

EFFECT OF FERMENTED CHUB MACKEREL EXTRACT ON LIPID METABOLISM OF DIABETIC RATS

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ABSTRACT

The present study was conducted to evaluate the effect of fermented chub mackerel extract (FCME) on lipid metabolism in diabetic rats. Four week-old male Wistar rats were divided into three groups based on weight. All rats were induced with diabetes mellitus by single intraperitoneal injection of streptozotocin at 45 mg/kg body weight. Thereafter, they were randomly distributed to three treatments with 7 rats assigned to each treatment. One group was the control with no additive, and two-treatment groups were given the purified diets supplemented with 1% or 2% FCME. Experimental results showed that in comparison to the control, diabetic rats fed FCME increased feed intake ($P < 0.01$) and body weight gain ($P < 0.05$). FCME inclusion significantly reduced the activities of acetyl-CoA carboxylase ($P < 0.01$) and fatty acid synthetase ($P < 0.05$) in diabetic rats. FCME significantly increased cholesterol 7 α -hydroxylase with no effect on HMG-CoA reductase activity. FCME had no effect on hepatic triglyceride, free cholesterol and phospholipid. FCME inclusion at 1% level significantly reduced serum triglyceride. FCME significantly increased HDL-cholesterol ($P < 0.05$) with no effect on LDL + VLDL-cholesterol, and significantly reduced atherogenic index. FCME did not significantly affect serum insulin and glucose concentration. In conclusion, FCME supplementation altered lipid metabolism in diabetic rats. FCME supplementation reduced the risk of atherosclerosis in diabetic rats.

Keywords: fermented chub mackerel extract, lipid metabolism, diabetic rat

INTRODUCTION

Diabetic mellitus is a state of nutritional state of nutritional starvation which frequently results in severe metabolic imbalance and pathological change in many tissues (Inui *et al.*, 2000). In relation to lipid metabolism, diabetic condition is characterized by increases both fatty acid and cortisol. Consequently this could account for why VLDL secretion is increased in ketotic type I diabetes, which is caused by insulin deficiency (Brindley, 1991). Hypersecretion of VLDL cause hypertriglyceridemia. Other dyslipidemia includes low HDL-cholesterol, and small, dense LDL. The incidence of atherosclerosis is 3-4 times greater in diabetics than non-diabetics at comparable plasma total cholesterol. Beyond total cholesterol concentration, lipid abnormalities in the plasma of diabetics include elevated triglyceride, decrease HDL-c levels, and the presence of small dense LDL. Dixon *et al.* (2002) found that the diabetic swine had a higher and broader IDL/LDL peak

and a significant higher LDL/HDL-c ratio. Furthermore, they stated that the difference in lipoprotein profile per se may be the cause of increased atherosclerosis in diabetic swines. In addition, the lack of insulin action combined with the effect of glucocorticoids also the LDL receptor in the liver (Schneider, 1991) resulting lower removing the major proportion of IDL and LDL from the circulation leading to hypercholesterolemia. Duchateau *et al.* (2000) found that there was significantly increases in plasma apo L level, cholesterol and triglyceride in subjects with type II diabetes.

Some investigations have been conducted to lower obesity in diabetic animals. Dixon *et al.* (2002) found that atorvastatin (80 mg/day) inclusion protected diabetic swine against coronary artery atherosclerosis. This protection was in part caused by reduced plasma triglyceride concentration since other lipid parameters, including the LDL/HDL ratio had not been affected. Georgopoulos *et al.* (1998) found that a

high monounsaturated fatty-acid enriched diet is not preferable to a high-carbohydrate diet in patients with type 1 Diabetes mellitus with regard to the occurrence of postprandial lipemia. Higashi *et al.* (2002) found that oleic acid enriched diet was associated with increased formation of postprandial chylomicron remnants compared with the linoleic acid enriched diet in patients with type 2 Diabetes mellitus. We recently found that fermented chub mackerel extract was effective to reduce lipid profiles in growing chicks (Tanaka *et al.*, 1990), broiler chicks (Tanaka *et al.*, 1992) and rats (Santoso *et al.*, 2000; 2001). This FCME was known to be rich in peptides. Peptides were known to promote cholesterol efflux from cholesterol-enriched cells, activate the plasma LCAT and to protect against atherosclerosis (Garber *et al.*, 2001). Therefore, it was assumed that FCME inclusion to the diet would reduce lipid profiles in diabetic animals. Therefore, the present study was conducted to evaluate effect of FCME on lipid metabolism in diabetic rats.

