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ORIGINAL ARTICLE

Effects of dietary fish oil on survival rate, plasma amino acid pattern, and inflammatory-related mediators in diabetic rats with sepsis

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Abstract—This study was designed to investigate the effects of dietary fish oil on survival rates, plasma amino acid profiles, and inflammatory-related mediators in diabetic rats with sepsis. Diabetes mellitus (DM) was induced in rats by streptozotocin. The DM rats were maintained for 4 weeks on medium fat (10%, w/w) diets containing either fish oil or safflower oil. After that, sepsis was induced by cecal ligation and puncture (CLP). There were 2 groups in this study: fish oil sepsis group (FOS) and safflower oil sepsis group (SOS). The survival rate was observed after CLP. Also, changes of the amino acid pattern as well as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, prostaglandin (PG) E_2 at 6, 12, and 24 h after CLP were investigated. The results demonstrated that survival rates were not significantly different between the 2 groups. Plasma arginine levels were significantly lower in sepsis groups than that in the DM-chow group, regardless of whether the diabetic rats were fed fish oil or safflower oil. No significant differences were observed in plasma valine, leucine, isoleucine, glutamine, or arginine concentrations between the FOS and SOS groups at different time points. Concentrations of IL-1β in peritoneal lavage fluid (PLF) at 6 h and TNF-α at 6 h as well as at 12 h after CLP in the FOS group were significantly higher than those in the SOS group. PGE_2 levels in PLF, by contrast, were lower in the FOS group at 6 and 12 h after CLP than in the SOS group. These results suggest that differences in IL-1β, TNF-α, and PGE_2 levels in PLF in the early period of sepsis did not influence the survival rates and plasma amino acid profiles of the FOS and SOS groups. Compared with safflower oil, feeding diabetic rats with fish oil had no beneficial effects on survival rates and muscle protein breakdown. The immunologic impact of dietary n-3 polyunsaturated fatty acids on diabetic rats with sepsis requires further investigation. © 2000 Harcourt Publishers Ltd.

Key words: diabetes mellitus; sepsis; fish oil; safflower oil; amino acid pattern; cytokines

Introduction

Dietary fish oil has attracted significant attention recently, as it has been shown to have beneficial clinical, immunologic, and biochemical effects in a number of disease states and animal models (1–5). The general anti-inflammatory benefits derived from a fish oil-containing diet is explained by the inhibitory effects of the cyclooxygenase and lipooxgenase pathways (5, 6). Mascioli et al. (7) reported that intravenous infusion of a lipid emulsion rich in fish oil significantly enhanced the survival of guinea-pigs to intraperitoneally injected lipopolysacchride compared with a safflower oil-rich emulsion. The diminished production of, and responses to, cytokines were thought to be responsible for the enhancement of the survival rate (7, 8). Billiar et al. (9) demonstrated that Kupffer cells produced less tumor necrosis factor (TNF) and interleukin (IL)-1 following fish oil feeding in rats. Studies also showed that supplementation of the diet with fish oil reduced in vitro cytokine production in patients with rhematoid arthritis, multiple sclerosis or type-1 diabetes (10–12). By contrast, some studies reported increased IL-1 and TNF-α production by mouse peritoneal macrophages with a fish oil-supplemented diet (13,14). Moreover, a study by Chang et al. (15) reported that laboratory animals fed fish oil showed lower survival rates to challenges with bacteria than those fed other types of fat. Some measurements of cell-mediated immunity made in vivo suggest immunosuppression as a result of fish oil feeding (16,17). Since studies have provided evidence both for and against the impact of pharmacologic use of n-3 polyunsaturated fatty acids, their use remains controversial.

Diabetes mellitus (DM) is a metabolic disorder with increasing mortality rates in Taiwan. DM is caused by an absolute or relative lack of insulin, and is characterized by hyperglycemia and hypertriglyceridemia (18). Many patients with diabetes have an increased risk of coronary heart disease, peripheral vascular diseases, and cerebrovascular diseases (19). Furthermore, the abnormalities in nutrient metabolism resulting from DM lead to impairment of wound healing and vulnerability to infection and sepsis. Previous studies concerned with the influence of fish oil on lipid

metabolism or immunologic response focused exclusively on the condition of infection and inflammatory diseases; no study so far, has investigated the effect of fish oil on DM with complication of sepsis. In this study we induced experimental sepsis after treating diabetic rats with fish oil or safflower oil to investigate the effect of different dietary fats on survival rate, plasma amino acid pattern, and inflammatory mediator production in diabetic rats with sepsis.

