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Dibenzoylmethane Altered Adipose Mass, Serum Lipid and Sex Steroid Hormone in Female Mice

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ABSTRACT

Dibenzoylmethane (DBM), a β-diketone structural analogue of curcumin, has been reported to exhibit anti-tumorigenic and chemopreventive activities in mouse mammary tumorigenesis. Our previous study indicated that DBM modulates estrogenic action both *in vitro* and *in vivo*. In this report, the potential role of DBM as modulator of lipid metabolism in two mouse models was investigated. Several biomarkers and intermediate metabolites related to lipid consumption were measured. The results indicated that the parametrial fat pad weight, the level of triglyceride in both CD-1 and Sencar mice fed with 1% DBM, was remarkably reduced. In addition, the estradiol level, uterus and mammary gland weights were significantly decreased in CD-1 and young Sencar mice at 55 days of age (during the estrous phase of estrous cycle). The reduction of both estradiol level and triglyceride in serum would explain the inhibitory effect of DBM on mammary tumorigenesis as a modulator in dietary lipid metabolism.

Key words: dibenzoylmethane, lipid metabolism, triglyceride, estradiol, mammary tumorigenesis

INTRODUCTION

Dibenzoylmethane (DBM), a β-diketone structural analogue of curcumin, has been reported to exhibit anti-tumorigenic and chemopreventive activities[(1-7)]. It modulates the Phase I/Phase II metabolic systems, induces apoptosis in various cancer cells, and exerts beneficial effects for the ischemic diseases as a metal-chelator[(1,3,5,8)]. Dibenzoylmethane has been recently reported to exhibit chemopreventive activities in mammary tumorigenesis. Particularly, dietary DBM inhibits the incidence of 7,12-dimethylbenzanthracene (DMBA)-induced mouse mammary tumorigenesis and decreases the number of breast tumors per mouse as well as the formation of DMBA-DNA adducts in mammary glands[(2)]. *In vitro* competitive estrogen receptor binding studies showed direct binding between DBM and estrogen receptor *in vitro*[(9)]. Further *in vivo* proliferation studies implicated the potential role of DBM as an anti-estrogenic agent[(2)]. In molecular level, we demonstrated that DBM strongly inhibited the E_2-induced proliferation in both the human breast cancer cell line MCF-7 and the mouse model, reduced the expression of *bcl-2*, *c-myc*, *Ha-ras* and, *hTERT* and acted as an anti-estrogenic agent by attenuating the ER-ERE binding within the regulatory regions of these oncogenes[(10)].

Dietary lipid has been the major focus in the search for dietary causes of breast cancer. In 1970, a report indicated that the occurrence of breast cancer in U.S. whites was six times higher than that in Asians[(11)]. This large variation in risk was not due to the genetic differences, but dietary factors. However, a relatively higher rate of breast cancer incidence among Japanese who have migrated to the United States was observed[(12)]. Epidemiological studies indicated that Asian women at low risk for breast cancer have been consistently shown to have lower urinary and blood levels of estrogens than Caucasian women at high risk[(13)]. Strong support for a role of post-menopausal estrogens and risk of breast cancer indicated that post-menopausal women who subsequently developed breast cancer showed a 15% higher mean concentration of serum estradiol than women who did not[(14)]. In

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1999, a meta-analysis of dietary fat intervention studies by Wu *et. al*. indicated that reducing fat consumption to below 20% of calories would reduce the risk of breast cancer and dietary lipid reduction could result in lower serum estradiol levels[15]. This is a substantial evidence to support the potential of breast cancer prevention through dietary factors, especially lipid consumption. In spite of that, the relationship between estradiol level and lipid consumption has not been elucidated, but is crucial to explain the way dietary lipid modulates mammary carcinogenesis.

