Effect of eicosapentaenoic acid ethyl ester on hypothyroid function

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Abstract

Thyroid hormones affect reactions in almost all pathways of lipid metabolism. It has been reported that plasma free fatty acid (FFA) concentration in hypothyroidism is generally within the normal range. In this study, however, we show that plasma FFA concentration in some hypothyroid patients is higher than the normal range. Symptoms of thyroid dysfunction in these individuals were less severe than those of patients with lower plasma FFA concentrations. From these findings we hypothesized that the change in FFA concentration must correlate with thyroid function. Using an animal model, we then examined the

effect of highly purified eicosapentaenoic acid ethyl ester (EPA-E), a n-3 polyunsaturated fatty acid derived from fish oil, on thyroid function in 1-methyl-2-imidazolethiol (MMI)-induced hypothyroid rats. Oral administration of EPA-E inhibited reduction of thyroid hormone levels and the change of thyroid follicles in MMI-induced hypothyroid rats. These findings suggest that FFA may affect thyroid functions and EPA-E may prevent MMI-induced hypothyroidism.

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Introduction

Thyroid hormones affect reactions in almost all pathways of lipid metabolism (Hoch 1988). Plasma free fatty acid (FFA) is released from triglyceride-rich lipoprotein by lipoprotein lipase and hepatic triglyceride lipase. Although it has been reported that plasma lipoprotein lipase and hepatic triglyceride lipase activities are increased in hypothyroidism (Valdemarsson 1983), several studies have shown that plasma FFA concentration in hypothyroidism is within the normal range (Hamberger *et al.* 1963, Tulloch *et al.* 1973, Saunders *et al.* 1980). Therefore, the relationship between plasma FFA concentration and the hypothyroid state remains unclear.

Fatty acids are important as an energy source and as essential components of the plasma membrane. Plasma membrane is composed of several different categories of lipids and functional proteins. Since fatty acid composition is a major factor influencing the biological functions of plasma membranes, structural and quantitative changes of fatty acids may alter the physical properties of the membrane. Fatty acids, especially polyunsaturated fatty acid (PUFA), may affect cellular functions such as membrane-bound enzymes (Neelands & Clandinin 1983), transport systems (Ekokoski *et al.* 1994) and receptors (Gould *et al.* 1982). In addition, several studies have shown that PUFAs are ligands for peroxisome proliferator activated receptor α

(PPAR α) (Forman *et al.* 1997, Kliewer *et al.* 1997). Moreover, PPAR α is related to cell proliferation and differentiation in various cells (Hanley *et al.* 1998, Kaplanski *et al.* 2000, Peters *et al.* 2000).

Eicosapentaenoic acid (EPA), an n-3 PUFA, found in various human tissues, has beneficial effects against several diseases (Kromann & Green 1980). To determine whether an increase in plasma FFA concentration might affect thyroid function, in the second part of this study we investigated whether eicosapentaenoic acid ethyl ester (EPA-E) might prevent the hypothyroid state in 1-methyl-2-imidazolethiol (MMI)-induced hypothyroid rats.

Subjects and Methods

Patients and treatment

The clinical study consisted of 15 unrelated Japanese subjects diagnosed with primary hypothyroidism on the basis of their symptoms and laboratory tests at Fujita Health University Hospital. After diagnosis and having obtained their consent to our study, patients were treated by oral administration of thyroxine at an initial dose of 25 μ g daily, and subsequently at 25 μ g every 2 weeks until plasma thyroid-stimulating hormone (TSH) concentration reached normal levels.

Table 1 Clinical and biochemical characteristics of 15 unrelated Japanese subjects with hypothyroidism. The high and low FFA groups were classified according to their fasting serum FFA levels Each value is the mean ± s.d.

