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Effects of 2-Acetylaminofluorene and Choline Deficiency on Lipid Peroxidation, Glucose 6-phosphatase and Glutathione S-transferase Activities in Rats Fed Different Dietary Fats

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ABSTRACT

This study examines the effects of choline deficiency and 2-acetylaminofluorene(2-AAF) on the lipid peroxide values, glucose 6-phosphatase(G6Pase) and glutathione S-transferase (GST) activities in rats fed different dietary fats.

Weanling Sprague Dawley male rats fed the diets containing 15% beef tallow or 15% corn oil with vitamin fortification mixture or choline free vitamin mixture for 10 weeks. At 3th and 5th week, 2-AAF was injected twice each week intraperitoneally. Total 2-AAF injection was four times.

2-AAF and choline deficiency increased lipid peroxidation in corn oil groups, so the role of 2-AAF and choline deficiency in lipid peroxidation was more important in corn oil groups than beef talllow groups. G6Pase activities tended to be decreased by 2-AAF in choline deficient groups, and in corn oil groups, the enzyme activities were decreased significantly in all subgroups compaired to beef tallow groups. GST activities were increased by 2-AAF in beef tallow groups and choline deficiency in corn oil groups, and might defence against carcinogen metabolism and lipid peroxidation.

KEY WORDS : choline deficiency · P/S ratio · lipid peroxidation · glutathione-S-transterase · glucose-6-phospharase.

INTRODUCTION

Choline, betaine and methionine serve as natural sources of labile methyl groups[1-3], and de novo synthesis of the methyl group is dependent upon adequate dietary supply of folic acid and vitamin B_{12}[1].

Several investigators[4-8] have shown that methyl donor deficiency is one of the few dietary interventions in which nutrient depletion alone can act as a complete carcinogen or as an effective tumor promoter after chemical initiation. It appears that choline deficient(CD) diet exerts modifying effects on the initiation of liver cells by certain carcinogens, and exerts promotional effects, and may even be a complete carcinogen.

The mechanisms by which CD diet exerts its promoting and/or carcinogenic effects are not

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known. But the possible mechanisms by which the CD diet modifies the induction of liver cancer implicate the following.

i) DAN hypomethylation[2)3)9)]

ii) Induction of liver proliferation[10-12)]

iii) Alteration in carcinogen metabolism[13)14)]

iv) Increased membrane lipid peroxidation[15-18)]

v) Depressed immune surveillance[5)6)]

Shinozuka et al[15)] demonstrated that the CD diet with a high-fat content exerted a stronger promoting action than diet with a low-fat content.

Since it is known that dietary fats, both in quantity and quality, play important roles in the genesis of cancer of many organs[19)20)], it is interesting to examine whether dietary fat composition in the CD diet modifies the promoting efficacy of experimental diet.

Metabolic alterations of phospholipids induced by choline deficiency led to the structural and fuctional changes of cell membranes[1)]. Membrane peroxidation may be one manifestation of the diet induced changes in membrane phospholipids[21)]. Peroxidation is believed to cause the loss of activity of membrane bound enzymes(eg. glucose 6-phosphatase(E.C. 3.1.3.9)[22)]).

Glutathione S-transferase(E.C. 2.5.1.18) plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic substances with reduced glutathione[23)24)].

In the present study, we investigated the effects of dietary fats and 2-acetylaminofluorene(2-AAF) injection on microsomal lipid peroxidation, microsomal glucose 6-phosphatase and cytosolic glutathione S-transferase activities in the choline deficient rat liver.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats(50~60g body wt.) were housed in plexiglas cages and were exposed to light during 07 : 00~19 : 00 hour daily.

They were fed the diets containing 15% beef tallow or 15% corn oil with vitamin fortification mixture(75.0g choline chloride/kg mixture, ICN, Cleveland, Ohio) or choline free vitamine mixture for 10 weeks.

At 3th and 5th weeks, 2-AAF(50mg/kg body wt.)was injected twice each week (1st and 3rd day of the week) intraperitonially. Total 2-AAF injection was four times. After 5 weeks from the last injection, animals were decapitated.