Our previous study indicated that DBM would modulate the estrogenic action as well as serum estradiol level found *in vivo*, and the potential role of DBM as a modulator of lipid metabolism was investigated. These results would provide some clues to explain the relationship among lipid, estrogen, and mammary tumorigenesis through understanding the mechanisms of DBM on lipid metabolism in animal models. In our studies, several biomarkers and intermediate metabolites related to lipid consumption were measured. These included parametrial fat pad weight, progesterone, cholesterol, triglyceride and fatty acid profile in serum. In addition, weekly food and water consumptions were recorded and compared. The information should provide substantial evidence to evaluate the potential role of DBM as a modulator in dietary lipid metabolism as well as its relation with estrogen-dependent mammary carcinogenesis.

MATERIALS AND METHODS

I. *Animals and Treatment*

Dibenzoylmethane (DBM) was purchased from Aldrich (Milwaukee, WI, U.S.A.). AIN-76A diet and 1% DBM in AIN-76A diet were purchased from Research Diets, Inc. (New Brunswick, NJ, U.S.A.). Female CD-1 mice (23 days old) or female Sencar mice purchased from Charles River Breeding Laboratories (Kingston, NY, U.S.A.) were given the AIN76A diet or 1% DBM in the AIN76A diet for several weeks. All experiments were conducted in the animal facility at Rutgers University (Busch Campus, Piscataway, NJ, U.S.A.) according to the NIH guideline. The mice were housed (ten mice per cage) in a room with controlled temperature (72 ± 2°F) and humidity (40 ± 10%). All experiments were done according to the animal protocol approved by the Rutgers University Animal Care and Use Committee. During the period of experiment, weekly water, food consumption, and body weight of mice were recorded. At the end of experiment, all mice were fasted overnight, sacrificed and parametrial fat pad weights were measured. Determination of estrous cycle was followed by the procedures recorded in reference[2]. The blood samples were collected and the serum was prepared by centrifugation of the collected blood and stored at -80°C. Concentrations of progesterone, cholesterol, triglyceride, HDL and estradiol were assayed by commercially available kits from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Oxford Biomedical Research Inc. (Oxford, MI, U.S.A.). Fatty acid profiles were determined by gas chromatography.

II. *Measurement of Hormone Levels in Serum*

Determination of the serum estradiol and progesterone concentrations was done by enzyme-linked immunosorbent assay (ELISA). The serum was extracted with ethyl ether (or petroleum ether for progesterone assay) and the concentrated residue was dissolved in extraction buffer provided by a commercial kit. The samples and enzyme conjugate (estradiol-horse radish peroxidase) were added to a microplate with the corresponding antibody pre-coated on each well and the mixture was incubated at room temperature for 1 h. During this time, competition for the limited binding sites should take place. After washing for 3 times with wash buffer to remove unbound material, the quantity of bound enzyme conjugate was determined by adding the substrate (3,3', 5,5'-tetramethylbenzidine and hydrogen peroxide). An optimal color was developed after incubation for 30 min and the absorbance reading of each well at 650 nm was measured by a microplate reader. The calculation of hormone concentration in samples was performed according to the manual, using a standard curve.

III. *Measurement of Lipid Metabolites in Serum*

The Sigma diagnostics triglyceride reagent was used for the measurement of the triglyceride levels. Briefly, the triglycerides in the sample were hydrolyzed to glycerol and fatty acids with lipoprotein lipase (Reagent B). After a series of coupled enzymatic reactions catalyzed by glycerol kinase, glycerol phosphate oxidase and peroxidase as well as appropriate substrates (Reagent A), a quinone imine was produced. The absorbance of both standard and samples at 540 nm was measured and the total glycerol content was calculated. For the determination of true triglycerides, the concentration of endogenous glycerol, measured by using reagent containing no lipase (Reagent A), was subtracted from the total glycerol content determined previously.

To measure the quantity of cholesterol in serum, a commercial diagnostic reagent containing esterase, enzymes and substrates for colorimetric determination was added to the samples. After 10 min of incubation at room temperature, the absorbance reading of both standard and samples at 500 nm was determined and the concentration of cholesterol in serum sample was calculated.

