The effects of diacylglycerol oil on bone metabolism of C57BL/6J mice

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Directed by Professor Sung-Kil Lim

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ABSTRACT

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In epidemiologic and animal studies, high fat diets (HFD) are associated with lower bone mineral density (BMD) and higher risk of osteoporotic fractures. Meanwhile, consuming HFD containing diacylglycerol (DAG) instead of triacylglycerol (TAG) has metabolically beneficial effects such as reduction in body weight and abdominal fat. This study investigated the effects of a HFD containing DAG on oxidative stress and bone metabolism in mice.

Four-week-old male C57BL/6J mice (n = 39) were divided into three weight-matched groups based on diet type: a chow diet group, a HFD containing TAG (HFD-TAG) group, and a HFD containing DAG (HFD-DAG)

group. After 20 weeks of treatment, body composition and bone microstructure were analyzed using dual energy X-ray absorptiometry and micro-computed tomography. Oxidative stress in bone marrow cells was measured using dihydroethidium staining. RT-PCR and quantitative real-time PCR of bone marrow cells were performed to investigate the expression of transcription factors for osteogenesis or adipogenesis.

It was found that the HFD-DAG group had lower body weight, higher BMD, and superior microstructural parameters of bone when compared to the HFD-TAG group. Oxidative stress in bone marrow cells was lower in the HFD-DAG group than in the HFD-TAG group. The HFD-DAG group also showed increased expression of Runx2 and decreased expression of PPARγ in bone marrow cells compared to the HFD-TAG group. Osteocalcin levels measured in plasma were higher in the HFD-DAG group than in the HFD-TAG group.

In conclusion, when compared to HFD-TAG, HFD-DAG induces less oxidative stress in bone marrow and has beneficial effects on bone and bone metabolism of C57BL/6J mice.

Key words: high fat diet, diacylglycerol oil, oxidative stress, bone metabolism

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I. INTRODUCTION

It is well known that high levels of dietary fat are metabolically deleterious to health. Several cardiometabolic disorders such as obesity, hyperlipidemia, diabetes, and cardiovascular disease have been associated with high fat diets (HFD)[1]. Recently, epidemiologic studies have shown that HFD are also associated with lower bone mineral density (BMD) and higher risk of osteoporotic fractures[2,3]. Animal studies have demonstrated that HFD also have deleterious effects on bone and bone metabolism[4-7]. Although the mechanism of this deleterious effect is unclear, metabolic derangements such as dyslipidemia or oxidative stress resulting from excess fat intake may mediate the deleterious

effects of dietary fat on bone[8-12].

The majority of dietary fat consists of triacylglycerol (TAG), a glyceride composed of three fatty acid chains covalently bonded to a glycerol molecule through ester linkages. Diacylglycerol (DAG) is a glyceride consisting of two fatty acid chains esterified to a glycerol molecule. DAG oil is similar in taste, appearance, and fatty acid composition to conventional edible oils that predominantly consist of TAG. In fact, various edible oils contain small quantities of DAG, ranging from 0.8% in rapeseed oil to 9.5% in cottonseed oil[13]. DAG can exist in two isoforms, 1,2 (or 2,3)-DAG and 1,3-DAG. The majority of DAG in edible oils consists of 1,3-isoform DAG due to the migration of the acyl group in an equilibrium reaction. This 1,3-DAG is believed to have metabolically beneficial effects compared to TAG due to differences in absorption and metabolism[14,15]. As demonstrated in both animal and human studies, DAG oil appears to be more effective than TAG oil for preventing hyperlipidemia and excess body fat accumulation[16-25].