Materials and methods

Animals

Male Wistar rats weighing 70–100 g were used in this study. All rats were housed in temperature and humidity controlled rooms, and allowed free access to a standard rat chow for 1 week prior to the experiment. After that, diabetes was induced in the rats by a single tail vein injection of streptozotocin (STZ, Sigma Chemical, St Louis, MO) at a dose of 60 mg/kg BW (20). STZ was dissolved immediately before use in 0.05 mol/L sodium citrate (pH 4.5) (21). Only those animals excreting more than 1% glucose in the urine were considered to be diabetic (21).

Study protocol

Experiment 1: Forty diabetic rats were divided into two experimental groups, and fed with a medium fat (10%, w/w) semipurified diet containing 0.1% cholesterol. The diets fed to the experimental groups were identical except for the sources of fat (Table 1); the fats used were safflower oil (Taiwan Sugar, ROC) or fish oil (TAMA Biochemical, Japan). The fatty acid compositions of the fish and safflower oils are shown in Table 2. After experimental diets were fed for 4 weeks, sepsis was induced in all rats by cecal ligation and puncture (CLP) according to the method of Wichterman et al. (22). Briefly, rats were lightly anesthetized with ether. The abdomen was opened through a midline incision, and the cecum was punctured twice with an 18-gauge needle and was replaced into the abdomen. The abdominal wound was closed in layers. There were two experimental groups: fish oil sepsis group (FOS, $n=20$) and safflower oil sepsis group (SOS, $n=20$). The animals were followed for survival after 12 h of CLP. Survival was noted every hour in the first 24 h, and then every 3 h until 84 h after sepsis induction.

Experiment 2: Sixty diabetic rats were divided into two experimental groups, with each group containing 30 rats. Rats in experimental groups were fed a fish oil or safflower oil diet for 4 weeks as described in experiment 1. After that, sepsis was induced in all rats by CLP. Ten rats in each experimental group were sacrificed at 6 h, 12 h, and 24 h after CLP, respectively, in order to compare the differences in amino acid patterns and cytokine production between the two septic groups at different time points. Eight diabetic rats fed with standard rat chow served as the control (DM-Chow). The control rats were sacrificed 4 weeks later without CLP. The plasma amino acid levels of the control rats were regarded as the data of diabetic condition without sepsis.

Table 1 Composition of the experimental diet

Ingredient	FO	SO
	% (w/w)	
Fish oil	10	
Safflower oil		10
Corn starch	62	62
Casein	20	20
Cholesterol	0.1	0.1
Salt mixture*	3.5	3.5
Vitamin mixture†	1	1
Methyl cellulose	3	3
Choline chloride	0.095	0.095
DL-methionine	0.3	0.3

FO: fish oil group, SO: safflower oil group.
*Salt mixture contains the following (mg/g): calcium phosphate dibasic 500 mg, sodium chloride 74 mg, potassium sulfate 52 mg, potassium citrate monohydrate 220 mg, magnesium oxide 24 mg, manganous carbonate 3.5 mg, ferric citrate 6 mg, zinc carbonate 1.6 mg, cupric carbonate 0.3 mg, potassium iodate 0.01 mg, sodium selenite 0.01 mg, and chromium potassium sulfate 0.55 mg.
†Vitamin mix contains the following (mg/g): thiamin hydrochloride 0.6 mg, riboflavin 0.6 mg, pyridoxine hydrochloride 0.7 mg, nicotinic acid 3 mg, calcium pantothenate 1.6 mg, D-biotin 0.02 mg, cyanocobalamin 0.001 mg, retinyl palmitate 1.6 mg, DL-α-tocopherol acetate 20 mg, cholecalciferol 0.25 mg, and menaquinone 0.005 mg.

Table 2 Fatty acid profiles of the lipid emulsions tested[a]

Fatty acid	Safflower oil	Fish oil
8:0		
10:0		
14:0		7.8
16:0	7.0	15.3
16:1 n-7		9.8
18:0	3.6	1.9
18:1 n-9	17.4	11.7
18:2 n-6	71.3	4.6
18:3 n-3	0.6	1.6
20:4 n-6		2.6
20:5 n-3		31.2
22:6 n-3		13.1

[a]Expressed as relative percent.