MATERIALS AND METHODS

Twenty one of four week-old male Wistar rats (body weight 110 ± 10 g) used in this experiment were purchased from Japan SLC Inc (Hamamatsu, Shizuoka, Japan). They were then weighed individually and divided into three groups based on weight. All rats were induced with diabetes mellitus by single intraperitoneal injection of streptozotocin at 45 mg/kg body weight {2-deoxy-2 [(methylnitrosoamino) carbonyl]amino]-D-glucopyranose (given Sigma) at 0.1 M citrate buffer solution, pH 4.5}. Thereafter, they were randomly distributed to three treatments with 7 rats assigned to each treatment. One group was the control with no additive, and two-treatment groups were given the purified diets supplemented with 1% or 2% FCME. The rats were raised to 7 weeks of age in individual cages in an air-conditional room (temperature $22 \pm 2^\circ\text{C}$ with humidity 50 to 60%) with the light on from 08:00 to 20:00. Rats were fed a commercial nonpurified diet (type CE-2, Japan Clea) for a week before the initiation of the experiment with purified diets. The composition of experimental diets is shown in Table 1. Feed and water were provided for *ad libitum* consumption. To confirm the induction of diabetes mellitus, the serum glucose level in the fasting state (16-hours starvation) was determined by using a commercial kit (Glucose CII-Test Wako

kit).

Commercial FCME was obtained from Kanzaki Company, Ltd., Takamatsu, Japan. The main constituents of this extract are peptides with 20-50 chain-length amino acids. This product contains 39,6% moisture, 51.1% crude protein, 0.0% crude fat, 0.0% crude fiber, 8.7% crude ash and 0.6% nitrogen free extract (NFE). Amino acid profiles of FCME are published elsewhere (Santoso *et al.*, 2000a).

Blood samples were drawn from tail arterial under ether narcosis before and every weeks after given streptozotocin, and left in an ice water to prevent glucose degradation. The serum was separated from each blood sample by centrifugation. Simultaneously glucose excretion to urine confirmed by test paper (Testepe, Shionogi Seiyaku Co., LTD).

At the end of experimental period, all rats were weighed individually. Thereafter, blood samples were drawn from heart and removed the liver under ether narcosis. The serum stored at -30°C until the determination of glucose, insulin, total cholesterol, HDL-cholesterol and lipid fractions.

Enzyme assay was prepared as previously described (Santoso *et al.*, 1995). The activities of key enzymes in fatty acid synthesis and cholesterologenesis were measured. Acetyl-Coenzyme A carboxylase (E.C. 6.2.1.3) activity was assayed by H^{14}CO_3 -fixation method (Qureshi *et al.*, 1980). Fatty acid synthetase (FAS) activity was assayed by the $1\text{-}^{14}\text{C}$ -acetyl-CoA incorporation method (HSU *et al.*, 1965). The 3-hydroxy-3-methylglutaryl-CoA reductase activity was assayed by the method of Shefer *et al.* (1973). The protein content of the solution used for enzyme assay was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. ACC and FAS activities were expressed as nanomole of substrate converted to product per minute per milligram of protein at 37°C . 3-hydroxy-3-methylglutaryl-CoA reductase activity was expressed as picomole of substrate converted to product per minute per mg protein at 38°C . Cholesterol 7α -hydroxylase was expressed as nmol/hour/mg protein. Samples were analyzed in triplicate.