Despite previous animal studies that demonstrated the effects of HFD on bone and bone metabolism[4-7], no studies have investigated the effects of HFD containing DAG (HFD-DAG) on bone. I hypothesized that a HFD-DAG may affect bone and bone metabolism differently than a HFD containing TAG (HFD-TAG), due to the beneficial effects of DAG on fat mass, lipid metabolism and/or oxidative stress. To test this hypothesis, body composition, bone microstructure, biochemical parameters, and bone turnover markers were measured in mice fed

three different types of diet including a chow diet, a HFD-TAG, and a HFD-DAG for 20 weeks. Intracellular oxidative stress of bone marrow cells was also investigated, as was gene expression for osteogenic or adipogenic differentiation of bone marrow cells.

II. MATERIALS AND METHODS

1. Animals

Four-week-old male C57BL/6J mice were purchased from Central Laboratory Animal Inc. (Seoul, Korea). During the study the mice were housed in standard cages placed in a room at 21 ± 2℃, 50 ± 5% relative humidity, with a 12-hour light-dark cycle. The animals used in this study were treated in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996), as approved by the Institutional Animal Care and Use Committee of Yonsei University.

2. Experimental protocol

Mice were fed a standard laboratory diet for one week in order to acclimatize them. They were then divided into three weight-matched groups (n = 13 per group) of three different diet types, the chow diet group, the HFD-TAG group, and the HFD-DAG group. The chow diet was a purified diet based on the AIN-93G rodent diet, which contains 7 gm% (16 kcal%) of fat[26]. The chow diet had 4.0 kcal per gm. Both the HFD-TAG and HFD-DAG contained 24 gm% (45 kcal%) of fat. Although the amount of fat in the HFD-DAG was identical to that of the HFD-TAG, the fat content was different. Seventy grams of soybean oil and 130 gm of lard were used for the HFD-TAG,

while 150 gm of DAG oil and 50 gm of lard were used for the same amount of HFD-DAG. Both diets were 4.8 kcal per gm. Detailed ingredients of each diet are listed in Table 1. All mice were allowed free access to food and water throughout the 20 week test period. The mice were weighed every week from the beginning of the study. Every four weeks, mice were anesthetized using a mixture of Zoletil and Rompun, and body composition was measured. At the end of the experimental period, mice were sacrificed following 12-h fasts. Blood was drawn by cardiac puncture from mice into EDTA-coated tubes. Plasma was obtained by centrifuging the blood at 2000 x g for 15 min at 4℃. The plasma was then frozen and stored at -20℃ until analysis. Femora and tibiae from the hind legs were dissected out and bone marrow cells were immediately isolated from the tibiae. The Yonsei University Institutional Animal Care and Use Committee approved all animal experiment protocols.

Table 1. Composition of experimental diets

Formula	Chow (AIN-93G)		HFD-TAG		HFD-DAG	
	gm	kcal	gm	kcal	gm	kcal
Casein, lactic	200.0	800	200.0	800	200.0	800
L-Cystine	3.0	12	3.0	12	3.0	12
Corn starch	397.486	1590	165.9	664	165.9	664
Sucrose	100.0	400	73.5	294	73.5	294
Dextrose	132.0	528	97.6	390	97.6	390
Cellulose	50.0	0	50.0	0	50.0	0
Soybean Oil	70.0	630	70.0	630	0	0
Lard	0	0	130.0	1170	50.0	450
DAG	0	0	0	0	150.0	1350
t-Butylhydroquinone	0.014	0	0.014	0	0.014	0
AIN-93 Mineral Mix	35.0	0	35.0	0	35.0	0
AIN-93 Vitamin Mix	10.0	40	10.0	40	10.0	40
Choline Bitartrate	2.5	0	2.5	0	2.5	0
Total	1000.0	4000	837.514	4000	837.514	4000

HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol.