	Low FFA level	High FFA level
Symptoms (%)		
Fatigue	100	100
Cold intolerance	63	29
Edema	75	43
Dry skin	75	29
Slow speech	75	29
Constipation	75	29
Delay of ATR	88	29*
Laboratory data		
GOT (mU/ml)	61.0 ± 60.5	30.9 ± 37.3
GPT (mU/ml)	63.9 ± 91.9	19.5 ± 16.6
CK (mU/ml)	331.5 ± 202.6	149.5 ± 260.4
Total cholesterol (mg/dl)	275.0 ± 95.5	261.7 ± 51.8
Triglyceride (mg/dl)	176.8 ± 39.7	126.7 ± 71.4
HDL cholesterol (mg/dl)	28.8 ± 9.3	59.3 ± 28.8
β-lipoprotein (mg/dl)	150.3 ± 188.9	498.3 ± 150.3
FFA (mEq/l)	0.17 ± 0.05	0.88 ± 0.24*
Thyroid function		
TSH (μU/ml)	141.9 ± 43.0	65.6 ± 38.1**
T ₃ (ng/ml)	0.45 ± 0.24	1.06 ± 0.46**
T ₄ (μg/dl)	1.23 ± 1.16	3.56 ± 2.47*
Free T ₃ (pg/ml)	1.16 ± 0.74	2.4 ± 1.08*
Free T ₄ (ng/dl)	0.18 ± 0.20	0.47 ± 0.25*

*P<0.05, **P<0.01; statistically significant, compared using Fisher's exact probability test (symptoms) or Mann-Whitney U test (laboratory data and thyroid function).

Animals and manipulation

Five-week-old male Wistar rats were used in the experiments described in this study. We obtained 12 rats, weighing from 100 to 110 g, from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. The rats were divided into two groups of six on the basis of their initial body weights. Hypothyroidism in rats was induced by subcutaneous injection of MMI (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) at a daily dose of 1 mg with concomitant oral administration of EPA-E (generously donated by Mochida Pharmaceutical Co., Ltd, Tokyo, Japan) at a daily dose of 300 mg/kg (or the same volume of saline as a control) for 4 weeks. After 4 weeks the rats were anesthetized with chloroform and blood samples and thyroid tissues were obtained. Part of the thyroid tissues were immediately frozen in liquid nitrogen and stored at - 80 °C until use. This work conformed to the guidelines on the handling of laboratory animals of our institution.

Determination of serum tri-iodothyronine (T₃), thyroxine (T₄) and TSH

Serum levels of T₃, T₄ and TSH (patients) and T₃ and T₄ (animals treated with and without EPA-E) were measured by an immunochemiluminescence method (Amersham Pharmacia Biotech, Little Chalfont, UK); normal range of

T₃, T₄ and TSH concentrations are 0.9–1.5 ng/dl, 5.0–9.7 μg/ml and 0.4–4.0 μg/ml respectively. Serum levels of rat TSH were measured using a rat TSH (rTSH) enzyme immunoassay (EIA) system (Amersham Pharmacia Biotech); normal range of rTSH concentration is 5–30 ng/ml.

Analysis of plasma lipid and FFA composition

At 9 weeks of age, after a 10-h fast, blood samples were taken from the tail veins of the rats without anesthesia. Plasma levels of total cholesterol, triglyceride and FFA were measured enzymatically with an autoanalyzer (Olympus Optical, Co., Ltd, Tokyo, Japan). FFAs were then separated and quantified by gas chromatography (model 663 Hitachi, Tokyo, Japan) using a capillary column (SP-2380).

Analysis of FFA composition in thyroid tissues

Thyroid tissues were obtained from the rats killed by exsanguination under anesthesia. Thyroid tissues were homogenized in a mixture of chloroform and methanol (2:1 vol./vol.), and total lipid extracts were prepared according to the method (Folch *et al.* 1957). The determination of FFA composition in the thyroid tissue extracts was performed as described for the determination of plasma FFA composition.

Figure 1 Change of serum TSH concentration in hypothyroid patients after T_4 treatment. (A) Seven hypothyroid patients with high plasma FFA levels. (B) Eight hypothyroid patients with low plasma FFA levels. The periods until normalization of TSH concentrations after the treatment of the patients with the high plasma FFA level were shorter than that of the patients with the low plasma FFA levels (38.1 ± 12.8 vs 62.6 ± 12.0 , $P < 0.01$, mean $\pm$ S.D.), and also the area under the curve was smaller (1031.0 ± 487.7 vs 5599.4 ± 3177.9 , $P < 0.01$, mean $\pm$ S.D.).