Dietary composition and experimental designs are shown in Table 1, Table 2, and Fig. 1.

Preparation of Microsome and Cytosol

At 11th week after feeding, animals were deca-

Table 1. Experimental design

Group	Sub group	Diet composition		Treatment
B(P/S ; 0.08) Beef tallow	B	Diet B	Vitamin fortification mix.	
	B -AAF			AAF [1]
	BCD	Diet B	Choline free Vitamin mix.	
	BCD-AFF			AAF
C (P/S ; 4.00) Corn oil	C	Deit C	Vitamin fortification mix.	
	C -AAF			AAF
	CCD	Diet C	Choline free Vitamin mix.	
	CCD-AAF			AAF

1) 50mg of 2-acetylaminofluorene in poly(ethylene glycol) 300/kg of body weight, intraperitoneal injection

Table 2. Composition of experimental diet (/100g diet)

Group	Diet B		Diet C	
ingradient	B	BCD	C	CCD
Corn starch	54.7	54.7	54.7	54.7
Casein	20.0	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0	5.0
Beef tallow	15.0	15.0	–	–
Corn oil	–	–	15.0	15.0
Vitamin mix.(1)	1.0	–	1.0	–
Vitamin mix. w/o choline(2)	–	1.0	–	1.0
Salt mix.(3)	4.0	4.0	4.0	4.0
DL-methione	0.3	0.3	0.3	0.3
P/S ratio(4)	0.08		4.00	

(1) Nutritional Biochemicals, ICN Life Science Group, Cleveland. Ohio. Vitamin mixture is composed of : Vit. A Acetate(500,000 IU per g) 1.8g, Vit. D conc. (850,000 IU per g) 1.125g,α-Tocopherol (250 IU per g) 22.0g, Ascorbic acid 45.0g, Inositol 5.0g, Choline Chloride 75.0g, Menadione 2.25g, p-Aminobenzoic acid 5.0g, Niacin 4.25g, Riboflavin 1.0g, Pyridoxine hydrochloride 1.0g, Calcium Pantothenate 3.0g, Biotin 0.02g, Folic acid 0.09g, Vitamin B-12 0.00135g and Dextrose to 1 kg.

(2) Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio. Vitamin mixture without choline chloride

(3) Composition of Salt mixture, g/Kg mixture. $CaPHO_4$ 500g, NaCl 74g, K_2SO_4 52G, Potassium Citrate Monohydrate 220g, MgO 24g, Manganous Carbonate(43–48% Mn) 3.5g, Ferric Citrate(16–17% Fe) 6.0g, Zinc Carbonate 1.6g, Cupric Car bornate(53–55% Cu) 0.3, KIO_3 0.01g, Chromium Potassium Sulfate 0.55g, Na_2SeO_3 $5H_2O$ 0.01g, sucrose finely powered 118.0g

(4) P/S ratio : Polyunsaturated/Saturated fatty acid ratio.

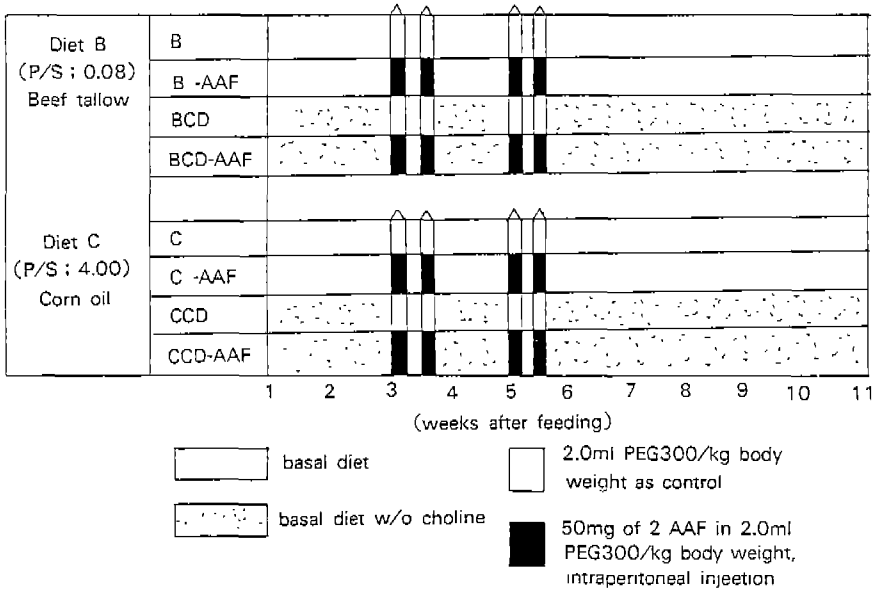

Fig. 1. Experimental design.