3. Body composition and bone microstructure

Body composition (lean soft tissue mass, fat mass, bone mineral content, and bone mineral density) of the mice was measured using dual X-ray absorptiometry (DXA) (QDR-4500A, Hologic, Waltham, MA, USA). The instrument was calibrated before scanning sessions using a phantom with known BMD, according to the manufacturer's guidelines. Body composition excluding the skull was assessed every four weeks from the fourth week of experimental period. All scans were performed with the animals positioned prone and spread, with tape attached to each limb on the platform. At the end of the study, the microarchitectures of the femora and the fifth lumbar vertebrae from sacrificed mice were analyzed using micro-computed tomography (micro-CT) scanning (SMX-90CT, Shimadzu, Kyoto, Japan). The cortical bone and trabecular bone were separated manually on each slice by a cursor line. The three dimensional structure was analyzed using the TRI 3D-BON (RACTOC System Engineering Co., Tokyo, Japan) program. In this study, the morphometric parameters calculated for both skeletal sites included bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, mm^{-1}), and trabecular separation (Tb.Sp, μm). Femoral images were also evaluated for cortical thickness (Ct.Th) and cortical cross-sectional area (CSa).

4. Total RNA isolation, RT-PCR, and quantitative real-time RT-PCR

The total RNA of tibial bone marrow cells (n = 5 for each group) was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and then reverse transcribed into cDNA using M-MuLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's recommendations. For reverse transcription-polymerase chain reaction (RT-PCR) analysis, one microliter of the RT product was used as a template for PCR amplification of Runx2, osterix, PPARγ, and β-actin using the following cycling conditions: 40 sec at 94℃, 40 sec at the respective annealing temperature and 1 min at 72℃ over the respective number of cycles presented in Table 2. The measured mRNA levels were normalized to the β-actin mRNA levels. The sequence information of oligonucleotide primers for RT-PCR is also presented in Table 2. PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining. Real-time RT-PCR quantitation of Runx2 and PPARγ mRNA was performed in an iCycler IQ detection system using SYBR® Green I as a double-strand DNA-specific binding dye. GAPDH primers were included in the reaction as internal controls. PCR runs consisted of an initial denaturation step at 94℃ for 5 min, 40 cycles consisting of 15 sec at 94℃, 30 sec at the respective annealing temperature (Table 2) and 30 sec at 72℃. The sequence information of oligonucleotide primers for quantitative real time RT-PCR is also presented in Table 2.

Table 2. Sequences of primers and PCR reaction parameters used in RT-PCR and quantitative real-time RT-PCR

RT-PCR			
Target gene	Primer sequence	AT (℃)	Cycles
β-actin	(F) 5’-GATAGCGATATCGCTGCGCT-3’	55	20
	(R) 5’-GCTCATTGCCGATAGTGATGACCT-3’		
Runx2	(F) 5’-CCGCACGACAACCGCACCAT-3’	55	28
	(R) 5’-CGCTCCGGCCCACAAATCTC-3’		
Osterix	(F) 5’-CACATCCCTGGTGCGGCAA-3’	55	30
	(R) 5’-CCGGGTGTGAGTGCGCACAT-3’		
PPARγ	(F) 5’-CACTTCACAAGAAATTACCAT-3’	58	30
	(R) 5’-GAAGGACTTTATGTATGAGTC-3’		
Quantitative real-time RT-PCR			
Target gene	Primer sequence	AT (℃)	Cycles
GAPDH	(F) 5’-AATGTGTCCGTCGTGGATCTG-3’	55	40
	(R) 5’-CAACCTGGTCCTCAGTGTAGC-3’		
Runx2	(F) 5’-CAGATGACATCCCCATCCATCC-3’	55	40
	(R) 5’-AAGTCAGAGGTGGCAGTGTC-3’		
PPARγ	(F) 5’-GCCCTGGCAAAGCATTTGTATG-3’	58	40
	(R) 5’-CCCATCATTAAGGAATTCATGTCGTAG-3’		

RT-PCR, reverse transcription-polymerase chain reaction; AT, annealing temperature.