For the determination of high density lipoprotein (HDL) level in serum, a precipitation procedure was used to separate the HDL from low density lipoprotein (LDL). Firstly, a reagent containing phosphotungstic acid and magnesium chloride ($PTA/MgCl_2$) was added to the

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sample to precipitate LDL fraction in the serum. After centrifugation at 3000 rpm, the supernatant containing HDL was subjected to the regular method for cholesterol determination as described previously. The level of LDL cholesterol could be calculated from the known concentrations of total cholesterol, HDL cholesterol and triglycerides by using an equation (LDL = Total cholesterol – HDL cholesterol – Triglycerides/5), provided that triglycerides are less than 400 mg/dL.

IV. *Determination of Fatty Acid Profile in Serum*

To gain insight into the effect of DBM on fatty acid compositions, the ratio of saturated to unsaturated fatty acids, and ω3/ω6 PUFA, the profile of fatty acids were was determined by gas chromatography. Fatty acids were extracted from serum with hexane and derivatized with 14% trifluoroborane in methanol. The methyl esters of fatty acids were analyzed by a Siemens Sichromat gas chromatograph fitted with an automatic injection system and a hydrogen flame ionization detector. A SP-2330 capillary column (30 m × 0.25 mm × 0.2 μm film thickness) from Supelco was used. The oven temperature was programmed from 70°C to 180°C at a rate of 25°C/min and held at 180°C for 30 min and then increased to 220°C for 3 min. The flow rate of the helium carrier gas was 1.2 mL/min. The injection port and detector temperatures were 250°C and 260°C, respectively. The autosampler was programmed to rinse the 10 μL syringe, once with sample, pumped three times to remove air bubbles, and then inject 5 μL of sample with a split ratio of about 10 : 1. The syringe was then rinsed five times with solvent. A Perkin-Elmer model LCI-100 integrator was used for the integration of peak areas and the concentration of each fatty acid methyl ester was calculated using standard curves from each reference.

RESULTS AND DISCUSSION

I. *Biological Effects of Dietary DBM in the Treatment of CD-1 and Sencar Mice*

The weekly body weight, food and water consumption of both CD-1 and Sencar mice were recorded and summarized in Table 1 and Table 2, respectively. CD-1 and Sencar mice were used in our previous study on the inhibitory effects of DBM on skin and mammary carcinogenesis. In the case of CD-1 mice, the body weight showed no change between the control diet

Table 1. Effect of oral administration of 1% DBM diet on body weight, food and water consumption in CD-1 mice

Group	Week 1	Week 3	Week 4
	Body weight (g/mouse)		
Control AIN 76A diet	12.2 ± 0.2	21.3 ± 0.3	23.0 ± 0.3
1% DBM in control diet	12.8 ± 0.2	19.4 ± 0.4	23.0 ± 0.4
	Food consumption (g/mouse/day)		
Control AIN 76A diet		3.3 ± 0.1	3.1 ± 0.1
1% DBM in control diet		4.2 ± 0.2*	3.8 ± 0.1*
	Water consumption (mL/mouse/day)		
Control AIN 76A diet		4.6 ± 0.3	4.8 ± 0.3
1% DBM in control diet		7.3 ± 0.4*	9.1 ± 0.6*

Female CD-1 mice were fed AIN 76A diet or 1% DBM in the AIN 76A diet starting at 23 days of age and ending at 55 days of age. Body weight, food consumption and water consumption were recorded weekly. Data are expressed as the mean ± S.E.

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

Table 2. Effect of oral administration of 1% DBM diet on body weight, food and water consumption in Sencar mice