pitated after 12 hour fasting. Liver was homogenized in tris-HCI buffer (pH 7.4), centrifugated at 12,000xg, 4°C for 20 minutes, and the supernatant was centrifugated again at 105,000xg, 4°C for 1 hour to obtain cytosolic upper fraction and the lower microsomal fraction. Microsome was resuspended in tris-HCI buffer and frozen with liquid nitrogen and stored at −20°C until using.

Biochemical Assay

-Microsomal lipid peroxides were determined by TBA method[25].

-Microsomal glucose 6-phosphatase (G6Pase) (E.C. 3.1.3.9) activities were determined by method of Baginski et al[26].

-Cytosolic glutathione S-transferase(GST) (E.C. 2.5.1.18) activities were determined by method of Habig et al[27].

Table 3. Effects of 2-AAF and choline deficiency on the liver wt./body wt. in rats fed different dietary fats

Group		(Liver wt./body wt.)×100
B (P/S ; 0.08)	B	$2.646 \pm 0.06_b$
Beef talllow	B -AAF	$2.577 \pm 0.08_b$
	BCD	$2.939 \pm 0.11_a$
	BCD-AAF	$2.963 \pm 0.07_a$
C (P/S ; 4.00)	C	$3.133 \pm 0.09_a$
Corn oil	C -AAF	$3.162 \pm 0.08_a$
	CCD	$3.017 \pm 0.10_a$
	CCD-AAF	$2.960 \pm 0.04_a$

Mean ± SE

Means with the same letter are not significantly different at $\alpha=0.05$ level by Duncan's multiple range test.

Average numbers of samples analyzed in duplicates are 8.

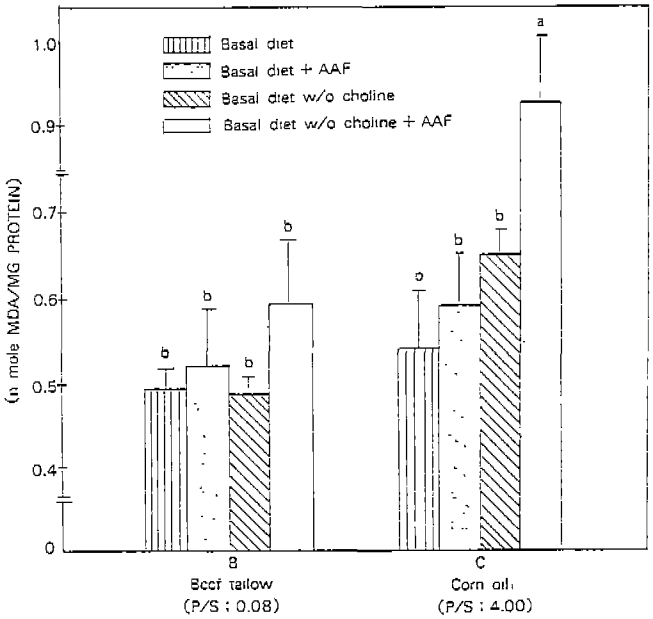

Fig. 2. Effects of 2-AAF and choline deficiency on the hepatic microsimal lipid peroxide values in rats fed different dietary fats.

Mean ± SE

Means with the same letter are not significantly different at $\alpha=0.05$ level by Duncan's multiple range test.

Average numbers of samples analyzed in duplicates are 5.

RESULTS AND DISCUSSION

As shown in Table 3, there was no significant difference between corn oil groups in liver/body wt.ratios. But in beef tallow groups, ratios were increased by CD diet. Generally, ratios in beef tallow groups were lower than in corn oil groups.

