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Fish (Bonito) Oil Supplementation Enhances the Expression of Uncoupling Protein in Brown Adipose Tissue of Rat

Keywords: *Fish oil; highly unsaturated fatty acids; uncoupling protein; brown adipose tissue; thermogenesis*

INTRODUCTION

The vertebrate body stores a large quantity of energy in adipose tissue. However, when the balance between calorific intake and energy expenditure is shifted in the direction of excess calorific intake or decline in energy expenditure, excessive accumulation of body fat, namely obesity, occurs. Recently, brown adipose tissue (BAT) has attracted a great deal of interest because of its important role in the regulation of whole body energy expenditure (Lowell et al., 1993; Kopecky et al., 1995; Silva and Rabelo, 1997). In BAT mitochondria, substrate oxidation is poorly coupled to ATP synthesis because of the presence of a specific protein named uncoupling protein (UCP) and its homologues , thereby leading to energy dissipation, that is, heat production (Klingenberg, 1990; Nicholls and Locke, 1984). The thermogenic capacity of BAT is determined by the level of expression of UCP. This has become evident from in vivo studies as well as from experiments with cells and isolated mitochondria (Himms-Hagen, 1985; Cannon and Nedergaar, 1985; Nicholls et al., 1986). We reported that some seasonings such as hot pepper, garlic, and mustard cause induction of UCP expression, resulting in enhancement of thermogenesis in animals during and after food intake (Kawada et al., 1986a,b, 1991; Oi et al., 1995). Recently, attention has been focused on differences among the effects of oils from various sources on nutritional and metabolic responses. In particular, fish oil containing highly unsaturated fatty acids (HUFA) has been reported to possess medically and pharmacologically useful properties on lipid metabolism (Corey et al., 1983; Hainault et al., 1993; Okuyama et al., 1997). In this study, we investigated the effects of fish oil, compared with those of animal fats and vegetable oils, rich in saturated fatty acids (SFA) or polyunsaturated fatty acids (PUFA), on the expression of UCP in rat BAT.

Table 1. Fatty Acid Composition of Dietary Fats[a]

fatty acid	lard	fish oil[b]	linseed oil	perilla oil
capric acid (10:0)	1.8			
lauric acid (12:0)	13.5			
myristic acid (14:0)	6.4	3.4		
palmitic acid (16:0)	19.5	18.1	5.2	6.2
palmitoleic acid (16:1)	2.2	4.1		
stearic acid (18:0)	10.6	4.7	2.9	2.2
oleic acid (18:1)	33.9	12.0	17.2	21.0
linoleic acid (18:2)	5.8	1.6	15.1	13.0
α-linolenic acid (18:3)	0.3	0.7	58.8	57.8
arachidic acid (20:0)	0.3	1.7	0.2	0.2
arachidonic acid (20:4)		2.0		
icosapentaenoic acid (20:5)		7.2		
docosapentaenoic acid (22:5)		1.2		
docosahexaenoic acid (22:6)		27.7		
nervonic acid (24:1)		1.6		

[a] Each value represents the mean proportion of the total methyl esters. [b] Fish oil derived from bonito.

MATERIALS AND METHODS

Animal Care. Male Sprague−Dawley rats (3 weeks old; Japan CREA Co., Tokyo, Japan) were individually housed in stainless steel wire-bottom cages, in a holding room maintained at 22−24 °C, 55 ± 5% relative humidity, and a 12-h light/dark cycle. Rats had free access to drinking water and were fed a commercial laboratory diet (F-2; Funahashi Farm Co., Chiba, Japan) for 1 week before the experiment was begun. The animal care and experimental procedures were approved by the Animal Care and Use Committee of the Kyoto University, Division of Applied Life Sciences.

Diets and Experimental Design. The experimental diets were prepared by adding 10% of either lard, fish oil, linseed oil, or perilla oil to the basic laboratory diet (F-2). The percentages of energy of experimental diets as protein, carbohydrate, and fat were 18.5, 54.5, and 27.0, respectively. The fish oil from bonito and other oils were supplied by NCF Co. (Tokyo, Japan). The fatty acid composition of each oil is shown in Table 1. Lard is rich in SFAs (palmitic acid and stearic