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	Body weight (g/mouse)					
Control AIN 76A diet	28.2 ± 0.4	28.0 ± 0.4	30.4 ± 0.5	31.6 ± 0.5	31.9 ± 0.6	32.9 ± 0.6
1% DBM in control diet	28.1 ± 0.5	29.3 ± 0.5	31.2 ± 0.5	32.5 ± 0.5	32.7 ± 0.6	33.3 ± 0.6
	Food consumption (g/mouse/day)					
Control AIN 76A diet	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.2	3.9 ± 0.2	
1% DBM in control diet	3.8 ± 0.1	4.2 ± 0.2	4.4 ± 0.1*	4.0 ± 0.0	4.3 ± 0.2	
	Water consumption (mL/mouse/day)					
Control AIN 76A diet	6.4 ± 0.3	5.9 ± 0.2	6.3 ± 0.4	6.2 ± 0.4	5.0 ± 0.5	
1% DBM in control diet	9.6 ± 0.8*	9.2 ± 1.2*	10.6 ± 0.8*	9.6 ± 0.7*	12.3 ± 1.3*	

Female Sencar mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 26 days of age and ending after 5 weeks. Body weight, food consumption and water consumption were recorded weekly. Data are expressed as the mean ± S.E.

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

and the 1% DBM group after four weeks of administration. Interestingly, the CD-1 mice that were fed with 1% DBM diet consumed more food (average of 4.0 ± 0.2 compared with 3.2 ± 0.1 g/mouse/day for control diet mice) and water (average of 8.2 ± 0.5 compared with 4.7 ± 0.3 mL/mouse/day for control diet mice) during week 3 and week 4. The 1% DBM group showed significant reduction ($p < 0.05$) in parametrial fat pad weight, uterus weight, while the liver weight was significantly increased ($p < 0.05$) at the end of the experiment (Table 3).

In the case of Sencar mice, the body weight showed no change between the control diet and 1% DBM diet groups after five weeks of administration. Similarly with the CD-1 mice, the mice fed with 1% DBM diet consumed more food (average of 4.1 ± 0.1 compared with 3.7 ± 0.1 g/mouse/day for control diet mice) and water (average of 10.3 ± 1.0 compared with 6.0 ± 0.4 mL/mouse/day for control diet mice). In addition, the 1% DBM diet group showed significant reduction ($p < 0.05$) in parametrial fat pad weight, mammary gland weight, but not in uterus weight ($p = 0.636$) while the liver weight significantly increased ($p < 0.05$) at the end of the experiment (Table 4). The unchanged body weight during the short-term treatment in both strains of mice suggested low toxicity for DBM.

II. *Serum Concentration of Hormones and Lipids in CD-1 and Sencar Mice*

The hormone and lipid levels in serum of both CD-1 and Sencar mice were examined and compared. As shown in Table 3, the concentration of estradiol in 1% DBM group (46.4 ± 5.4 pg/mL) was significantly reduced to 40% that of the control diet group (77.8 ± 11.6 pg/mL) in CD-1 mice. However, no significant difference in the progesterone level ($p = 0.688$) was observed. The effect of DBM on lipid metabolism was examined by measuring the concentrations of triglyceride, cholesterol and HDL. 1% DBM group showed marked reduction (approximately 50%) in both triglyceride total glycerol levels, compared with the control diet group. Inversely, the concentrations of cholesterol and HDL in 1% DBM group were more than two folds higher than those in the control group. These significant fat biomarker changes implied an important role of DBM on the modulation of lipid metabolism.

In the case of Sencar mice, the concentration of estradiol in the 1% DBM group (77.8 ± 7.0 pg/mL) was not significantly different ($p = 0.433$) from that of the control diet group (85.4 ± 6.1 pg/mL) (Table 4). Inversely, significant difference was observed in the progesterone level ($p < 0.005$). The effect of DBM on lipid metabolism was examined by measuring the concentrations of triglyceride, cholesterol and HDL. Similar to the case of CD-1 mice, the 1% DBM group showed marked reduction (approximately 50%) in both

Table 3. Biological effects of dietary DBM and measurements of serum concentrations of hormones and lipids in CD-1 mice

