Relationship between serum pseudocholinesterase and triglycerides in experimentally induced diabetes mellitus in rats

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Summary. This study was designed to understand the reasons for the increase in serum pseudocholinesterase activity in diabetes mellitus. Streptozotocin-induced diabetic rats were used for the study. Serum pseudocholinesterase activity increased with the induction of diabetes ($381.5 \text{ units/l} \pm 11.8$) compared to the non-diabetic rats ($243.1 \text{ units/l} \pm 7.2$). Serum triglycerides, total low density lipoprotein and glycerol also increased concurrently with the development of diabetes. Insulin treatment of the diabetic rats normalized serum glucose concomitant with the reduction of pseudocholinesterase activity, triglycerides, total low density lipoprotein and glycerol. Heparin injection appeared to activate lipoprotein lipase in the diabetic rats by showing a marked fall in serum triglyceride and total low density lipoprotein levels but not in pseudocholinesterase activity. Administration of tetraisopropylpyrophosphoramidate a specific pseudocholinesterase inhibitor, inhibited serum and adipose tissue pseudocholinesterase activity by $> 80\%$ and liver $> 50\%$.

Concurrent with the inhibition of pseudocholinesterase activity serum triglyceride, low density lipoprotein and glycerol decreased significantly. In normal rats treatment with tetraisopropylpyrophosphoramidate also reduced serum lipoproteins markedly, while glycerol only showed a marginal decrease. Glycerol was used as a marker of adipose tissue lipolysis and total low density lipoprotein which is defined as lipoproteins of density < 1.063 (LDL + VLDL). Our findings suggest (a) that the increase in serum pseudocholinesterase activity in diabetic rats is due to the overproduction of very low density lipoprotein triglyceride in the liver, and (b) decreased lipolysis in the adipose tissue of the diabetic rats treated with tetraisopropylpyrophosphoramidate is accompanied by an inhibition of pseudocholinesterase activity.

Key words: Pseudocholinesterase, diabetes, triglyceride, glycerol, lipoproteins.

Pseudocholinesterase (PChE: EC 3.1.1.8) is an unusual enzyme which has no known cholinesters as biological substrates in the mammalian body [1]. Further, PChE has a ubiquitous distribution and is found mainly in the plasma and to a lesser extent in the adipose tissue, small intestine, smooth muscle cells and others [2]. It is also unclear at the present time what the biological inducers of this enzyme are. However, PChE is found to be increased in a significant number of patients with hyperlipoproteinaemia [3, 4] and obesity [5]. The increase in serum PChE is more common in patients with types IIb and IV hyperlipoproteinaemia [6, 7].

These observations have been extended in animal models. For example, Zucker fat rats that exhibit a marked increase in very low density lipoprotein (VLDL) have increased serum PChE activity [8]. Besides, genetically obese hyperglycaemic mice (ob/ob) and diabetic (db/db) mice also have increased serum PChE activity [9].

The common features of these animals are that they are hyperphagic, have increased lipogenesis and synthesize more triglycerides and VLDL [10].

Both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus in humans are associated with hyperlipoproteinaemia [11] especially when hyperglycaemia is uncontrolled. The major abnormality in lipoprotein metabolism appears to be associated with both increased serum VLDL and triglycerides (TG) and hypertriglyceridaemia. Diabetic patients also exhibit increased levels of serum PChE [12] which may also be related to abnormal lipid metabolism. Alloxan-induced diabetic rats have also been reported to have increased serum PChE activity concomitant with hyperlipoproteinaemia [13].

The objective of the present study was to discover the reasons for the increase in serum PChE activity in diabetes mellitus using the streptozotocin-induced diabetic rat as a model.

