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Effect of Essential Fatty Acid Deficiency on Blood and Tissue Lipid Compositions*

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필수 지방산 결핍이 성장기 쥐의 혈액 및 조직의 지방조성에 미치는 영향

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이유후의 Sprague Dawley종의 숫컷 쥐에게 10% 옥수수기름 혹은 10% 야자경화유를 함유하는 식이를 5주간 주었다. 야자경유화를 주어 필수지방산을 결핍시킨 식이군의 쥐와 다른 식이군의 쥐 사이에, 본 연구에서 조사한 범위내에서는 성장발달에 차이가 없었으며, 필수지방산 결핍증세를 관찰할 수 없었다. 실험군의 쥐의 조직중에서 지방조성의 변화를 살펴보면 (1) 야자 경화유를 준 쥐는 다른 군에 비하여 3주, 5주에 혈장의 TG농도, 5주에 간의 TG농도가 증가하였다. (2) 야자경화유를 준 쥐는 다른 군에 비하여 3주에 혈장 Phospholipid농도가 감소했다. 반면 생식기는 3주에 PL농도가 증가하였으며, 5주에는 PL농도 변화는 없었다. (3) 야자경화유를 준 쥐는 3주에 생식기의 콜레스테롤의 농도가, 5주에 신장, 부신의 콜레스테롤의 농도가 감소하였다. 이로써 성장기 쥐는 필수지방산 결핍시기에 대해, 각 조직에 따라, 또 식이기간에 따라 각기 달리 영향을 받음을 알 수 있다.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are aliphatic monocarboxylic acids with two or more double bonds in any positional arrangement or geometric configuration. Those PUFAs that prevent or relieve the symptoms of dietary deficiency in humans and animals are named, therefore, the essential fatty acids (EFAs). The essentiality of the PUFAs is related to their capability to incorporate into lipids and act as a precursor in the formation of prostaglandins[1)-4)]. The physical properties of PUFAs differ in many ways from those of long-chain saturated fatty acids. The essential fatty acids are principally found in phospholipids, and so they have an accepted structural function in lipoproteins of cell membranes and enzymes[1)]

The weanling period has been shown to be

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considerably important to further development [5] As far as lipids are concerned, there is no doubt that they play an important role in that period. It is noteworthy that many functions attain a much more mature level on about day 18, including such diverse ones as thermo-regulation, secretion of adrenal cortical hormones, thyroid function, muscle innervation, brain maturation and enzymes in the gut. It seems likely that critical periods of development in the same individual differ for different tissues and even cells, since development of the body is not a uniform process. There are many indications that show differences in enzyme development in liver, kidney, brain, and so on. Biochemically, we know little in that respect. As far as lipids, it might be worth while to look into the possibility of early nutrition affecting subsequent development in various tissues.

The recent stimulus to study in this field comes to clinical investigations which suggest that levels of serum lipids may be related to the dietary intake of unsaturated fatty acids. Kinsell and Michaels [6] reported that the feeding of coconut oil (a highly saturated fat) produced higher levels of serum lipid than the feeding of a highly unsaturated oil. It has been claimed that ingestion of EFAs decreases the concentration of lipids in the serum and that an adequate intake of these acids may be the cause of the high serum lipid levels [7]. Since these high levels are allegedly involved in the pathogenesis of atherosclerosis and coronary disease, it has been tempting to implicate lowered intake of unsaturated fatty acids in the rising incidence of these diseases.