Histopathological examination

The portions of thyroid tissues were immediately dissected and immersed in neutralized 10% formalin for 5 days. Specimens were then embedded in paraffin by routine procedures. Paraffin sections of the specimens were deparaffinized with xylene and stained with hematoxylin and eosin for light microscopy examination.

Statistical analysis

Data are expressed as the mean $\pm$ S.D. The significance of differences versus the control group was analyzed by Fisher's exact probability test (symptoms in hypothyroidism patients) or Mann-Whitney U test (laboratory data in hypothyroidism patients and in animal study), and a statistically significant difference was defined at P less than 0.05.

Results

Clinical experiments

Clinical and biochemical characteristics of hypothyroid patients Some hypothyroid patients had a higher plasma FFA concentration than normal range. The severity of hypothyroid symptoms and abnormal values of biochemical markers was elevated in those patients with

low levels of plasma FFA (Table 1). Serum T_3 and T_4 concentrations were significantly decreased, and serum TSH concentrations were significantly increased, in patients with low plasma levels of FFA when compared with patients with high levels of plasma FFAs (Table 1).

Changes in TSH concentrations in patients with hypothyroidism after treatment Patients with high plasma levels of FFA exhibited more rapid normalization of TSH than patients with low plasma FFA (Fig. 1).

Animal experiments

Animal condition There were no significant differences in food intake and body weight of the MMI-induced hypothyroid animals during the experimental period (Table 2). Thyroid weight was, however, noticeably greater in animals treated without EPA-E than in animals treated with EPA-E (Table 2).

Lipid levels in thyroid tissues and plasma in hypothyroid rats Plasma FFA levels showed no statistically significant differences between animals treated with and without EPA-E during the experimental period (Table 2), nor was any significant difference observed between the groups in total cholesterol and triglyceride levels (Table 2). MMI-induced hypothyroid rats treated with EPA-E

Table 2 The change of animal condition and lipid for rats with MMI-induced hypothyroidism with or without EPA. Each value is the mean ± S.D.

	Treatment without EPA-E (days)					Treatment with EPA-E (days)				
	0	7	14	21	28	0	7	14	21	28
Body weight (g)										
Heart rate (beats/min)	145 ± 9	221 ± 13	231 ± 12	252 ± 9	272 ± 8	146 ± 3	220 ± 14	243 ± 16	269 ± 17	283 ± 9
Thyroid weight (mg)	445 ± 33		418 ± 23		439 ± 21	457 ± 43		444 ± 36		445 ± 33
Heart weight (mg)					36.5 ± 5.6					29.7 ± 1.9*
					980.3 ± 90					930.2 ± 77.3
Total cholesterol (mg/dl)					61.75 ± 2.63					65.75 ± 5.56
Triglyceride (mg/dl)					26 ± 6.98					25.75 ± 15.65
FFA (mEq/l)	1.898 ± 0.27				0.582 ± 0.161	1.754 ± 0.244				0.662 ± 0.26

*P<0.05; statistically significant, compared using Mann–Whitney U test.

showed elevated level of EPA (C20 : 5) in plasma and thyroid tissues compared with hypothyroid rats treated without EPA-E (Table 3).

Changes in serum T_3 , T_4 and rTSH levels in MMI-induced hypothyroid rats Hypothyroid rats treated with EPA-E exhibited significantly higher levels of serum T_3 and T_4 throughout the experimental period than rats treated with EPA-E (Fig. 2).

After these results were obtained, this experiment was reconfirmed by the same methods using seven rats from each group. Serum T_3 , T_4 and rTSH levels of rats treated with EPA-E versus rats treated without EPA-E were 0.82 ± 0.08 vs 0.72 ± 0.06 mg/ml ($P=0.0181$), 4.63 ± 0.38 vs 4.10 ± 0.34 µg/dl ($P=0.0253$), and 34.58 ± 8.43 vs 62.26 ± 11.40 ng/ml ($P=0.017$) respectively.