Materials and methods

Male Sprague Dawley rats weighing 200–250 g were used for all the experiments. The animals were housed in individual cages in a controlled atmosphere at the Animal Care Unit of the Health Science Centre, Memorial University of Newfoundland. All the animals had free access to food and water unless otherwise mentioned. The blood from the experimental rats was collected after an overnight fast to prevent interference from chylomicron TG. Polyacrylamide gel electrophoresis confirmed the absence of chylomicrons in the experimental rats. Hence, TG represents VLDL-TG unless otherwise mentioned. Serial blood samples were collected by cardiac puncture under mild anaesthesia with Ethrane (2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether). Serum was used for all the measurements described below. Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (100 mg/kg body weight) dissolved in 0.01 mol/l disodium citrate buffer pH 4.5 [14]. Diabetes was controlled by the administration of 3 IU of protamine zinc insulin/day for one week. The higher dose of tetraisopropylpyrophosphoramide (iso-OMPA) i.e. 17.6 mg/rat was used to obtain PChE inhibition > 90%. The duration of six days treatment was selected to approximate the time scale used in our previous study [1]. The other seven diabetic rats were given about half of the higher dose to obtain an idea of the dose response effect at a lower dose of iso-OMPA.

Serum PChE was measured with butyrylthiocholine iodide as the substrate and 5,5'-di-thiobis-(2-nitrobenzoic acid) (DTNB) as the colouring agent as described by Dietz [15]. Serum triglyceride (TG) [16] and cholesterol [17] were determined using enzymatic methods in a discrete analyser (Monarch – Spokane, Wash. USA). Serum total low density lipoprotein (TLDL) was measured by a turbidimetric procedure using manganese chloride and heparin at 660 nm [18]. This measures the apo-B containing lipoproteins such as LDL and VLDL in the serum. Serum lipoprotein fractions were monitored using polyacrylamide gel electrophoresis [19]. HDL cholesterol (HDL-C) was estimated after precipitation with phosphotungstic acid and magnesium chloride and measurement of the cholesterol in the supernatant after centrifugation [17]. The degree of adipose tissue lipolysis was monitored by measuring serum glycerol using an enzymatic procedure [Glycerol measured on the Monarch 2000 chemistry autoanalyzer using Test-Combination Triglycerides Kit (cat # 125032), Boehringer Mannheim GmbH Diagnostica Kit.]. Liver and adipose tissue PChE was measured as described earlier [8]. For the measurement of liver TG, lipids were extracted using chloroform/methanol (2:1) [20] and the TG in the extract was estimated as described for the serum by employing the manual technique [16].

The measure of the precision of the methods used in this study in terms of mean $\pm$ standard deviation (SD) and coefficient of variation (CV) was monitored using the quality control serum "Precilip" (Boehringer Mannheim GmbH, Mannheim, FRG). Day-to-day variation calculated over a period of one month for the analytes are as follows. Cholesterol (4.06 mmol/l $\pm$ 0.15 SD, CV 3.6%) TG (2.3 mmol/l $\pm$ 0.08, CV 3.27%), HDL Cholesterol (1.68 mmol/l $\pm$ 0.07, CV 4.23%), total LDL (1.77 g/l $\pm$ 0.12, CV 6.8%), glycerol (1.64 mmol/l $\pm$ 0.07, CV 5.26%) and PChE (2175 units $\pm$ 65, CV 2.98%).

Statistical analysis

Results are presented as mean $\pm$ SEM. The statistical analysis was done using the Student's *t*-test.

Results

Induction of diabetes with streptozotocin

Table 1 shows a comparison of serum and tissue values in rats before and after the induction of diabetes with streptozotocin. The reduced number of rats in the diabetic

Table 1. Comparison of serum values before and 72 h after streptozotocin-induced-diabetes in rats

Variables	Before induction (n = 38)	After induction (n = 30)
Glucose (mmol/l)	5.09 $\pm$ 0.21	27.7 $\pm$ 1.33 ^a
PChE (units/l)	243.1 $\pm$ 7.2	381.5 $\pm$ 11.8 ^a
TG (mmol/l)	0.63 $\pm$ 0.05	2.43 $\pm$ 0.24 ^a
Glycerol (mmol/l)	0.33 $\pm$ 0.01	0.52 $\pm$ 0.03 ^a
Chol (mmol/l)	1.62 $\pm$ 0.06	1.58 $\pm$ 0.09 ^b
TLDL (g/l)	0.17 $\pm$ 0.01	0.27 $\pm$ 0.02 ^a
HDL-C (mmol/l)	1.4 $\pm$ 0.07	1.19 $\pm$ 0.07 ^a
Weight (g)	259 $\pm$ 1.49	229.7 $\pm$ 1.36 ^a

n = Number of animals; PChE = Pseudocholinesterase, TG = Triglycerides, Chol = Cholesterol, TLDL = Total low density lipoprotein, HDL-C = High density lipoprotein cholesterol