The lipid fractions were separated by thin-layer chromatography on silica gel chromarod using hexane-diethylether-formic acid (60:10:1) and hexane-benzene (1:1) as developing solvent and quantified by IATROSCAN TH-10 TLC/FID Analyzer (Iatron Laboratories, Inc., Tokyo,

Table 1. Composition of Experimental Diet (%)

Ingredients	0% FCME	1% FCME	2% FCME
Corn starch	45	45	45
Sucrose	23.9	23.9	23.9
Soybean Oil	3	3	3
Casein	23	22	21
Mackerel extract	0	1	2
Mineral mixture ¹	4	4	4
Vitamin A & D mixture ²	0.1	0.1	0.1
Vitamin B mixture ³	1	1	1
Total	100	100	100
Chemical Composition			
Protein (%)	23	22.4	21.8
ME (kcal/kg)	3751.6	3733.6	3715.6
ME-protein ratio	163.1	166.7	170.4

1) Supplied 650.0 g CaHPO₄, 160.0 g NaCl, 140.0 g K₂CO₃, 32.7 g MgCO₃, 10.0 g FeSO₄ · 7H₂O, 3.0 g MnSO₄ · H₂O, 1.0 g CoCl₂ · 6H₂O, 1.0 g CuSO₄, 2.0 g ZnCO₃, 0.1 g KI and 0.2 g NaF per 1 kg. mixture

2) Supplied 0.10 g retinyl acetate, 0.00005 g cholecalciferol and 0.8995 g corn starch per 1 gram mixture.

3) Supplied 0.083 g thiamine-HCl, 0.233 g riboflavine, 0.833 g niacin, 0.75 g Ca-pantothenate, 0.1 g pyridoxine-HCl, 0.058 g folic acid, 15 g inositol, 1.667 g p-aminobenzoic acid, 0.005 g biotin, 0.004 g cyanocobalamin, 33.333 g choline-HCl, 0.333 g menadione and 47.599 g corn starch per 100 g mixture.

Japan).

Concentrations of serum total cholesterol, HDL-cholesterol were measured with commercial kits (Cholesterol E Test Wako Kit and HDL-cholesterol E Test Wako Kit from Wako Junyaku Kogyo Co. LTD). The difference between the total cholesterol and HDL-cholesterol was assumed to be LDL+VLDL cholesterol (Nishizawa and Fudamoto, 1995). An atherogenic index was measured using equation published by Nishizawa and Fudamoto (1995).

Treatment effects were assessed for all response variables using one-way ANOVA in which the overall treatment differences were represented by single orthogonal contrasts between control and treatment groups (Shinjo, 1990).

RESULTS

FCME inclusion significantly increased feed intake ($P < 0.01$) and body weight gain ($P < 0.05$) FCME had no effect on liver weight (Table 2). FCME inclusion did not influence the concentration of serum glucose (Table 3).

The activities of acetyl-CoA carboxylase and

fatty acid synthetase were significantly lower in diabetic rats fed FCME, whereas cholesterol 7 α -hydroxylase was significantly increased ($P < 0.01$). The activity of HMG-CoA reductase was not significantly affected (Table 4).

FCME had no effect on hepatic triglyceride, free cholesterol and phospholipid. Serum triglyceride was significantly lower in rats fed diet with FCME ($P < 0.01$), but total cholesterol and free cholesterol were not significantly influenced. Serum HDL-cholesterol was significantly increased ($P < 0.05$) with lower atherogenic index ($P < 0.05$). Mackerel extract had no effect on LDL+VLDL-cholesterol concentration (Table 5). Dietary FCME had no effect on serum insulin. However, 2% FCME inclusion tended to increase insulin concentration at 24.4% level.