Biological effects or substances assayed	Control diet (n = 20)	1% DBM diet (n = 20)
Body weight (g)	23.0 ± 0.3	23.0 ± 0.4
Abdominal fat weight (g)	0.35 ± 0.04	0.26 ± 0.02*
Uterus weight (g)	0.28 ± 0.02	0.21 ± 0.01*
Liver weight (g)	1.26 ± 0.04	2.11 ± 0.01*
Estradiol (pg/mL)	77.8 ± 11.6	46.4 ± 5.4*
Cholesterol (mg/dL)	93.6 ± 7.0	244.0 ± 14.6*
HDL (mg/dL)	74.6 ± 0.2	150.6 ± 8.9*
Total glycerol (mg/dL)	82.1 ± 2.5	41.3 ± 3.5*
Triglyceride (mg/dL)	59.3 ± 3.4	24.2 ± 5.5*
Progesterone (ng/mL)	12.3 ± 1.3	13.4 ± 2.3

Female CD-1 mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 23 days of age and ending at 55 days of age. The mice were killed and the body, abdominal, uterus and liver weights were determined. Concentrations of serum hormones and lipids were measured by commercially available kits. Data are expressed as the mean ± S.E.

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

Table 4. Biological effects of dietary DBM and measurements of serum concentrations of hormones and lipids in Sencar mice

Biological effects or substances assayed	Control diet (n = 26)	1% DBM diet (n = 25)
Body weight (g)	32.9 ± 0.6	33.3 ± 0.6
Abdominal fat weight (g)	0.97 ± 0.07	0.76 ± 0.07*
Uterus weight (g)	0.16 ± 0.01	0.17 ± 0.02
Mammary gland weight (g)	0.15 ± 0.01	0.12 ± 0.01*
Liver weight (g)	1.91 ± 0.07	2.94 ± 0.04*
Estradiol (pg/mL)	85.4 ± 6.1	77.8 ± 7.0
Cholesterol (mg/dL)	107.0 ± 13.7	231.9 ± 7.5*
HDL (mg/dL)	72.4 ± 9.5	112.6 ± 7.3*
Total glycerol (mg/dL)	92.1 ± 13.1	58.3 ± 3.3
Triglyceride (mg/dL)	60.0 ± 11.6	24.2 ± 6.3*
Progesterone (ng/mL)	9.41 ± 0.92	4.36 ± 0.29*

Female Sencar mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 26 days of age and ending after 5 weeks. The mice were killed and the body, abdominal, uterus and liver weights were determined. Concentrations of serum hormones and lipids were measured by commercially available kits. Data are expressed as the mean ± S.E.

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

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Table 5. Biological effects of dietary DBM and measurements of serum concentrations of hormones and lipids in immature Sencar mice

Biological effects or substances assayed	Control diet (n = 15)	1% DBM diet (n = 17)
Body weight (g)	29.0 ± 1.0	28.0 ± 0.9
Abdominal fat weight (g)	0.58 ± 0.06	0.41 ± 0.05*
Uterus weight (g)	0.20 ± 0.14	0.15 ± 0.02
Mammary gland weight (g)	0.09 ± 0.01	0.07 ± 0.01*
Liver weight (g)	1.64 ± 0.06	2.51 ± 0.09*
Estradiol (pg/mL)	558.1 ± 100.0	221.7 ± 9.6*
Cholesterol (mg/dL)	111.3 ± 4.0	271.1 ± 12.6*
HDL (mg/dL)	73.0 ± 0.4	133.9 ± 2.4*
Total glycerol (mg/dL)	161.7 ± 3.4	66.2 ± 3.7*
Triglyceride (mg/dL)	119.5 ± 3.4	39.5 ± 3.6*
Progesterone (ng/mL)	5.84 ± 1.66	5.75 ± 0.88

Female Sencar mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 21 days of age until 50 days of age. The mice were killed and the body, abdominal, uterus and liver weights were determined. Concentrations of serum hormones and lipids were measured by commercially available kits. Data are expressed as the mean ± S.E.

