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Effects of Exercise and Dietary Polyunsaturated Fatty Acid on Blood Lipid Profiles of Streptozotocin-induced Diabetes in Rats

Hasliza, A.H.*, Noordin, M.M. and Goh, Y.M.
Department of Veterinary Preclinical Sciences,
Faculty of Veterinary Medicine, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia
**E-mail: haslizaabu@putra.upm.edu.my*

ABSTRACT

The efficacy of exercise and dietary polyunsaturated fatty acid (PUFA) on streptozotocin-induced diabetes in rats was investigated based on blood lipid profiles, with respect to triglycerides, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol. A total of 32 Sprague-Dawley male rats were used and divided into eight groups. Four groups were exercised daily for 8 weeks, while the other four were sedentary. Treatment diets were defined as follows: rat chow diet only (Control diet), rat chow added with 2:1, menhaden oil:soybean oil (Diet 1), rat chow added with 1:2, menhaden oil:soybean oil (Diet 2), and rat chow added with 10% (w/w) butter (Diet 3). Blood plasma was collected at the end of 8 weeks for blood lipid profiles determination. The results showed that a combination of exercise and dietary PUFA significantly improved lipid abnormalities by lowering the triglycerides, total cholesterol, and LDL-cholesterol levels.

Keywords: Blood lipids, diabetes, exercise, polyunsaturated fatty acid, rat

INTRODUCTION

Diabetes mellitus is a serious chronic metabolic disorder which is becoming a major global health problem nowadays. It has significant impacts on the health, quality of life and life expectancy of patients, as well as on the health care system (Dey *et al*., 2002). The incidence and prevalence of diabetes are escalating, particularly in developing countries. The risk for diabetes mellitus results from a combination of genetic predisposition and lifestyle changes. The most important lifestyle changes are related to the changes in dietary habits and physical activities (Cockram, 2000).

Diabetic patients showed clinical characteristics, such as hyperglycaemia, elevated low-density lipoprotein (LDL) cholesterol and reduced high-density lipoprotein (HDL) cholesterol and high triglyceride levels. These hyperglycaemia and lipid abnormalities significantly contribute complications of diabetes mellitus, as well as increase cardiovascular risk (O'Keefe and Bell, 2007). Previous studies have shown that diets containing omega-3 and omega-6 may play a role in preventing or delaying the complication of diabetes mellitus. Meanwhile, beneficial effects of polyunsaturated fatty acid (PUFA) supplementation are probably mediated by their lowering blood lipid and preserves pancreas from some later complications of diabetes mellitus (Gvozdjáková *et al*., 2008). There are also studies that have indicated that low fitness increases the risk of diabetes and increased physical activity is effective in

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*Corresponding Author

preventing diabetes (Helmrich *et al*., 1994). The benefits of exercise performed by the diabetic patients include increased insulin sensitivity, improved glycaemic control, weight loss, lower blood pressure, and improved blood lipid profile (Wheeler, 1999). Thus, attention to diet and weight management, combined with physical activity such as exercise, may help to improve glycaemic control and lipid profiles (Wolever *et al*., 1999). This study was carried out to determine the efficacy of exercise and dietary PUFA intervention on blood lipid profiles, with respect to triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol.

MATERIALS AND METHODS

Experimental Animals

Thirty-two, 6 weeks old, male Sprague-Dawley rats with an average weight of between 250-300 grams were used in this study. Two rats were housed in stainless steel mesh cages in an air-conditioned room, with temperature-control of 23°C–25°C and maintained on a 12:12 h of light-dark cycle (Krinke, 2000). All rats were acclimatized for one week and the baseline values were taken as a control group of the normal, non-diabetic rats prior the commencement of the experiment. Standard rat chow pellet and water were provided *ad libitum* throughout the acclimatization period. Diabetes mellitus was induced by giving a single intraperitoneal injection of streptozotocin (STZ) at the dosage of 40 mg STZ/kg body weight. Prior to the injection, STZ was freshly prepared in a 0.10 M citrate buffer solution (pH 5.0). Forty eight hours following the injection, the blood glucose level was determined and rats exhibiting hyperglycaemia (fasting blood glucose level > 13.0 mmol/L) were considered as diabetic and used for the experiment.