It is known that glucose 6-phosphatase(G6Pase) (E.C. 3.1.3.9) activities were decreased in neoplastic nodule of liver[28] and associated to lipid peroxidation. Wills[22] reported that induction of the formation of lipid peroxide in suspensions of liver microsomal preparations by incubation with ascorbate or NADPH, or by treatment with ionizng radiation, led to a marked decrease of the activities of G6Pase, and led conclusion that the loss of G6Pase activities resulting from peroxidation was a consequence of loss of membrane structure essential for the activities of enzyme.

In present study, there were significant increase of G6Pase activities in beef tallow groups(Fig. 3) compaired to corn oil groups, and MDA contents(Fig. 2) in beef tallow groups tended to be decrease compaired to corn oil groups, but not significantly different. In corn oil groups, G6Pase activities tended to be decreased and MDA contents tended to be increased by CD diet. Especially, MDA contents were increased significantly in CCD-AAF group compaired to other groups. So lipid peroxidation was increased and G6Pase activities tended to be decreased by 2-AAF and CD diet. These results were supported by the report[15] in which conjugated dienes were increased by CD

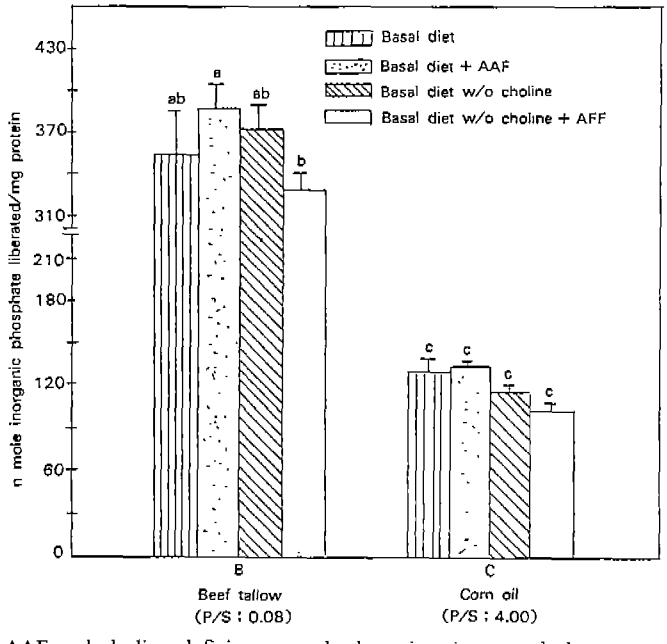

Fig. 3. Effects of 2-AAF and choline deficiency on the hepatic microsomal glucose 6-phosphatase activities in rats fed different dietary fats.
Mean ± SE
Means with the same letter are not significantly different at $\alpha=0.05$ level by Duncan's multiple range test.
Average numbers of samples analyzed in duplicates are 5.

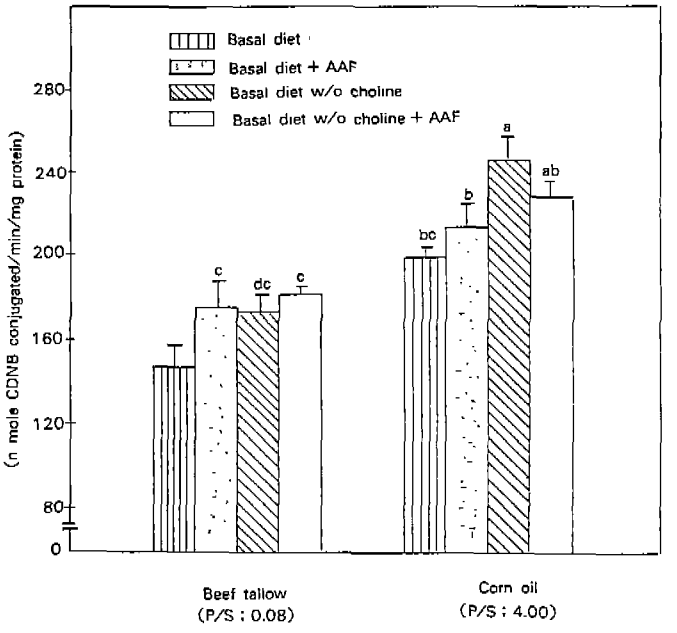

Fig. 4. Effects of 2-AAF and choline deficiency on the hepatic cytosolic glucose S-transferase activities in rats fed different dietary fats.
Mean ± SE
Means with the same letter are not significantly different at $\alpha=0.05$ level by Duncan's multiple range test.
Average numbers of samples analyzed in duplicates are 7.

diet.