A possible indication that the deficiency leads to a metabolic difficulty is seen in the findings of Alfin-Slater, et. al.[8] that the deficient state is accompanied by production of a fatty liver in which the major lipids deposited are cholesterol esters with abnormally saturated fatty acids. This condition is often accompanied by gross and histological evidence of kidney damage [9]. The deficiency affects the reproductive organs of male and female rats somewhat differently. Deficient females conceive but either resorb the fetuses or deliver pups that shows severe symptoms of deficiency, including caudal necrosis and soon die. Male, on the other hand, become sterile and the sexual organs atrophy [10]. The high concentration of cholesterol as well as of other lipids in the adrenal suggests that the metabolism of adrenal lipids is related to the formation and secretion of adrenocortical hormones. It has been well established that cholesterol is a precursor of the adrenal cortical hormones [11][12]. Alfin-Slater and Bernick [13] indicated the necessity of EFA for maintaining adrenal activity in the rat. However, little work has been done in determining the lipid composition of adrenal. As one aspect of our interest in the metabolism of lipid, studies have been undertaken to evaluate the effect of essential fatty acid deficiency on the adrenal lipids.

The present report concerns the effect of EFA deficiency on the growth and tissue lipid composition of weanling rats and the effect of kinds of dietary fat source (corn oil, hydrogenated coconut oil) on tissue lipid composition. The changes in body weight, tissues lipid compositions of the experimental diet fed rats were measured.

EXPERIMENTAL

1) Animal and Diets

Male weanling Sprague-Dawley rats weighing about 70g, supplied by Animal Breeding Laboratory of Seoul National University, were used in this study. Thirty weanling rats were first raised on the commercial diet (obtained from Jeil Fodder Co.) for two days. And then ten rats were decapitated for sampling. Twenty rats were divided into two groups of ten rats and housed in plexiglass cages. They were maintained on their respective diets ad libitum.

Two kinds of experimental diet were used ; a hydrogenated coconut oil (HCO) diet containing 10 % hydrogenated coconut oil, a corn oil diet identical to the HCO diet except the substitution of 10 % corn oil for 10 % HCO (Table 1).

Table 1. Composition of experimental diets

(g/100g diet)

Ingredient \ Group	Corn oil	Hydrogenated coconut oil
Cornstarch (1)	59	59
Casein	20	20
Cellulose	6.5	6.5
Corn oil (2)	10	–
Hydrogenated coconut oil (3)	–	10
Vitamin mixture (4)	1	1
Salt mixture (5)	3.5	3.5
Total	100	100

(1) Obtained from Mi Won Co.
(2) Du San Cereal Industry Co.
(3) Gift from Lotte Sam Gang Co.
(4) Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals, Cleveland, Ohio, USA.
(5) AIN Mineral Mixture 76. ICN Nutritional Biochemicals.

The body weight and diet consumption of ex perimental animals were checked every other day. At three and five weeks rats were killed by decapitation after an overnight fast. Plasma, liver, heart, testis, kidney, epididymal fat pad, and adrenal gland were obtained and stored at −20℃ until analysis.

2) Experimental Methods

Each tissue was homogenized in 19 volumes of 2 : 1 chloroform : methanol per g wet tissue. Lipid extraction was carried out by the method of Folch et al.[14]. Total lipids were determined by the method of the sulfo-phospho-vanillin reaction [15]. Cholesterol was determined by the method of Parekh & Jung[16], phospholipid by the method of Baginski et. al.[17], and triglyceride by the method of Biggs[18]. Data were analyzed by Student's t-test for difference between the means.

RESULTS

1) Body Weight and Physical Appearance

No significant differences were found in body weight of each group and in the amount of food consumed by each group. In this study, diminished growth and scaly skin were not observed in HCO – fed rats.

2) Organ Weights

Organ weights of rats at 3 and 5 weeks are shown in Table 2. The liver weight of HCO group were significantly lower than those of corn oil group ($P < 0.05$) at 3rd week. In the case of other organ (testis, kidney, epididymal fat pad, adrenal gland, heart), there was no significant difference in weights between the groups.