Diet and Exercise Regime

The three treatment diets were formulated by adding 10% of specific fat sources, either in mixtures of oil (menhaden oil and soybean oil, omega-3 and omega-6, respectively) or butter only. The treatment groups were defined as follows: rat chow added with 2:1, menhaden oil:soybean oil, with exercise (D1E) and sedentary (D1XE); rat chow added with 1:2, menhaden oil:soybean oil, with exercise (D2E) and sedentary (D2XE); rat chow added with 10% (w/w) butter, with exercise (D3E) and sedentary (D3XE). DCE and DCXE were used as a control for standard diet with exercise and sedentary, respectively. The control diet used in this experiment was the standard rat chow diet. The rats were fed with daily prepared diet at the rate of 0.05 mg/kg of body weight.

Swimming was the exercise regimen used in this study. The swimming protocol was adapted from Molly and Catherine (2002), with a slight modification. In more specific, rats were trained to swim in a pool with a depth of 60 cm. Water and room temperature were maintained at 34°C to 35°C to eliminate cold-induced stress. Before the start of the experiment, rats were acclimatized to swimming for 10 minutes daily for 3 days. Training was accomplished in 8 weeks, starting with 5 minutes of swimming in the first week and this was gradually increased to 30 minutes in weeks 6, 7, and 8. Rats were continuously monitored during swimming so as to prevent drowning. At the end of each daily swimming session, each rat was dried using hair dryer and towel-dried. The sedentary rats remained in their cages during this exercise period.

Biochemical Analysis

Blood was collected at the end of the experiments. Blood plasma were assayed for their total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels using a commercial diagnostic kit (Pointe Scientific, Inc, Michigan, USA) and their values were colorimetrically determined in a Roche Cobes Mira Plus chemistry analyzer (Roche Diagnostic Systems, Basel, Switzerland).

Statistical Analysis

In this study, the collected data were analyzed using SPSS version 15.0. The blood lipid

profiles were analyzed using one-way ANOVA. The level of significance was set at 0.05 and only differences of $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The selected blood lipid profiles of triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol of rats at week 8 of the experiments are tabulated in Table 1. The highest level of triglyceride was seen in the D1XE, D2XE, D3XE and DCXE, whereas the rats in D1E, D2E, D3E had the lowest level with a significant higher reduction in triglycerides observed from the D1E group which had received more omega-3 diet and D2E group that had received more omega-6. This finding is similar to that described by Harris (1999) who reported a significant reduction in serum triglycerides by ingestion of omega-3 and omega-6 fatty acids. The possible mechanism for this phenomenon was the reducing effect of dietary omega-3 fatty acids on plasma lipids in plasma triglycerides by 20–50%. Furthermore, the triglycerides-lowering effect has been attributed mostly to a suppressed hepatic lipogenesis and partially to increased β-oxidation (Harris and Bulchandani, 2006).

The lowest ($P<0.05$) cholesterol concentration in the D2E group was similar to that of the DCE group only. The cholesterol concentration in the D3E, D2XE, D3XE, and DCXE groups were the highest ($P<0.05$), whereas the cholesterol concentration of rats in the D1E group was similar to that of the D1XE group. Some of the possible reasons of the lower concentration of the total cholesterol in the exercise group may be attributed to the increased muscular exercise or improvement of cholesterol catabolism. Agte and Tarwadi (2004) suggested a promising potential for exercise as a complementary treatment for patients with diabetes after they had observed a significant reduction by exercise on the total cholesterol and triglycerides. The hypocholesterolemic effect of PUFA-enriched diets on cholesterol levels (Sirtori and Galli, 2002) could also be due to the capability of dietary PUFA to attenuate hyperlipidaemia and reduced oxidative stress. Furthermore, the dietary PUFA may improve hypercholesterolemia by modifying lipoprotein metabolism, which then enhanced the uptake of LDL-cholesterol by increasing LDL-cholesterol receptors and increasing the LCAT activity which may contribute to the regulation of blood lipids (Khanna *et al*., 2002).