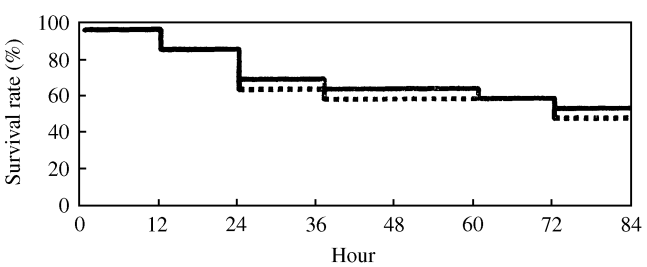

Fig. 1 Effect of dietary fish oil and safflower oil on survival rates in diabetic rats with sepsis. FOS (▪▪▪): fish oil sepsis group, SOS(—): safflower oil sepsis group. No significant difference was observed between the two sepsis groups.

Measurements and analytical procedures

Rats were sacrificed by drawing arterial blood from the aorta of the abdomen. The abdomen was opened and the peritoneal cavity was lavaged with 10 mL of normal saline. The peritoneal lavage fluid (PLF) was collected for further analysis. Blood samples were collected in tubes containing EDTA-Na_2 and immediately centrifuged at 3000 rpm for 10 min to separate the plasma. All PLF and plasma samples were stored at $-70^{\circ}C$ until the assay. Amino acid was analyzed by the standard ninhydrin technology (Beckman Instrument, model 6300, Palo Alto, CA), after deproteinization of the plasma with 50% salicylic acid (23). IL-1β and TNF-α levels in plasma and PLF were measured using commercially available enzyme-linked immunosorbent assay (ELISA) in microtiter plates. Antibodies specific for rat IL-1β and TNF-α were coated onto the wells of the microtiter strips provided (BioSource International, Nivelles, Belgium). Prostaglandin (PG) E_2 levels in peritoneal lavage fluid were also measured by ELISA. The surface of the microtiter plates was precoated with mouse monoclonal antibody. Acetylcholinesterase covalently coupled to PG E_2 was used as the enzymatic tracer (Cayman Chemical, Ann Arbor, MI). The cytokines and PGE_2 concentrations in peritoneal lavage fluid were expressed in pg/mg protein. Protein level was measured by Lowry's method (24).

Statistics

Data are expressed as mean ± SD. Differences among groups were analyzed by analysis of variance using Duncan's test. A P value <0.05 was considered statistically significant.

Results

There were no differences in initial body weights and the weights after experimental diets for 4 weeks between the two experimental groups, either in experiments 1 or 2 (data not shown). There were 10 survivors among the 20 rats in the FOS group, and 11 survivors in the SOS group after observation for 84 h following CLP. The survival rate did not significantly differ between the two sepsis groups. The results of plasma amino acid profiles in experiment 2 demonstrated that concentrations of branch-chain amino acids (BCAAs), including valine, leucine, and isoleucine in the SOS group at 6 h after CLP were significantly higher than those in the DM-Chow group. Glutamine (GLN) levels were significantly lower in the FOS group at 6 h and 12 h after CLP than in the DM-Chow group; however, it returned to a level comparable to that of the DM-Chow group at 24 h after CLP. Arginine (Arg) levels were significantly lower in sepsis groups than in the DM-Chow group, regardless of whether the diabetic rats were fed fish or safflower oils. No significant differences were observed in plasma BCAAs, GLN, or Arg concentrations between the FOS and SOS groups at different time points (Table 3). Most of the plasma concentrations of IL-1β and TNF-α were undetectable in both the FOS and SOS groups. IL-1β and TNF-α concentrations in PLF, however, were measurable. IL-1 β and 6 h and TNF-α at 6 h as well as at 12 h after CLP in the FOS group were significantly higher than those in the SOS group. PGE_2 levels in PLF, by contrast, were lower in the FOS group at 6 h and 12 h after CLP than in the SOS group. No significant differences in IL-1β concentrations at 12 h and 24 h were seen between the SOS and FOS groups. Neither difference in TNF-α concentrations at 24 h in PLF was observed between the two sepsis groups (Table 4).