5. Intracellular oxidative stress of bone marrow cells

Intracellular oxidative stress of marrow cells was measured with the dihydroethidium (DHE) (Sigma, St Louis, MO, USA). DHE is rapidly oxidized by reactive oxygen species (ROS) to yield red fluorescent ethidium which intercalates with the cell's DNA so that bright red nuclei can be visualized in the fluorescence microscope. Bone marrow cells were isolated from the tibiae and pooled for each group of mice (n = 5 per each group). Pooled marrow cells resuspended in phosphate buffered saline (PBS) were stained with 10 μM DHE for 30 min at 37°C. The cells were then centrifuged for 10 min at 1500 rpm, and washed twice with PBS in order to remove the extracellular fluorescent indicators. Finally, they were resuspended in PBS. DHE fluorescence was visualized by confocal microscopy (LSM 510, Zeiss, Gottingen, Germany). Flow cytometric analysis was used to quantify DHE fluorescence. Fluorescence intensity resulting from DHE oxidation was measured using a FACS Calibur (Becton Dickinson, San Jose, CA, USA). Signals were obtained using a 575 ± 13 nm band pass filter (FL-2 channel) for ethidium.

6. Biochemical analyses

Plasma concentrations of glucose, total cholesterol, triglyceride, and HDL-cholesterol were measured using an ADVIA 1650 Chemistry system (Siemens, Tarrytown, NY, USA). Plasma insulin levels were measured with an enzyme-

linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden), and the inter-assay and intra-assay variations were 4.8 ± 1.3% and 2.6 ± 0.6%, respectively. Mouse cross-linked N-telopeptide of type I collagen (NTX) (Cusabio Biotech Co., Wuhan, China) and mouse osteocalcin (Biomedical Technologies Inc., Stoughton, MA, USA) levels were also measured using a commercially available ELISA kit.

7. Statistical analyses

Statistical analyses were performed using SPSS 15 (SPSS, Inc., Chicago, IL, USA). Data were reported as mean ± standard error (S.E.). Comparisons across groups were performed by a one-way analysis of variance (ANOVA) with a post-hoc Duncan's test. A repeated measures ANOVA was performed to test for significant differences in the means of body weight, percent fat mass, and BMD over time between groups. P <0.05 was considered statistically significant.

