the dose of streptozotocin given was smaller than in the present study.

When insulin was given to rats with streptozotocin-induced diabetes no significant difference in serum HDL cholesterol concentration or in diabetic-controls was produced by a dose of 1 unit of insulin/day when compared with that produced by 3 units/day. However, when the rats with diabetes given insulin were grouped according to the degree of diabetic control, as judged by blood glucose, those with blood glucose concentrations in the range 20-30 mmol/l had higher concentrations of serum HDL cholesterol than those with higher or lower blood glucose. This resulted in a curvilinear relationship between blood glucose and serum HDL cholesterol concentrations.

In man improvement in diabetic control with either insulin or oral hypoglycaemic drugs is associated with an increase in serum HDL cholesterol concentration (Paisey, Elkeles, Hambley & Magill, 1978) and higher than normal concentrations of serum HDL cholesterol have been reported by some workers in patients with diabetes receiving insulin treatment (Nikkilä & Hormila, 1978; Durrington, 1980). There are some reports that the level of serum HDL cholesterol in such patients is inversely related to the level of glycosylated haemoglobin (Calvert et al., 1978; Durrington, 1980) and is thus lower in those whose control is poor than in those with better control. However, it is not, as yet, known whether in man there is any tendency for the concentration of serum HDL cholesterol to return to normal in insulin-treated patients with exceptionally good control.

Serum HDL apolipoproteins are secreted by the liver and gut, either as nascent HDL or as components of triglyceride-rich lipoproteins and thus serum HDL concentration is, in part, determined by the rate at which HDL apolipoproteins are transferred to the HDL fraction during the metabolism of circulating triglyceride-rich lipoproteins, which is itself dependent on the activity of lipoprotein lipase (Havel, 1978). Insulin is important for the activity of lipoprotein lipase and low activities of adipose tissue lipo-
protein lipase have been found in patients with diabetic ketoacidosis (Nikkila, 1978). In patients treated with insulin supra-physiological values of adipose tissue lipoprotein lipase activity were found and it was suggested that this might explain the raised concentrations of serum HDL cholesterol present in these patients (Nikkila, 1978).

However, the results of the present study suggest that in the rat factors other than lipoprotein lipase activity are important since this hypothesis would predict that the highest concentrations of serum HDL cholesterol would occur in the best controlled rats given insulin with the lowest level of serum triglycerides. An alternative proposal is that diabetes produces an increased secretion of HDL precursors either within the triglyceride-rich lipoproteins or as nascent HDL. When adipose tissue lipoprotein lipase activity is low in uncontrolled insulin-deficient diabetes, there may be no significant alteration in serum HDL cholesterol concentration since the HDL precursors are either held back within chylomicrons or very-low-density lipoproteins or, if within the HDL fraction, receive little cholesterol. When insulin is given, the increase in lipoprotein lipase activity may reveal the state of HDL over-secretion as triglyceride-rich lipoproteins are metabolized and produce HDL particles rich in cholesterol. When the dose of insulin becomes sufficient to control diabetes, serum HDL may return to normal despite the increased activity of lipoprotein lipase because the secretion of HDL precursors is restored to normal.

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References