DISCUSSION

An increase in body weight gain resulted from higher feed intake in FCME group. It is possible that streptozotocin eliminated the advantage of FCME in improving feed conversion ratio. It is unknown why feed intake of diabetic rats fed FCME was higher. Although, there is

Table 2. Effects of Fermented Chub Mackerel Extract on Feed Intake, Body Weight Gain and Feed Conversion Ratio of Diabetic Rats

Variables	0% FCME	1% FCME	2% FCME
Final body weight, g/rat	194.28±6.2 ¹	209.12±5.6	218.92± 5.4
Body weight gain, g/day	3.01±0.60	3.54±0.65*	3.89±0.59*
Feed intake, g/day	17.80±1.2	23.80±1.8***	24.60±2.2**
Feed conversion ratio	5.91±0.05	6.72±0.06	6.32±0.05
Liver weight, g/100 g BW	4.78±0.43	4.87±0.21	4.82±0.31

Mean±SD for 7 rats

* Significantly different (P<0.05) from the control group.

** Significantly different (P<0.01) from the control group.

*** Significantly different (P<0.001) from the control group.

Table 3. Effects of Fermented Chub Mackerel Extract on Concentration of Glucose in the Serum of Diabetic Rat

Weeks after administration	0% FCME	1% FCME	2% FCME
0	148.9±11.5 ¹	148.9±11.5	148.9±11.5
1	347.6±29.6	364.8±35.0	318.9±53.3
2	468.1±28.9	514.3±29.4	462.2±68.7
3	475.1±23.5	461.5±25.0	448.2±37.2
4	554.2±82.9	536.0±41.6	520.8±29.4

Mean ± SD for 7 rats

correlation between serum glucose and feed intake ($r = -0.93$), it appear that the higher feed intake could not fully be explained by lower serum glucose concentration. It was known that FCME rich in glutamic acid, one of an active taste compounds in feed that may also improve the palatability of diet, and therefore it increased feed intake. As far as growth efficiency concerned, the inclusion of FCME in diabetic rats had no beneficial effect. The present results agree with the observation of Santoso *et al.* (2000) who found that FCME inclusion had a little value on improving feed efficiency in rats fed cholesterol-containing diet.

Acetyl-CoA carboxylase was suggested as a rate limiting enzyme in fatty acid synthesis (Brindley, 1991). Therefore, the reduction of acetyl-CoA carboxylase activity in diabetic rats fed FCME would result in lower fatty acid synthesis. Our previous studies (Santoso *et al.*, 2000, 2001) also found that FCME inclusion resulted in lower acetyl-CoA carboxylase and fatty acid synthetase activities of rats fed diet

without cholesterol or those fed high-cholesterol containing diet. A reduced in hepatic fatty acid synthesis is a major factor which caused lower hepatic triglyceride synthesis (Scorve *et al.*, 1993), and resulting in lower triglyceride secretion into circulation. This may explain lower serum triglyceride in diabetic rats fed 1% FCME. It is unknown however, although feeding 2% FCME reduced hepatic acetyl-CoA carboxylase and fatty acid synthetase activities it resulted in higher serum triglyceride concentration and no change in hepatic triglyceride. It was known that a major site of fatty acid synthesis in rats was in adipose tissues. Therefore, it is needed to evaluate the activity of acetyl-CoA carboxylase and fatty acid synthetase in adipose tissue to elucidate the mechanism of different change in serum triglyceride. In addition, triglyceride clearance from the circulation by lipoprotein lipase may also contribute to this phenomenon.

A major advance in the understanding of cholesterol metabolism emerged from the observation that in rats the liver exhibits high rates of cholesterol synthesis whereas nonhepatic tissues other than intestine show rates that are less than 5% of those in the liver (Balasubramaman *et al.*, 1976). FCME inclusion did not reduce the activity of hepatic HMG-CoA reductase, a rate limiting enzyme in cholesterol synthesis. Therefore, FCME inclusion might have no effect on hepatic cholesterol synthesis. However, FCME inclusion might increase hepatic bile acid synthesis as indicated by higher activity of hepatic cholesterol 7 α -hydroxylase activity, a rate limiting enzyme in bile acid synthesis. An increase in hepatic bile acid synthesis, however,