Discussion

Dietary fish oil supplementation interferes with eicosanoids production and appears to influence the secretion of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. Despite the key protective role cytokine plays in the response to infection and injury, an exaggerated secretion may cause muscle proteolysis and other damaging effects on the host, as in the condition of sepsis (25). DM is a metabolic disorder with high

Table 3 Plasma amino acid concentrations among the chow-fed group and sepsis groups at different time points

Group	Val	Leu	Ile	Gln	Arg
			nmol/mL		
DM-Chow	237.7 ± 38.4	168.6 ± 20.6	100.4 ± 16.1	516.3 ± 121.6	128.7 ± 47.6
6 h after CLP					
FOS ($n=10$)	315.1 ± 244.4	237.7 ± 187.9	133.1 ± 107.2	305.5 ± 83.2*	88.4 ± 16.2*
SOS ($n=10$)	381.8 ± 150.3*	297.0 ± 119.9*	167.3 ± 69.8*	391.6 ± 76.8	107.9 ± 17.4
12 h after CLP					
FOS ($n=10$)	158.1 ± 26.5	117.9 ± 18.8	60.3 ± 8.5	332.2 ± 37.6*	61.7 ± 30.2*
SOS ($n=10$)	272.9 ± 116.7	206.5 ± 87.2	111.7 ± 50.5	403.7 ± 138.7	92.8 ± 28.9*
24 h after CLP					
FOS ($n=10$)	160.6 ± 27.6	119.9 ± 21.3	61.7 ± 11.8	476.6 ± 142.5	95.4 ± 34.6*
SOS ($n=10$)	148.3 ± 34.0	110.3 ± 32.2	57.7 ± 15.5	450.4 ± 64.5	85.1 ± 22.0*

Values are mean ± SD.
*Significantly different from DM-chow group at $P<0.05$ as determined by Duncan's multiple range test.
Abbreviations: FOS: fish oil sepsis group; SOS: safflower oil sepsis group; Val: valine; Leu: leucine; Ile: isoleucine; Gln: glutamine; Arg: arginine.

Table 4 Interleukin (IL) 1-β, tumor necrosis factor (TNF)-α, and prostaglandin (PG) E_2 concentrations in peritoneal lavage fluid at different time points between the two sepsis groups

Group	IL-1β	TNF-α pg/mg protein	PGE_2
6 h after CLP			
FOS	92.5 ± 52.8*	21.7 ± 10.6*	214.0 ± 170.0*
SOS	45.9 ± 24.7	10.3 ± 5.2	585.2 ± 254.8
12 h after CLP			
FOS	23.2 ± 12.0	18.4 ± 12.7*	207.2 ± 188.3*
SOS	18.8 ± 11.2	7.4 ± 4.8	533.2 ± 202.4
24 h after CLP			
FOS	14.0 ± 6.6	15.3 ± 6.2	ND
SOS	24.5 ± 19.3	16.6 ± 14.4	ND

Values are means ± SD.
*Significantly different from the SOS group at 6 h and 12 h after CLP, respectively, as determined by Duncan's multiple range test.
Abbreviations: FOS: fish oil sepsis group; SOS: safflower oil sepsis group; ND: not determined.

incidence of infection; in this study we use an animal model of DM with sepsis to investigate whether fish oil supplementation has a beneficial effect on immunologic response, and consequently reduces mortality and tissue protein breakdown. STZ-induced diabetic rats are a model that is frequently used to stimulate non-insulin-dependent diabetes in animal studies (26), and CLP is considered to be a simple and reproducible model of sepsis in rats (22).