III. RESULTS

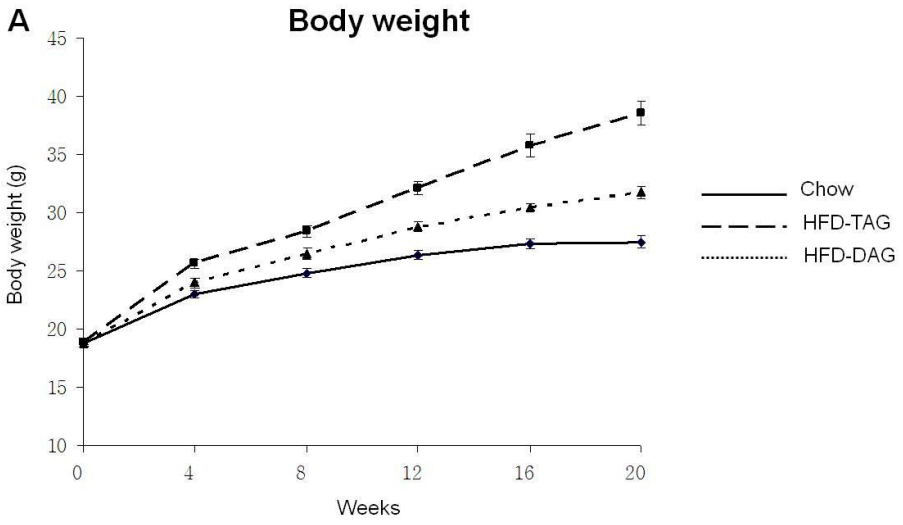
1. Body weight and composition

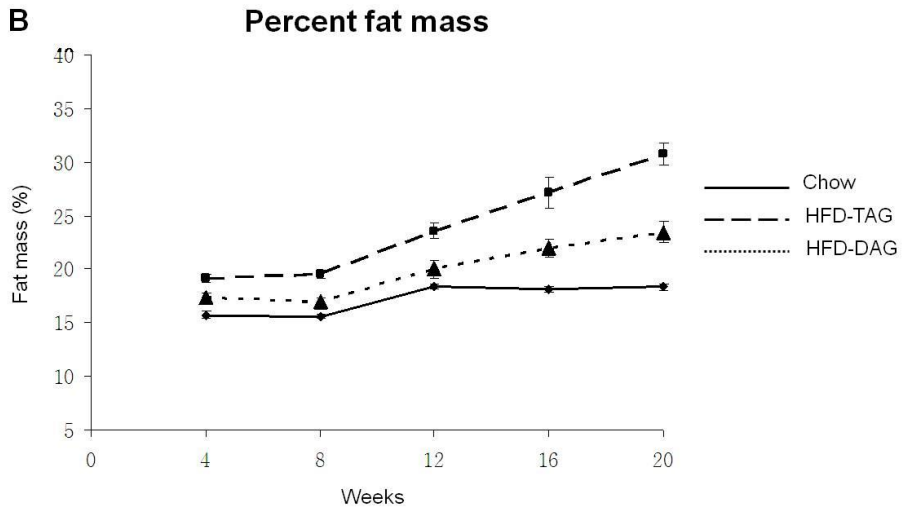
The body weights and compositions measured at each time point for the three diet groups are shown in Figure 1. The body weight and percent fat mass of mice fed the HFD containing either TAG or DAG were greater than those of mice fed the chow diet at each time point. Among mice that were fed the HFD, the HFD-TAG group had greater body weight and percent fat mass than the HFD-DAG group. The difference between the groups increased from the beginning of the experimental period. At the end of the 20-week study, mice in the HFD-TAG group had significantly higher body weight and fat mass than those in the HFD-DAG ($P < 0.0001$).

2. BMD and bone microstructure

BMD measured by DXA at each time point is presented in Figure 1. BMD was higher in mice fed the HFD containing either TAG or DAG than in those fed the chow diet, which is probably due to the greater body weight of mice fed the HFD. Among mice fed the HFD, however, mice fed the HFD-DAG had a greater BMD than those fed the HFD-TAG despite having lower body weight. Micro-CT was conducted after sacrificing the animals to analyze the microstructures of cortical and trabecular bone. The femur of the posterior limb and the fifth lumbar vertebra were used for micro-CT analysis.

Compared to mice fed the HFD-TAG, mice fed the HFD-DAG had increased cortical thickness and cross-sectional area in their femoral bone (Figure 2A and Figure 3), and increased trabecular thickness in their vertebrae (Figure 2B and Figure 4) despite having lower body weight.


A
Body weight
Body weight (g)
45
40
35
30
25
20
15
10
0
4
8
12
16
20
Weeks
Chow
HFD-TAG
HFD-DAG


B
Percent fat mass
Fat mass (%)
40
35
30
25
20
15
10
5
0
4
8
12
16
20
Weeks
Chow
HFD-TAG
HFD-DAG