At 5th week in kidney, testis, and heart, there

Table 2. Effects of experimental diets on organ weight

(g)

Group \ Weeks on Diet \ Organ	Testis	Kidney	Adrenal gland	Liver	Heart
Corn 0	0.81 ± 0.074	0.96 ± 0.048	0.022 ± 0.0029	3.46 ± 0.44	0.35 ± 0.05[a]
Corn 3	2.35 ± 0.57	1.60 ± 0.24	0.037 ± 0.012	7.72 ± 0.44	0.76 ± 0.05
Corn 5	3.66 ± 0.45	2.20 ± 0.24	0.044 ± 0.0022	9.74 ± 0.51	0.95 ± 0.07
HCO 3	2.28 ± 0.58	1.66 ± 0.19	0.043 ± 0.0015	7.29 ± 0.32+	0.75 ± 0.06
HCO 5	3.48 ± 0.38	2.22 ± 0.22	0.051 ± 0.0061+	10.25 ± 0.48+	0.94 ± 0.08

a : Mean ± S. D. Five animals were sacrificed
+ : $P < 0.05$ Significantly different from C group
Corn : Corn oil diet
HCO : Hydrogenated coconut oil diet

Table 3. Effects of experimental diets on plasma lipids

(mg/100ml)

Group \ Weeks on Diet \ Lipid class		Total lipid	Total chol.	Phospholipid	Triglyceride
Corn	0	259.3 ± 98.6[a]	127.3 ± 25.4	79.6 ± 14.7	78.2 ± 4.8
	3	273.5 ± 2.1	113.6 ± 14.3	87.4 ± 19.0	64.3 ± 5.1
	5	243.8 ± 42.7	132.4 ± 10.4	91.4 ± 16.8	63.9 ± 5.7
HCO	0	259.3 ± 98.6	127.3 ± 25.4	79.6 ± 14.7	78.2 ± 4.8
	3	236.0 ± 15.6[+]	127.7 ± 15.4	60.0 ± 3.3[+]	81.7 ± 3.3[+]
	5	277.8 ± 47.2	121.6 ± 25.2	61.4 ± 19.7[*]	83.5 ± 28.5

a : Mean ± S.D.
+ : $P < 0.1$ Significantly different from corn oil group
* : $P < 0.05$ Significantly different from corn oil group

Table 4. Effects of experimental diets on tissue lipids

(mg/g wet weight)

Organ	Group / Weeks on Diet / Lipid class		Corn oil		Hydrogenated coconut oil	
		0	3	5	3	5
Testis	Total Lipids	14.4 ± 3.9[a]	17.4 ± 11.2	10.4 ± 5.6	8.9 ± 4.8	7.8 ± 2.1
	Cholesterol	2.9 ± 0.7	2.9 ± 0.9	2.2 ± 0.6	1.4 ± 0.5[+]	2.0 ± 0.6
	Phospholipid	10.8 ± 3.6	7.3 ± 1.1	8.2 ± 1.9	10.6 ± 1.0[+]	8.6 ± 3.7
Kidney	Total Lipids	15.1 ± 5.3	22.6 ± 9.4	19.4 ± 2.4	17.9 ± 3.0	21.1 ± 2.3
	Cholesterol	4.1 ± 0.7	3.8 ± 2.0	6.1 ± 1.2	5.3 ± 0.4	3.6 ± 1.3[*]
	Phospholipid	12.7 ± 2.3	9.9 ± 3.0	13.1 ± 1.5	9.3 ± 3.8	14.2 ± 3.3
Adrenal Gland	Total Lipids	68.4 ± 1.8	112.3 ± 5.0	124.1 ± 1.7	104.4 ± 10.4	91.5 ± 0.6[++]
	Cholesterol	4.1 ± 0.1	10.5 ± 1.9	16.5 ± 2.2	10.6 ± 1.0	12.3 ± 2.0[+]
	Phospholipid	12.7 ± 2.3	21.3 ± 4.2	17.1 ± 7.8	20.6 ± 4.6	14.5 ± 2.3
Liver	Total Lipids	56.1 ± 0.59	58.2 ± 2.6	60.8 ± 2.5	59.8 ± 3.7	62.3 ± 3.3
	Cholesterol	2.6 ± 0.01	2.7 ± 0.31	3.0 ± 0.12	2.7 ± 0.39	3.1 ± 0.18
	Phospholipid	25.1 ± 0.38	28.0 ± 1.6	30.1 ± 2.0	27.9 ± 1.7	30.1 ± 2.1
	Triglyceride	16.0 ± 0.25	17.1 ± 2.7	17.2 ± 2.1	18.0 ± 2.2	19.3 ± 2.1[+]
Heart	Total Lipids	25.2 ± 0.46	25.8 ± 2.5	27.8 ± 2.5	25.4 ± 2.5	28.2 ± 2.3
	Cholesterol	0.53 ± 0.01	0.59 ± 0.23	0.61 ± 0.48	0.53 ± 0.48	0.72 ± 0.34
	Phospholipid	14.2 ± 0.08	15.0 ± 2.7	14.2 ± 2.8	14.9 ± 2.8	15.7 ± 2.9
	Triglyceride	2.0 ± 0.02	2.0 ± 0.09	2.05 ± 0.13	2.24 ± 0.13	2.5 ± 0.31[*]