Table 4. Effects of Fermented Chub Mackerel Extract on Activities of Lipogenic Related Enzymes in the Liver of Diabetic Rats

Enzymes	0% FCME	1% FCME	2% FCME
Acetyl-CoA carboxylase (nmol/min/mg protein)	1.61±0.511	1.08 ±0.23**	1.07 ±0.58*
Fatty acid synthetase (nmol/min/mg protein)	1.15 ±0.44	0.71 ± 0.29*	0.69 ±0.45*
HMG-CoA reductase (pmol/min/mg protein)	13.72 ± 4.25	11.76 ± 2.08	12.64 ±3.05
Cholesterol 7-hydroxylase (nmol/hr/mg protein)	10.07± 1.23	11.69 ± 0.65**	17.09 ±4.29**

¹Mean ± SD for 7 rats; *Significantly different (p<0.05) from the control group.

** Significantly different (p<0.01) from the control group.

Table 5. Effects of Fermented Chub Mackerel Extract on Various Lipid Fractions and Insulin of Diabetic Rats

Variables Liver (mg/g)	0% FCME	1% FCME	2% FCME
Triglyceride	4.3±1.0 ¹	3.8±1.5	3.6±0.8
Free cholesterol	1.7±0.1	1.9±0.2	1.9±0.2
Phospholipid	41.1±7.8	42.2±7.0	37.1±3.4
Serum (mg/100 ml)			
Total cholesterol	90.7±16.6	98.6±12.8	98.0±12.3
Free cholesterol	19.9±5.0	18.4±2.6	18.1±1.8
HDL-cholesterol	59.4±14.6	72.9±6.9*	72.7 ± 5.3*
LDL+VLDL- cholesterol	31.1±5.1	25.7±2.5	25.2 ± 3.5
Triglyceride	86.8±19.2	53.0 ± 14.5*	81.4±17.8
Phospholipid	224.8±35.1	212.3±17.9	222.4±25.6
Atherogenic index ²	0.50±0.10	0.35±0.05**	0.39±0.07*
Insulin (µU/ml)	16.0±6.7	15.2±7.5	19.9±14.9

¹Mean±SD for 7 rats

²(Total cholesterol – HDL-cholesterol)/HDL-cholesterol

*Significantly different (p<0.05) from the control group

** Significantly different (p<0.01) from the control group

was not accompanied by neither lower hepatic cholesterol nor serum total cholesterol. No change in hepatic or serum cholesterol in rats fed FCME was in agreement with the observation of Santoso *et al.* (2001).

A protective effect against the development of atherosclerotic disease is attributed to HDL-cholesterol on the basis of their key roles in reverse cholesterol transport and also their antioxidant properties. Badimon *et al.* (1990) also found that injection of HDL into rabbits fed on atherogenic diet has been shown to inhibit atherosclerotic lesion formation. Therefore, an increase in HDL-cholesterol with tendency of lower LDL-cholesterol by FCME in the present study might have beneficial impact on reducing

the risk of atherosclerosis. In our previous results (Santoso *et al.*, 2000) also showed that when FCME was supplemented to a high-cholesterol containing diet, an increase in serum HDL-cholesterol with lower LDL-cholesterol without any change in total cholesterol was observed. Lower atherogenic index found in rats fed FCME indicated that FCME inclusion might reduce the risk of atherosclerosis in diabetic rats.

FCME had no antidiabetic properties as indicated by no effect of FCME on serum glucose and insulin.

CONCLUSION

As far as growth efficiency concerned, the inclusion of FCME in diabetic rats had no beneficial effect. FCME supplementation reduced the activities of hepatic acetyl-CoA carboxylase and fatty acid synthetase, but it increased the activity of hepatic cholesterol 7 α -hydroxylase in diabetic rats. FCME supplementation reduced the risk of atherosclerosis as indicated by lower atherogenic index.

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