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Table 2. Effects of Lard, Fish, and Linseed and Perilla Oil Diets on the Body and Fat Pad Weights of Rats[a]

	diet group			
	lard	fish oil	linseed oil	perilla oil
body wt gain, g	329.8 ± 2.7	326.2 ± 8.6	297.7 ± 14.7	319.7 ± 22.9
abdominal fat pad wt,[b] g/100 g of BW	4.6 ± 0.3[a]	3.3 ± 0.2[b]	3.6 ± 0.1[bc]	4.1 ± 0.4[ac]
IBAT, mg/100 g of BW	102.4 ± 0.8[a]	114.2 ± 1.5[b]	89.0 ± 0.5[c]	90.4 ± 1.3[c]
IBAT mitochondrial protein, mg/organ	1.46 ± 0.15	1.84 ± 0.26	1.89 ± 0.20	1.43 ± 0.11

[a] The values are means ± SEM for five rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. [b] Abdominal fat pads are made up of epididymal and perirenal adipose tissues.

acid) and monounsaturated fatty acids (oleic acid), fish oil is rich in HUFAs (docosahexaenoic acid and icosapentaenoic acid), and linseed and perilla oils are rich in a vegetable-derived PUFAs (α-linolenic acid). The rats, weighing 56.0 ± 0.2 g, were separated into four groups of five rats each and given, ad libitum, lard diet, fish oil diet, linseed oil diet, or perilla oil diet for 9 weeks. The rats were killed under ether anesthesia, 12 h after the last experimental diet. The interscapular brown adipose tissue (IBAT), abdominal fat pads composed of perirenal and epididymal adipose tissues, were immediately excised, weighed, and stored at −20 °C for further analysis.

Western Blot Analysis. Each IBAT sample (five individual samples in each group) was subjcted to Western blot analysis for UCP as described previously (Tsukazaki et al., 1995). A rabbit antiserum against rat UCP purified from IBAT in cold-exposed rats as described elsewhere (Kawada et al., 1991) was used as primary antibody. Antibody bound to the blotting membrane was visualized using the chemiluminescence system (NEN Life Science Products, Boston, MA).

Statistical Analysis. The data are presented as means ± SEM and were statistically analyzed by means of the unpaired *t* test or the Welch *t* test when variances were heterogeneous. Group means were tested by using standard one-way ANOVA and Duncan's multiple-range test. Differences were considered significant when p was <0.05.

RESULTS AND DISCUSSION

Effects of Dietary Oils on Body and Adipose Tissue Weights. Throughout the experimental period of 9 weeks, no significant differences were observed among the four groups of rats given the four different oil diets in either the mean body weight gain (Table 2) or the mean calorific intake (in kJ day^{-1} rat^{-1}: lard, 327.2; fish oil, 340.6; linseed oil, 323.4; perilla oil, 346.4). The weights of the liver, and other organs, other than white and brown adipose tissues, were not different among the different groups (data not shown). The weight of the abdominal fat pads, composed of perirenal and epididymal adipose tissues, was significantly lower in fish oil diet-fed rats and linseed oil-fed rats ($p < 0.001$ and < 0.01, respectively) than in the lard diet-fed group (Table 2). In perilla oil-fed rats, the weights of the abdominal fat pads were observed to be slightly lower than in the lard-fed group. The weight of the IBAT was significantly greater ($p < 0.001$) in fish oil diet-fed rats than in the lard-fed group (Table 2). However, the weights of the IBAT of the linseed oil- or perilla oil-fed rats were significantly lower than those of the lard-fed rats. It was observed that brown adipocytes in IBAT exhibited good development, histologically, in fish oil-fed rats compared to in the lard-fed rats and that the IBAT of lard-fed rats was composed of a mixture of brown adipocytes and white adipocytes. The development of IBAT in fish oil fed-rats resulted in enhancement of thermogenesis. On the other hand, the reduction of weight of the IBAT observed in linseed oil- or perilla oil-fed rats presumably resulted from moderate activation of BAT thermogenesis and the subsequent reduction in the number of white adipocytes in IBAT,