a : Mean ± S.D. * : Significant at $P < 0.1$ + : Significant at $P < 0.05$ ++ : Significant at $P < 0.01$

is no significant difference in weight between the groups. But the weight of liver, adrenal gland, and epididymal fat pad of HCO group were significantly higher than those of corn oil group($P < 0.05$).

3) Plasma Lipid Composition

The content of individual major lipid fraction of plasma from experimental rats is summarized in Table 3.

During 3 week period, it was noticeable that lipid content and phospholipids (PL) in rat plasma of HCO group were significantly lower than those of corn oil group. But the plasma TG of HCO group was significantly higher than those of corn oil group.

During 5 week period, a little increase in the concentration of lipid of HCO group was observed but this difference was not significant. In the level of plasma PL, decrease in the concentration of plasma PL of HCO group was noticeable. The cholesterol level remained relatively constant throughout the 5 week period.

4) Tissue lipid composition

Table 4. Shows the effect of experimental diets on lipid compositions of liver, heart, testis, kidney, adrenal gland.

At 3 weeks, there were significant differences in various testis lipids. Cholesterol level in testis from HCO group was significantly lower than that of corn oil group, but the phospholipid level of testis from HCO group was higher than that of corn oil group. In other tissues, there was no significant difference between the groups.

At 5 weeks, the level of cholesterol in kidney from HCO group was significantly lower than that of corn oil group ($P < 0.1$). The liver TG content of HCO group was significantly higher than corn oil group. In adrenal gland, the lipid level of HCO group was lower than that of corn oil group ($P < 0.01$), and cholesterol level of HCO group was lower than that of corn oil group ($P < 0.05$). No significant differences in other lipid compositions of tissues were found between the groups.

DISCUSSIONS

The most remarkable changes found in the lipids of rats in experimental groups were :(1) An increase in the concentration of plasma TG and liver TG in HCO group. (2) A decrease in the concentration of plasma PL and an increase in the concentration of testis PL of HCO group. (3) A decrease in the cholesterol level of testis, kidney and adrenal of HCO group.

The rats fed the EFA-free diet grew similary to the control group and did not show any symptoms of EFA deficiency. Panos & Finerty [19] demonstrated enlargement of the kidneys as well as of the brain, adrenals, liver, and heart in EFA deficiency. In our studies, liver and adrenal weight of HCO group at 5 weeks was also increased. But these effects did not appear in the kidney, testis, heart. Studies of the excised testis from the EFA deficienct group and corn oil group rats revealed no delay in maturity of the testis judging from the weight of the testis.