In addition, 5-week-old male Wistar rats were orally administrated with EPA-E at a daily dose of 300 mg/kg ($n=4$) or the same volume of saline as a control ($n=4$) for 4 weeks. The rTSH levels in rats treated with EPA-E were not significantly different from rats treated with saline: 9.58 ± 6.36 vs 13.76 ± 6.88 ng/ml.

Light microscopic findings Histopathological features in the thyroid tissues of hypothyroid rats are shown in Fig. 3. Although the size of the thyroid follicular lumens varied in MMI-induced hypothyroid rats treated without EPA-E (Fig. 3A), the size of the thyroid follicular lumens were uniform in MMI-induced hypothyroid rats treated with EPA-E (Fig. 3B).

Discussion

While several studies have shown that plasma FFA concentration in hypothyroidism is within normal range (Hamberger *et al.* 1963, Tulloch *et al.* 1973, Saunders *et al.* 1980), some hypothyroid patients have higher plasma FFA concentrations than the normal range in our investigation. In the clinical part of our study, we confirmed that symptoms of thyroid dysfunction in hypothyroid patients with high plasma FFA concentrations were less severe than those of patients with low plasma FFA concentration. Severely hypothyroid patients had lower FFA concentrations in this study, correlating with the fact that activities of lipoprotein lipase and hepatic triglyceride lipase, key enzymes of regulation in plasma FFA, are increased in hypothyroidism (Valdemarsson 1983). Hypothyroidism in patients with high plasma FFA concentrations was less severe, therefore we hypothesized that the increase of plasma FFA concentration might affect thyroid function in the hypothyroid state. To test our hypothesis, we examined the influence of the n-3 PUFA EPA-E on rats with MMI-induced hypothyroidism. In the animal experiments, the decrease in plasma T_3 and T_4 concentrations and increase in plasma TSH concentration

Table 3 Changes in serum and thyroid FFA compositions for rats with MMI-induced hypothyroidism with or without EPA. Each value is the mean $\pm$ S.D.

	Plasma		Thyroid	
	EPA-E (-)	EPA-E (+)	EPA-E (-)	EPA-E (+)
C14:0	0.32 $\pm$ 0.05	0.37 $\pm$ 0.09	1.85 $\pm$ 0.30	2.00 $\pm$ 0.21
C16:0	18.92 $\pm$ 0.71	19.60 $\pm$ 1.32	26.77 $\pm$ 1.04	28.077 $\pm$ 0.83
C16:1	0.82 $\pm$ 0.17	1.27 $\pm$ 0.17*	2.95 $\pm$ 0.58	3.75 $\pm$ 0.41
C18:0	14.25 $\pm$ 1.66	13.32 $\pm$ 1.22	9.45 $\pm$ 0.83	7.75 $\pm$ 0.75
C18:1	6.90 $\pm$ 0.82	6.97 $\pm$ 0.69	24.30 $\pm$ 2.61	24.6 $\pm$ 0.79
C18:2	20.67 $\pm$ 1.05	21.02 $\pm$ 1.43	24.22 $\pm$ 2.13	24.47 $\pm$ 2.30
C18:3	0.35 $\pm$ 0.05	0.35 $\pm$ 0.05	0.75 $\pm$ 0.12	0.95 $\pm$ 0.17*
C20:0	0.12 $\pm$ 0.05	0.10 $\pm$ 0.00	0.17 $\pm$ 0.05	0.20 $\pm$ 0.00
C20:1	0.12 $\pm$ 0.05	0.10 $\pm$ 0.00	0.52 $\pm$ 0.05	0.50 $\pm$ 0.00
C20:2	0.22 $\pm$ 0.00	0.22 $\pm$ 0.00	0.30 $\pm$ 0.00	0.32 $\pm$ 0.05
C20:3	0.77 $\pm$ 0.09	1.1 $\pm$ 0.18	0.42 $\pm$ 0.12	0.35 $\pm$ 0.05
C20:4	29.02 $\pm$ 1.61	25.17 $\pm$ 3.52	4.25 $\pm$ 1.33	2.87 $\pm$ 0.33
C20:5	1.15 $\pm$ 0.17	3.22 $\pm$ 0.66**	0.20 $\pm$ 0.08	0.62 $\pm$ 0.09**
C22:0	0.17 $\pm$ 0.05	0.25 $\pm$ 0.05	0.17 $\pm$ 0.05	0.22 $\pm$ 0.05
C22:1	ND	ND	ND	ND
C22:5	0.85 $\pm$ 0.20	1.77 $\pm$ 0.69	0.92 $\pm$ 0.15	1.55 $\pm$ 0.28
C22:6	5.05 $\pm$ 0.26	4.80 $\pm$ 0.76	2.50 $\pm$ 1.08	1.60 $\pm$ 0.29
C24:0	0.10 $\pm$ 0.00	0.17 $\pm$ 0.15	0.01 $\pm$ 0.00	0.17 $\pm$ 0.00
C24:1	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.05 $\pm$ 0.00	0.10 $\pm$ 0.00