The lowest ($P<0.05$) LDL-cholesterol level seen in the D3XE group was comparable to those of the D1E, D2E, DCE, D1XE, and D2XE groups and the rats from the exercise and dietary PUFA intervention groups showed a significant decrease in the LDL-cholesterol levels after eight week of the study as compared to the control. It

TABLE 1
Blood lipid profiles (mmol/L) in exercise and non-exercise rats during the experimental period (Mean ± SE)

Group	Triglyceride	Cholesterole	LDL-chole	HDL-chole
D1E	0.52 ± 0.09^{a}	1.23 ± 0.12^{b}	0.36 ± 0.06^{ab}	0.84 ± 0.11^{ab}
D2E	0.35 ± 0.05^{a}	0.85 ± 0.19^{a}	0.39 ± 0.14^{ab}	0.57 ± 0.13^{a}
D3E	0.44 ± 0.01^{a}	1.86 ± 0.05^{c}	0.51 ± 0.02^{b}	1.33 ± 0.06^{c}
DCE	0.82 ± 0.06^{b}	0.92 ± 0.03^{ab}	0.37 ± 0.02^{ab}	1.03 ± 0.07^{bc}
D1XE	1.25 ± 0.11^{c}	1.22 ± 0.16^{b}	0.43 ± 0.09^{ab}	0.92 ± 0.06^{b}
D2XE	1.42 ± 0.18^{cd}	1.56 ± 0.09^{c}	0.30 ± 0.04^{ab}	1.06 ± 0.09^{bc}
D3XE	1.58 ± 0.07^{d}	1.78 ± 0.08^{c}	0.28 ± 0.02^{a}	1.14 ± 0.16^{bc}
DCXE	1.41 ± 0.02^{cd}	1.81 ± 0.05^{c}	0.80 ± 0.05^{c}	1.87 ± 0.03^{d}

Note: a, b, c, and d: values between rows bearing the same superscript/s do not differ at $P<0.0$

is well recognized that the hypotriglyceridaemic effect of omega-3 may influence the LDL-cholesterol levels by improving the LDL-cholesterol catabolism. The LDL-cholesterol levels are not influenced by the diet only, but it could be enhanced by a combination of aerobic exercises. Previous study has shown that the combination of dietary PUFA and 12 week of exercise training was able to reduce LDL-cholesterol levels more than the dietary PUFA alone (Hill *et al*., 2007).

The level of HDL-cholesterol in the D2E group was the lowest ($P<0.05$) but it remained comparable to that of the D1E group, whereas that of the DCXE group was the highest ($P<0.05$). Surprisingly, the HDL-cholesterol of these intervention groups showed a decrement when they were expected to register an increment. Stone (2008) reported that although dietary fat is effective in reducing microvascular and macrovascular complications in diabetes mellitus, these may be less effective in improving mixed dyslipidaemia after three months of experiment, where they found only the total cholesterol and LDL-cholesterol were lower but no changes in HDL-cholesterol and triglycerides levels were observed. This report has annotatively explained the findings of the low HDL-cholesterol observed in the exercise and the dietary intervention groups of this study, whereby the experiment was only carried out for eight weeks.

CONCLUSIONS

In conclusion, this study has demonstrated the interactions between exercise and dietary PUFA in improving the blood lipid profiles. This would further give better effects in alleviating the detrimental effects of diabetes mellitus in relation to lipid abnormalities.

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