BCAAs are oxidized primarily in skeletal muscle (27). Previous studies have shown the leucine flux from muscle and plasma leucine disappearance was significantly increased in stress and catabolic diseases (28). In a catabolic state, BCAAs are produced as the result of muscle protein breakdown and the amino groups of BCAAs transfer to other amino acids for the *de novo* synthesis of alanine and GLN (29). The results demonstrate that the plasma of BCAAs levels did not differ between the FOS and SOS groups at different time points may indicate that the extent of muscle BCAAs oxidation and protein breakdown were similar in the two septic groups. The plasma BCAA levels were significantly higher at 6 h after CLP than at 12 h and 24 h in the SOS group, and returned to levels comparable to the DM-Chow group at 12 h and 24 h. This result suggests that BCAAs were released from muscle protein early in infection to be used as energy, and the homeostasis of the body amino acids was maintained 12 h after CLP. In this study we observed that GLN levels in the FOS group were significantly lower at 6 h and 12 h after CLP than in the DM-Chow group, whereas the SOS group showed no difference from the DM-Chow group at that time. This finding may indicate that in the early stage of sepsis, hepatic GLN uptake increased in the FOS group, possibly due to an increase in hepatic blood flow and in GLN extraction from the blood stream (30). Arg is the substrate of nitric oxide synthase and is used as a nitrogen donor to produce nitric oxide (NO) (31). In this study plasma Arg levels were significantly lower in sepsis groups than in those without sepsis at 12 h and 24 h after CLP, regardless of being fed fish oil or safflower oil. Study showed that large amount of NO are produced by inducible NO synthase in response of inflammatory cytokines and bacterial endotoxin (32). Major sites of NO synthesis induction in lipopolysaccharide-treated rats are liver, spleen, lung and circulating macrophages (33). A metabolic tracer study by Hibbs et al. (34) demonstrated that endogenous NO production in the body was derived from L-arginine in the plasma. It is possible that in the condition of DM-sepsis, inducible NO synthase is induced which may consequently result in NO production and lower plasma Arg.

The results in this study demonstrated that diabetic rats fed fish oil had higher IL-1β at 6 h and higher TNF-α at 6 h and 12 h in PLF after CLP than those fed safflower oil. These findings can be explained by the lower PGE_2 in the FOS than the SOS group. Previous reports have shown that dietary n-3 fatty acids decrease the synthesis of PGE_2 by peritoneal macrophages or peripheral blood mononuclear cells when stimulated by mitogens (35, 36); and lowered PGE_2 synthesis may consequently lead to enhanced production of TNF (36, 37). This result is inconsistent with a report by Billiur et al. (9) which showed that production of the cytokines by Kupffer cells was significantly decreased after 6 weeks of a fish oil-enriched diet. Endres et al. (38) and Caughey et al. (39) also reported that dietary fish oil supplementation resulted in decreased in vitro production of IL-1β and TNF-α by stimulated peripheral blood mononuclear cells. However, our results are similar with those reported by Blok et al. (13) and Lokesh et al. (14) which showed that IL-1β and TNF-α synthesis by mouse peritoneal macrophages is enhanced by dietary n-3 PUFAs. Blok et al. (40) also reported that circulating TNF-α was increased when lipopolysaccharide was administered intraperitoneally in mice fed with fish oil. The discrepancies on cytokine production after fish oil administration may be explained by the different study design and infectious state of the individual study. Besides, the results of in vitro studies may not accurately reflect in vivo situations. Although an exaggerated or prolonged secretion of these cytokines can be detrimental to the host (24, 41, 42), an overproduction of endogenous IL-1 and TNF in resident peritoneal macrophages early in infection induced by dietary fish oil supplementation may have a protective effect in infections (13). In this study we observed no significant differences in survival rates or plasma amino acid profiles between the two sepsis groups. These results suggest that differences in IL-1β, TNF-α and PGE_2 levels in PLF might not be responsible for the catabolic responses of muscle protein and survival rates in DM-septic rats. Because of short half-lives and rapid degradation of these cytokines in plasma, the dynamic changes of systemic cytokines was not observed. The current explanation for the beneficial effects of fish oil

in a variety of autoimmune diseases focuses on their inhibitory effect of the cyclooxygenase and lipooxgenase pathways. We did observe an inhibitory effect on the cyclooxygenase pathway as shown by a lower PGE_2 level in the FOS group than in the SOS group. Whether the effects of a fish oil diet on host resistance to infection are related to interference of the lipooxgenase pathway remains to be established.

In conclusion, this study demonstrates that diabetic rats fed fish oil had lower PGE_2 and higher IL-1β, and TNF-α than those fed with safflower oil. However, compared with safflower oil, rats fed with fish oil had no beneficial effects as to survival rate and catabolic response of muscle protein. The immunologic impact of dietary n-3 polyunsaturated fatty acids on diabetic rats with sepsis requires further investigation.

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