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

Table 6. Effect of dietary DBM on the fatty acid profile in serum in CD-1 mice

Fatty acid	Concentration (μg/mL)	
	Control diet	DBM diet
Palmitic acid (C16 : 0)	129 ± 25	132 ± 69
Stearic acid (C18 : 0)	203 ± 25	221 ± 59
Oleic acid (C18 : 1)	27 ± 3	30 ± 13
Linoleic acid (C18 : 2)	6 ± 0.2	3 ± 0.6*
Linolenic acid (C18 : 3)	4 ± 0.8	6 ± 2
Arachidonic acid (C20 : 4)	6 ± 0.4	7 ± 1
Eicosapentaenoic acid (C20 : 5)	9 ± 1	12 ± 0.1
Total saturated fatty acids (S)[a]	332 ± 50	353 ± 128
Total unsaturated fatty acids (U)[b]	52 ± 5	58 ± 17
Total polyunsaturated fatty acids (PU)[c]	25 ± 2	28 ± 4
S/U	6.6	6.1
S/PU	13.3	12.6

Female CD-1 mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 23 days of age and ending at 55 days of age. The mice were killed and the serum was extracted with hexane. The fatty acids were derivatized with 14% trifluoroborane in methanol and analyzed by gas chromatography. The concentration of each fatty acid methyl ester was calculated using standard curves from each reference. Data are expressed as the mean ± S.E. from three determinations.

[a] C16 : 0, C18 : 0

[b] C18 : 1, C18 : 2, C18 : 3, C20 : 4, C20 : 5

[c] C18 : 2, C18 : 3, C20 : 4, C20 : 5

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

triglyceride total glycerol levels, compared with the control diet group. The concentrations of cholesterol and HDL in the 1% DBM group were more than two folds higher than those in the control group. In another set of experiment shown in Table 5, immature Sencar mice were fed either control or 1% DBM diet until 50 days of age, when the biological effects and biomarker changes were the same as observed in the matured Sencar mice as described previously, except the concentration of estradiol showed significant difference between the control diet group (558.1 ± 100.0 pg/mL) and 1% DBM group (221.7 ± 9.6 pg/mL).

From the breast cancer research, it has been found that dietary lipid reduction can lower serum estradiol level, which in turn, results in reducing the mitogenic effect of estradiol toward tumor promotion[15,16]. For the mechanisms involved, dietary lipid has been shown to modulate carcinogenic process through the regulation of prostaglandin biosynthesis[17]. The possible mechanisms include PGE_2-induced expression of estrogen biosynthesis, the inter-relation of metabolic pathways between lipid and estradiol. However, they all need to be further investigated and confirmed.

In this study, the remarkable reduction on parametrial fat pad weight and the level of triglyceride in both CD-1 and Sencar mice explained the potential role of DBM as a modulator of lipid metabolism. In addition, the decrease in estradiol level, uterus and mammary gland weights in CD-1 and Sencar mice at 55 days of age (during the estrous phase of estrous cycle) indicated that DBM would either interfere with the generation of estrogen through the lipid metabolic pathway in fat tissue or influence the metabolism of estradiol by the induction of enzymes, as implicated in the increased liver weight. The high cholesterol and HDL levels would lead to further investigation on the effect of DBM on the pathway involved in the generation of cholesterol, especially the reductase HMG-CoA, a rate-limiting enzyme in this process.

III. *Fatty Acid Profile in Serum*

In order to investigate the effect of DBM on fatty acid compositions in serum, the fatty acid profiles of both the control and 1% DBM groups in CD-1 and Sencar mice were examined and compared (Tables 6 and 7). For

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Table 7. Effect of dietary DBM on the fatty acid profile in serum in Sencar mice

Fatty acid	Concentration (μg/mL)	
	Control diet	DBM diet
Palmitic acid (C16 : 0)	86 ± 14	53 ± 13
Stearic acid (C18 : 0)	149 ± 6	139 ± 16
Oleic acid (C18 : 1)	23 ± 1	9 ± 1*
Linoleic acid (C18 : 2)	5 ± 0.1	3 ± 1
Linolenic acid (C18 : 3)	3 ± 0.1	3 ± 0.5
Arachidonic acid (C20 : 4)	6 ± 0.3	8 ± 1
Eicosapentaenoic acid (C20 : 5)	8 ± 2	8 ± 0.0
Total saturated fatty acids (S)[a]	235 ± 20	192 ± 29
Total unsaturated fatty acids (U)[b]	45 ± 4	31 ± 4
Total polyunsaturated fatty acids (PU)[c]	22 ± 3	22 ± 3
S/U	5.2	6.2
S/PU	10.7	8.7