^a p < 0.05; ^b not significant

group is a result of rats that died after streptozotocin injection. Diabetes was induced by a single intraperitoneal injection of streptozotocin (100 mg/kg body weight). Induction of diabetes was established by qualitative test of urine for glucosuria. A significant increase in serum glucose and PChE was noted within 72 h of streptozotocin treatment with a concomitant increase in TG, glycerol and TLDL levels. There was no significant change in the serum cholesterol concentration, while the decrease in HDL-C was significant (*p* < 0.05). A decrease of 12% in the average body weight was noted in streptozotocin-treated rats.

The influence of insulin treatment and withdrawal on diabetic rats

Figure 1 illustrates the effect of insulin treatment and withdrawal on diabetic rats. The diabetic rats were given 3 IU s.c. insulin/day for one week. Treatment of diabetic rats with insulin resulted in a significant decrease in the serum level of glucose (*p* < 0.001).

With the diabetes under control, serum levels of PChE, glycerol and TG decreased significantly (*p* < 0.05) while there was no significant change in the TLDL concentration. In contrast cholesterol and HDL-C showed a significant increase (*p* < 0.05). Glucosuria reappeared when insulin treatment was discontinued. The levels of serum glucose, TG, glycerol, PChE, cholesterol, TLDL and HDL-C reverted to those observed in the diabetic state, when measured at the end of the third day after stopping the insulin treatment.

Effect of heparin treatment of the diabetic rats

Table 2 shows the effect of heparin treatment on serum PChE activity, TG, cholesterol, glucose, HDL-C and TLDL levels. Heparin was injected (500 U) through the tail vein and blood was collected (as before) 30 min after the injection. In the heparin-treated animals serum TG showed a significant (*p* < 0.001) reduction but the treatment did not affect PChE activity, LDL and cholesterol levels (Table 2). A non-significant rise in serum glucose

Fig. 1. The mean $\pm$ SEM of the variables (y-axis) and time intervals (x-axis) among rats under different treatment conditions; non-diabetic rats ($n = 12$) 3 days after the injection of streptozotocin ($n = 12$) (▨), 7 days after the treatment of the diabetic rats with insulin ($n = 8$) (■), and 3 days after the withdrawal of insulin treatment ($n = 6$) (■). Abbreviations as in Table 1. (n represents the number of rats)

was observed ($p < 0.630$). Administration of an equal volume of NaCl (0.15 mol/l) dissolved in sterile distilled water to the diabetic rats had no effect on serum PChE activity or lipid levels. However, serum glucose showed a small but significant increase ($p < 0.05$).

Effect of iso-OMPA treatment of diabetic and normal rats

Two dose levels (17.6 and 8.3 mg/rat) of iso-OMPA (tetraoisopropyl pyrophosphoramidate), a specific PChE inhibitor was administered to the diabetic rats intraperitoneally for a period of 6 days. This was to determine whether the inhibition of PChE activity in general and specifically in the adipose tissue can decrease lipolysis and thereby reduce the efflux of fatty acids necessary for the synthesis of TG and VLDL. Treatment of the diabetic rats with iso-OMPA (Table 3) at both the higher dose

(17.6 mg/rat) and lower dose (8.3 mg/rat) resulted in a significant reduction of serum TG, cholesterol, glycerol and TLDL when compared to the diabetic rats. However, the lower dose of iso-OMPA treatment caused a lesser reduction in the serum levels of TG, cholesterol and TLDL compared to the diabetic rats treated with the higher dose of iso-OMPA. PChE inhibition was significant at both the dose levels of iso-OMPA i.e. $> 90\%$ with the higher dose and $> 74\%$ with the lower dose in the diabetic rats. The higher dose of iso-OMPA also caused a significant reduction in the serum glucose level ($p < 0.05$), while the lower dose had no effect. Table 4 shows the tissue levels of TG and PChE activity. Liver TG and PChE activity in the liver and adipose tissue were significantly decreased in the iso-OMPA-treated rats ($p < 0.05$). The inhibition of PChE activity in the normal rats after treatment with iso-OMPA also resulted in a significant reduction ($p < 0.05$) in the serum glycerol and TG levels (Fig. 2). Liver TG also decreased significantly ($p < 0.05$) in the iso-OMPA-treated normal rats (Table 4).