Glutathione S-transferase(GST) plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic substances with reduced glutathione[24]. In basal diet groups, GST activities were increased by 2-AAF in beef tallow group, but not significant in corn oil group(Figure 4). These results were supported by the report[29] in which GST activities were increased by carcinogens such as 2-AAF. But in other report[30], GST activities were significantly increased by 2-AAF in soybean oil group, but not in beef tallow group. It was also reported that GST activities were increased significantly by high dosage of 2-AAF(100mg/kg body wt.) administration in beef tallow and corn oil group[31]. Increase of GST activities by 2-AAF in basal diet groups may have an effect on the metabolism of 2-AAF. In beef tallow groups, GST activities were increased by 2-AAF in basal groups, but not significantly different in choline deficient groups.

GST activities tended to be increased by CD diet in both oil groups. Especially in corn oil group, there were significant increase of enzyme activities by CD diet. These results were supported by the report[32] in which increased GST activities were examined 5 weeks after feeding a methionine-choline deficient diet. But Murry et al[33] demonstrated that the CD diet produced a relatively uniform decrease in GST activities in male liver to 37-59% of CS(choline-supplement)-control, and said that the enzyme activities were changed through out the tumor differentiation process. From these studies, we led the conclusion that although, GST activities might be increased in carcinogenesis in general, the degree of tumor differentiation might

be an important determinant of the enzyme activities.

In this study, 2-AAF and CD diet increased lipid peroxidation in corn oil group, so the role of 2-AAF and CD diet in lipid peroxidation was more important in corn oil group than in beef tallow group. G6Pase activities tended to be decreased by 2-AAF in choline deficient groups (but not significantly different) and in corn oil groups, the enzyme activites were decreased significantly in all subgroups compared to beef tallow groups. GST activities were increased by 2-AAF in beef tallow groups and CD diet in corn oil groups, and might defence against carcinogen metabolism and lipid peroxidation.

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2-Acetylaminofluorene과 Choline결핍이 서로 다른 지방을 섭취한 쥐 간의 지질 과산화 반응 및 Glucose 6-phosphatase, Glutathione S-transferase활성도에 미치는 영향

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국문초록

발암물질인 2-acetylaminofluorene(2-AAF)과 Choline 결핍이 서로 다른 지방을 섭취한 쥐간의 지질과산물, Glucose 6-phosphatase (G6Pase)와 Glutathione S-transferase(GST) 활성도에 미치는 영향을 연구 하였다. 식이 지방은 쇠기름과 옥수수유를 사용하였으며 각 식이 지방군을 Choline 결핍군과 대조군으로 나누어 실험하였다. 식이섭취 3주와 5주째, 2-AAF 처리군과 비처리군으로 나누어 처리군에 매주 2회씩 총 4회 2-AAF를 주사 한 뒤 식이섭취 10주 후 동물을 희생 시켰다.

Microsome의 지질과산화물 함량은 2-AAF와 Choline결핍(CD)식이에 의해 옥수수유 군에서 증가하여 지질과산화 반응에 있어 2-AAF와 CD식이의 역할이 쇠기름을 섭취 하였을때보다 옥수수유를 섭취한 경우 더 중요함을 알 수 있었다. Microsome의 G6Pase활성은 유의적이지는 않으나 2-AAF와 CD식이에 의해 감소하는 경향을 보였으며 옥수수유의 섭취에 의해서는 유의적으로 감소하였다. GST활성은 식이지방에 따라 2-AFF나 CD식이에 의해 증가하였으며 이때 증가된 GST는 발암물질의 대사와 지질과산화물 형성에 대해 방어 작용을 한것으로 보인다.