Female Sencar mice were fed AIN 76A diet or 1% DBM in AIN 76A diet starting at 26 days of age and ending after 5 weeks. The mice were killed and the serum was extracted with hexane. The fatty acids were derivatized with 14% trifluoroborane in methanol and analyzed by gas chromatography. The concentration of each fatty acid methyl ester was calculated using standard curves from each reference. Data are expressed as the mean ± S.E from three determinations.

[a] C16 : 0, C18 : 0

[b] C18 : 1, C18 : 2, C18 : 3, C20 : 4, C20 : 5

[c] C18 : 2, C18 : 3, C20 : 4, C20 : 5

* Statistically different from the control AIN 76A diet group ($p < 0.05$) as determined by Student's *t*-test.

saturated, unsaturated and polyunsaturated fatty acids, no significant change was observed between the two groups in both mouse strains. The ratios of total saturated fatty acids to total unsaturated fatty acids (S/U), and total saturated fatty acid to total polyunsaturated fatty acids (S/PU) were not significantly different.

Both epidemiological data and experimental animal studies have indicated the relationship between dietary lipid intake as well as the type of lipid consumed with the incidence of cancers[18-20]. Several studies indicated that diets high in ω-6 polyunsaturated fatty acids (PUFA) are associated with increased breast cancer risk. On the contrary, consumption of oleic acid, a monounsaturated fatty acid found in olive oil, and ω-3 PUFAs, which are present in certain fish and fish oils, may reduce risk of breast cancer. In experimental animal carcinogenic and *in vitro* models, the development of tumors has been found to be enhanced by ω-6 PUFA, but inhibited by ω-3 PUFA[21,22]. Studies indicated that most eicosanoids,

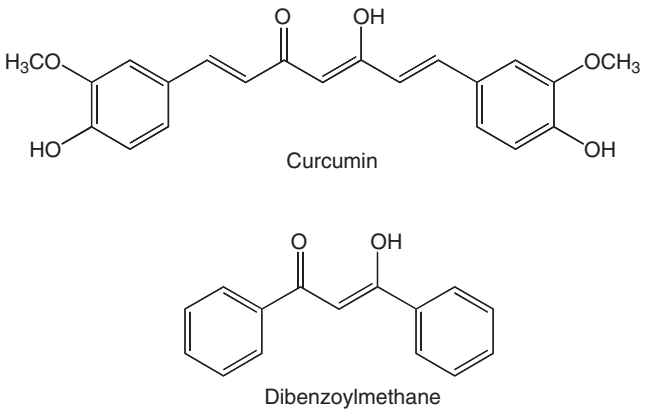

Figure 1. Structures of curcumin and dibenzoylmethane

metabolites of arachidonic acid (ω-6 PUFA), are biologically active and act as inflammatory inducers involved in the process of tumor promotion[23]. Nevertheless, our results ruled out the possibility that DBM inhibits the formation of mammary tumor by modulating the type of fatty acid formed in serum.

In summary, our previous study indicated that DBM would modulate the estrogenic action both *in vitro* and *in vivo*. In this report, the results indicated that the mice that were fed dietary DBM showed reduced parametrial fat pad weight and lower level of triglyceride. In addition, the estradiol level, uterus and mammary gland weights were significantly decreased in CD-1 and young Sencar mice at 55 days of age (during the estrous phase of estrous cycle), indicating that DBM would either interfere with the generation of estrogen through the lipid metabolic pathway or influence the metabolism of estradiol by the induction of enzymes, as implicated in the increased liver weight. The reduction of both estradiol level and triglyceride in serum would explain the inhibitory effect of DBM on mammary tumorigenesis in part from its role as a modulator in dietary lipid metabolism.

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