Table 2. Comparison of the serum variables (units of measure as in Table 1) between untreated diabetic rats vs control rats, heparin-treated diabetic rats vs untreated diabetic rats, and diabetic rats treated with NaCl (0.15 mol/l) vs untreated diabetic rats

Variables	Control rats ($n = 10$)	Untreated diabetic rats ($n = 10$)	Treated diabetic rats	
			Heparin ($n = 5$)	NaCl ($n = 5$)
Glucose	6.34 ± 0.33	29.81 ± 1.66^a	31.12 ± 2.06^c	35.84 ± 1.69^b
PChE	231.6 ± 18.8	417.0 ± 26.7^a	407.4 ± 29.2^c	420.8 ± 41.5^c
TG	0.79 ± 0.08	4.47 ± 0.8^a	0.65 ± 0.17^a	5.67 ± 1.54^c
Chol	1.71 ± 0.11	1.83 ± 0.17^c	1.59 ± 0.18^c	1.79 ± 0.13^c
HDL-C	1.33 ± 0.08	1.13 ± 0.08^c	1.04 ± 0.1^c	1.07 ± 0.14^c
TLDL	0.13 ± 0.02	0.33 ± 0.04^a	0.24 ± 0.06^c	0.36 ± 0.08^c

Abbreviations as in Table 1. ^a $p < 0.001$; ^b $p < 0.05$; ^c not significant

Discussion

Treatment of rats with streptozotocin creates a useful experimental model in insulin-dependent diabetes mellitus [21, 22] for investigating the relationship between PChE and VLDL. Our results show clearly that serum PChE activity in streptozotocin-induced diabetic rats increases in concert with the development of hyperlipaemia. In general, this model confirms observations in diabetic rats and in patients with hypertriglyceridaemia [6, 13].

Insulin deficiency is associated with increased adipose tissue lipolysis [22] which provides the liver with excess

Table 3. Comparison of the serum variables (units of measure as in Table 1) between untreated diabetic rats vs normal control rats, iso-OMPA (higher dose) treated diabetic rats vs untreated diabetic rats and iso-OMPA (lower dose) treated diabetic rats vs untreated diabetic rats

Variables	Control rats (n = 14)	Untreated diabetic rats (n = 14)	Treated diabetic rats	
			Iso-OMPA (n = 7) (17.6 mg/rat)	Iso-OMPA (n = 7) (8.3 mg/rat)
Glucose	4.19 ± 0.09	32.77 ± 1.33 ^a	25.88 ± 1.92 ^a	32.74 ± 1.98 ^b
PChE	230 ± 7.01	440 ± 18.5 ^a	35.83 ± 0.83 ^a	69.43 ± 1.94 ^a
TG	0.99 ± 0.08	4.62 ± 0.62 ^a	0.51 ± 0.08 ^a	0.62 ± 0.1 ^a
Chol	1.25 ± 0.07	1.28 ± 0.12 ^b	0.38 ± 0.09 ^a	0.7 ± 0.22 ^a
Glycerol	0.32 ± 0.02	0.72 ± 0.07 ^a	0.39 ± 0.06 ^a	0.37 ± 0.07 ^a
TLDL	0.12 ± 0.01	0.56 ± 0.12 ^a	0.05 ± 0.02 ^a	0.13 ± 0.02 ^a