* $P < 0.05$, ** $P < 0.01$; statistically significant, compared using Mann-Whitney U test.

in the MMI-induced hypothyroid state was inhibited by administration of EPA-E. In addition, the sizes of the thyroid follicles without administration of EPA-E were varied and contained large of quantities of colloid. These findings indicate that thyroid hormone secretion in the rat in the absence of EPA-E administration is elevated.

The regulation of cell functions by PUFA, including EPA, might occur on two general levels: modulation of signal transduction via manipulation of membrane fatty acid composition; and rapid, direct modulation of gene transcription. Several studies have provided evidence indicating that plasma membrane lipids in thyroid glands

Figure 2 Changes in serum T_3 (ng/dl) and T_4 (μ g/ml) concentration for MMI-induced hypothyroid rats treated with (●) or without (○) EPA-E. Each value represents the mean $\pm$ S.D. ** $P < 0.01$, * $P < 0.05$.

Figure 3 Histopathological analysis of the thyroid glands of MMI-induced hypothyroid rats treated with (A) or without (B) EPA-E.

modulate the TSH receptor and its ability to bind TSH (Bashford *et al.* 1975, Mehedi *et al.* 1977, Omodeo-Sale *et al.* 1978, Aloj *et al.* 1979). Furthermore, an important role for phospholipids has been indicated in the TSH-responsive adenylate cyclase system (Macchia *et al.* 1970, Yamashita *et al.* 1970, Yamashita & Field 1973, Moore & Wolff 1974, Amir *et al.* 1976).

EPA and arachidonic acid (AA), constituents of the plasma membrane, are metabolized via the cyclooxygenase, lipoxygenase or epoxygenase pathways to generate eicosanoids. Eicosanoids produced from EPA and AA have different effects in their actions (Yu *et al.* 1995). These differences may affect receptor and signal transduction, resulting in the changes of thyroid hormone biosynthesis or release.

PUFAs are ligands for PPAR α (Forman *et al.* 1997, Kliewer *et al.* 1997), a member of the nuclear receptor superfamily. PPAR α is related to cell proliferation and differentiation in hepatocytes (Peters *et al.* 2000), keratinocytes (Hanley *et al.* 1998) and oval cells (Kaplanski *et al.* 2000). Although a relationship between PPAR α and thyroid follicular cells is not apparent in cellular growth and function, it is possible that PPAR α affects thyroid follicular cell proliferation and differentiation.

It is clear that administration of EPA-E inhibits the decrease in thyroid hormone levels. It will be interesting to determine whether other PUFAs, such as AA and docosapentaenoic acid, have the same effect. Our findings suggest that FFA may affect thyroid functions, and that EPA-E may prevent MMI-induced hypothyroidism.

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