The effects of EFA deficiency on plasma lipids and on plasma cholesterol were conflicting with some other studies done before. Studies on the effect of dietary cholesterol and PUFAs on the plasma cholesterol in infants have also yielded conflicting result [20]. The ingestion of PUFAs by man has been shown to reduce plasma cholesterol [21] and triglyceride concentration [22] and to increase their proportion in lipoprotein lipids [23]. Our data are consistent with this report. Plasma phospholipid in corn oil group was higher than in HCO group. Plasma triglyceride level in HCO group was higher than in corn oil group. From our results, an increase of plasma TG level and liver TG level in HCO group was noticeable. From the data of Nath et. al. [24], it appears that after 10 weeks of deficiency the amounts of cholesterol, PL, and total fat were greater in the livers of deficient rats than in those of normal rats by 60, 5, and 2200mg/100g liver, respectively, indicating that the accumulation of triglycerides

was the most pronounced.

A little decrease of lipid level in tissues agrees with the work of Paoletti & Galli[25]. He reported that during the fat free diet brain weight is decreased, total lipid and PL concentration decreased. Unlike the concentration of plsma cholesterol, the concentration of testis cholesterol from HCO group was lowered in our studies. The increased concentration of pentaenoic acid in testicular tissue of rats with age (3 weeks to 3 months) observed by Kirshman & Coniglio[26] suggests that lipids may have an essential role in the maturation of the testis. And Holman & Greenberg [27] suggested that the large quantity of PUFAs in gonadal tissue may have been biochemical significance. This significance may lie, for the rat at least, in the fact that tissue PUFAs belong to the linoleic acid series and possess EFA properties.

Adrenal cholesterol levels are affected by the fatty acid composition of the diet and most of the variation results from changes in the cholesterol ester component[28]. Compared to adrenal from corn oil group, adrenal from HCO group contains a lower level of total cholesterol. This result agrees with the work of Treadwell, et al.[29]. But others have reported that EFA deficiency results in an increase in esterified cholesterol and a decrease in PL[30] in whole adrenal gland of rat. Alfin-Slater and Bernick[13] indicated the necessity of EFA for maintaining adrenal activity in the rat. Although the functional role of EFA in the adrenal are not clear, EFA deficiency affects some oxidative enzymes[31] and uncouples the oxidative phosphorylation in the liver mitochondria[32]. Because the synthesis of adrenocortical steroids must include certain oxidation – reduction reaction, it is possible that the decreased adrenocortical secretion in EFA deficiency is caused by a defect in those enzyme system.

Present data showed that the EFA deficient diet feeding for 5 weeks did not result in profound EFA deficiency in weanling rats. That trend is considered that in our experimental design fat content is lower than other reported studies. Thus, symptoms of EFA deficiency were not shown within experimental period. The age of the animals appears to be an important factor in EFA deficiency. And failure to induce deficiency symptom in rats may be due to the adipose tissue in part. Brown[33] reported adipose tissue in the animal provided store of EFA.

From our result, it seems to require method of assessing the nutritional status with respect to EFA which are valid in the marginally deficient state, as well as in florid deficiency. In addition to the analysis of lipid composition of various tissues, the analysis of the fatty acid composition of tissue lipids as predicators of EFA intake and metabolism is necessary.

SUMMARY

Weanling male rats were fed a semi – synthetic diet containing either 10 % corn oil, or 10 % hydrogenated coconut oil (HCO) for 5 weeks. They were analyzed for plasma, liver, heart, testis, kid ney and adrenal lipid compositions ; total lipids, total cholesterol, phospholipid, and triglyceride.

The rats fed essential fatty acids deficient diet grew comparably with corn oil group and did not any dermal symptoms of EFAs deficiency.

The most remarkable changes found in lipids of rats in experimental groups were ; (1) an increase in the concentration of plasma TG and liver TG in HCO group. (2) a decrease in the concentration of plasma PL and an increase in the concentration of testis PL of HCO group. (3) A decrease in cholesterol level of testis, kidney and adrenal of HCO group.

Although this study did not show the profound EFA deficiency in weanling rats, this study showed the possibility of early nutrition affecting sub sequent development in various tissues.

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