Abbreviations as in Table 1. ISO-OMPA = tetraisopropylpyrophosphoramidate ^a p < 0.05; ^b not significant

non-esterified fatty acids. The excess fatty acids thus available in diabetes can result in either ketoacidosis and/or increased synthesis of TG [23] which can be reversed with insulin treatment [22]. Insulin treatment of the diabetic rats (Fig. 1) caused a parallel reduction in both serum PChE and TG. However, the proportional decrease in TG was far greater than PChE. Insulin is known to activate lipoprotein lipase [22]. Both decreased synthesis and increased clearance could account for the reduction in serum TG in the insulin-treated diabetic rats. However, reduction in serum PChE may be related only to the decreased production of VLDL. Heparin is also known to activate lipoprotein lipase causing rapid clearance of VLDL-TG (27). However, heparin treatment of the diabetic rats (Table 2) very rapidly decreased serum TG without affecting the serum PChE activity. This suggests that the increase in serum PChE activity in diabetic rats is not a function of reduced clearance from the blood in the insulin deficient state but reflects PChE overproduction.

The source of serum PChE is the liver [2] and serum levels may be an indication of its rate of synthesis [24]. However, the hepatic levels of PChE were not elevated in diabetic rats relative to control rats. PChE is a glycoprotein [25] and it is probably exported directly into circulation along with VLDL at a rate matching its synthesis.

Clitherow et al. [28] have suggested that PChE may be involved in hepatic fatty acid metabolism. Other possibilities include a role as a hormone sensitive lipase in rat aorta and adipose tissue [29, 30]. To determine whether elevated serum PChE had any direct role in VLDL metabolism we treated both normal and diabetic rats with

Table 4. Comparison of the variables in tissues; liver TG, liver and adipose tissue PChE. Control rats vs control rats treated with iso-OMPA and diabetic rats. Diabetic rats vs diabetic rats treated with iso-OMPA

Variables	Control rats (n = 12)	Control rats treated with iso-OMPA (n = 6)	Diabetic rats (n = 8)	Diabetic rats treated with iso-OMPA (n = 7)
Liver TG (μmol/g wet wt)	19.9 ± 0.68	16.9 ± 0.9 ^a	23.4 ± 1.2 ^a	13.5 ± 2 ^a
Liver PChE (μmol min ⁻¹ .g wet wt ⁻¹)	0.9 ± 0.04	0.51 ± 0.01 ^a	1.01 ± 0.03 ^b	0.4 ± 0.01 ^a
Adipose tissue PChE (μmol.min ⁻¹ .g wet wt ⁻¹)	0.35 ± 0.01	0.04 ± 0.01 ^a	0.43 ± 0.01 ^b	0.13 ± 0.01 ^a

Abbreviations as in Table 1. ISO-OMPA = tetraisopropylpyrophosphoramidate ^a p < 0.05; ^b not significant

iso-OMPA, a specific inhibitor of PChE [31]. We have previously observed (unpublished data) that iso-OMPA decreased adrenaline-mediated lipolysis in isolated rat adipocyte. Our present findings that iso-OMPA significantly decreased serum glycerol suggest that iso-OMPA may have a similar role as an inhibitor of lipolysis in vivo. Iso-OMPA-treated diabetic rats exhibited decreased serum and liver TG, suggesting that this anti-PChE blocks the increased fatty acid mobilisation-VLDL synthesis cycle characteristic of diabetes. In the normal rat it does not appear that fatty acids from the adipose tissue contribute significantly to the synthesis of VLDL in the liver. In the normal rat, the serum level of LDL is very low and is known to be derived from VLDL and this transformation is initiated by the action of lipoprotein lipase [32]. Iso-OMPA-treated normal rats showed a marked reduction in TLDL. We had made similar observations in a patient [33] who accidentally ingested an organophosphate (Parathion) anti-cholinesterase and in rats [34] treated with a carbamate (neostigmine) anti-cholinesterase. Parathion and neostigmine inhibit both PChE and acetylcholinesterase.

In conclusion, although the changes in PChE activity and lipid metabolism documented here in diabetic and normal animals are highly suggestive of an important relationship, they are still circumstantial.

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Fig. 2. The effect of tetraisopropylpyrophosphoramidate (iso-OMPA) treatment of the serum variables (y-axis) in normal rats before (0 days) and after treatment (6 days) with the phosphocholinesterase inhibitor. The results are the mean ± SEM. Abbreviations as in Table 1. (n represents the number of rats)

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