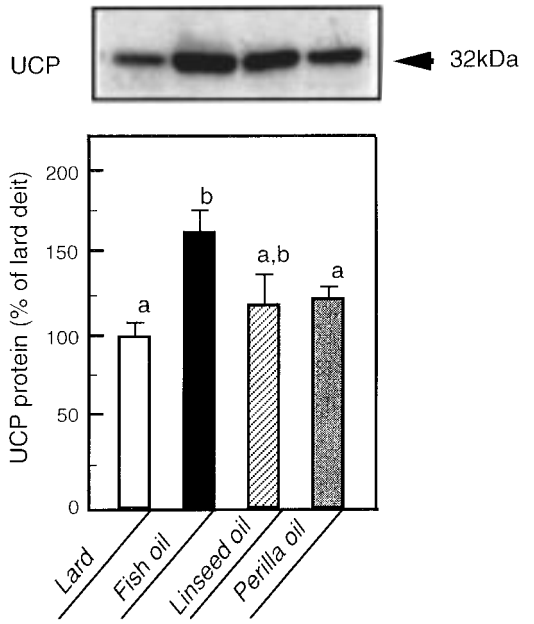

Figure 1. UCP expression in IBAT of lard-, fish oil-, linseed-, and perilla oil-fed rats. Rats were fed each diet for 9 weeks, and subsequently fat-free extracts (50 μg of protein), prepared from IBAT, were subjected to Western blot analysis. The values are expressed as relative to that of lard-fed rats and are means ± SEM for five rats. Means not sharing a common superscript letter are significantly different at $p < 0.05$. Other experimental details are under Materials and Methods.

as previously reported in the case of sweetener diet-fed rats (Kawada et al., 1991). IBAT mitochondrial protein content in rats fed fish oil and linseed oil diets was slightly greater than that in lard-fed rats (Table 2). The capacity of BAT thermogenesis depends on the weight of the organ and the mitochondrial expression of UCP; hence, it was suggested that the development of IBAT and its mitochondria in fish oil- or linseed oil-fed rats was associated with a reduction of abdominal fat pad weights via the activation of thermogenesis.

Effects of Dietary Oils on the Expression of UCP in IBAT. BAT exhibits a unique property of possessing a high heat production capacity. We have reported that some sweeteners and seasonings cause induction of UCP expression in BAT, resulting in enhancement of thermogenesis in animals during and after food intake (Kawada et al., 1986a,b, 1991; Oi et al., 1995). In this study, the expression of UCP in IBAT was significantly higher in the fish oil diet-fed rats ($p < 0.001$) compared to that in the lard-fed or perilla oil-fed group (Figure 1). Furthermore, the expression of UCP in rats given linseed or perilla oil diets was slightly higher than that in rats given lard diet. Interestingly, the degree of augmentation of expression of UCP in the rats fed fish oil rich in HUFA was much greater than that in those fed vegetable oil rich in PUFA or animal fat rich in SFA. Furthermore, it should be noted that the extent of

induction of UCP expression correlated with the reduction of abdominal fat pad weight.

The prime activator of BAT thermogenesis is regulated by sympathetic nerves distributed to this tissue, principally through the β-adrenergic action of norepinephrine (Himms-Hagen, 1989). There are three isoforms of the β-adrenergic receptor (AR) in brown adipocytes: β1-, β2-, and β3-AR (Lafontan and Berlan, 1993). Because the β3-AR is expressed primarily, but not exclusively, in brown and white adipocytes, it is expected that an agonist of the β3-AR, which would be a selective stimulant of lipolysis and BAT thermogenesis, might be useful as an antiobesity drug (Arch and Kaumann, 1993). On the other hand, we have reported that some dietary components, especially sweeteners and the pungent ingredients of seasonings (hot pepper, mustard, and garlic), induced UCP expression in IBAT by the mediation of the sympathetic nervous system (Kawada et al., 1991; Watanabe et al., 1988; Oi et al., 1995).

PUFA and HUFA from vegetable or fish oils suppressed the excessive accumulation of adipose tissue, as compared to SFA from animal fats (Shimomura et al., 1990; Hainault et al., 1993; Okuno et al., 1997). Our present study supports these previous results in terms of the antiobesity functions of PUFA and HUFA. Although it was reported that perilla oil, rich in PUFA, prevents the excessive growth of abdominal fat pads in rats by suppressing the late phase of adipocyte differentiation (Okuno et al., 1997), the mechanism of the antiobesity functions of PUFA are not fully understood, especially with respect to the following: How can the extra energy or fat, which could be not used in abdominal fat pads, be utilized? In this study, it is suggested that the intake of unsaturated fatty acids, especially of a HUFA-rich diet, causes UCP induction and enhancement of thermogenesis, resulting in suppression of the excessive growth of abdominal fat pads. Fish oil might be useful in functional foods for obesity. Further investigation is necessary on the UCP induction effect of fish oil. Such studies are now in progress.

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