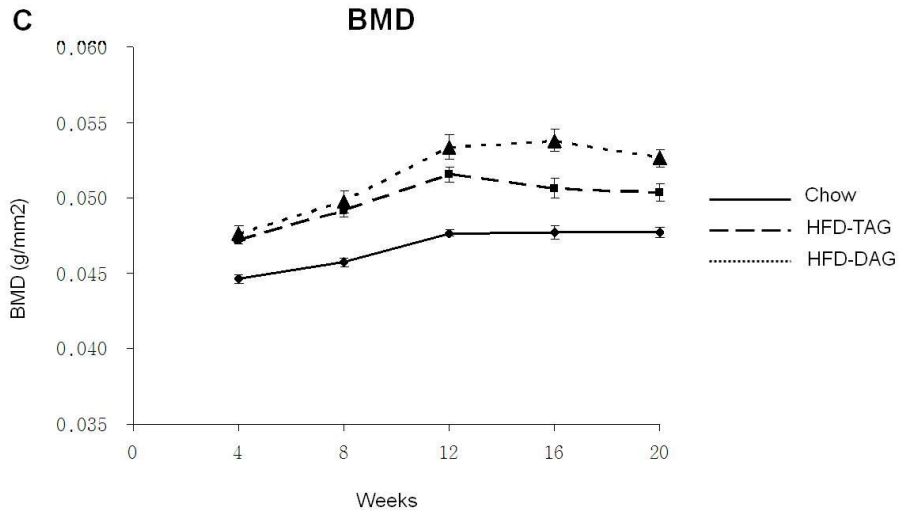
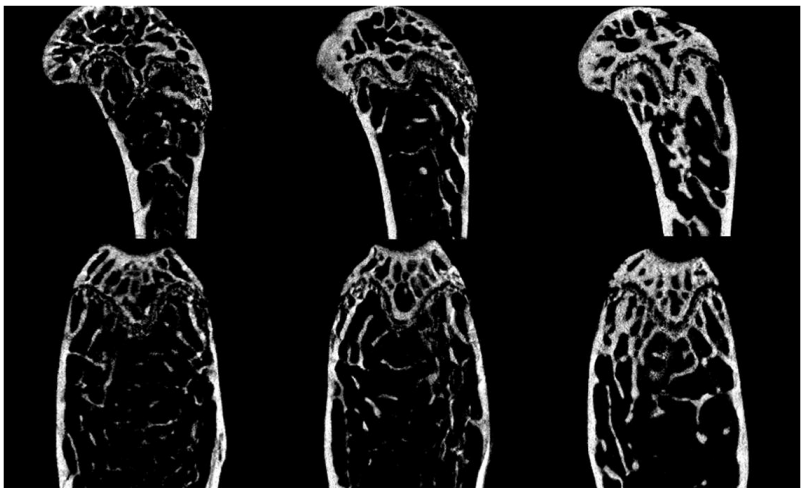


Figure 1. Changes in body weight, percent fat mass, and BMD in mice. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; BMD, bone mineral density. Repeated measures ANOVA, P <0.0001 for body weight (A), percent fat mass (B), and BMD (C).

A



B

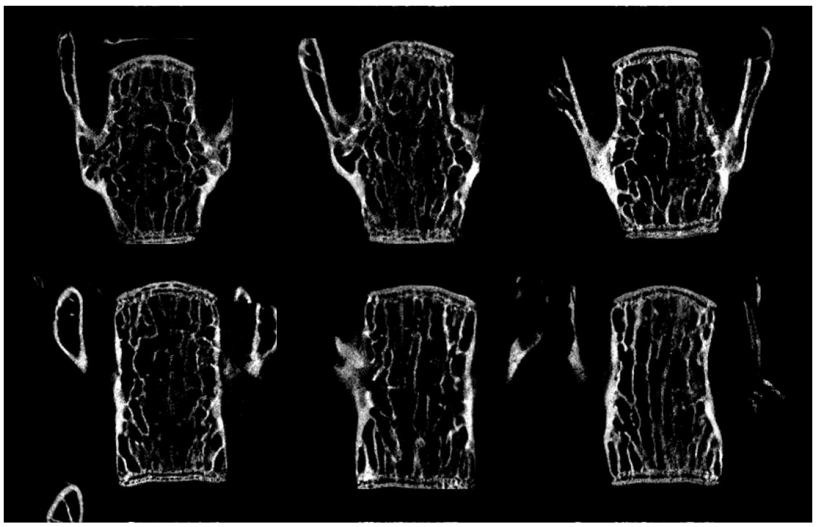


Figure 2. Microstructure images of femoral bones (A) and fifth lumbar vertebrae (B) in mice. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol.

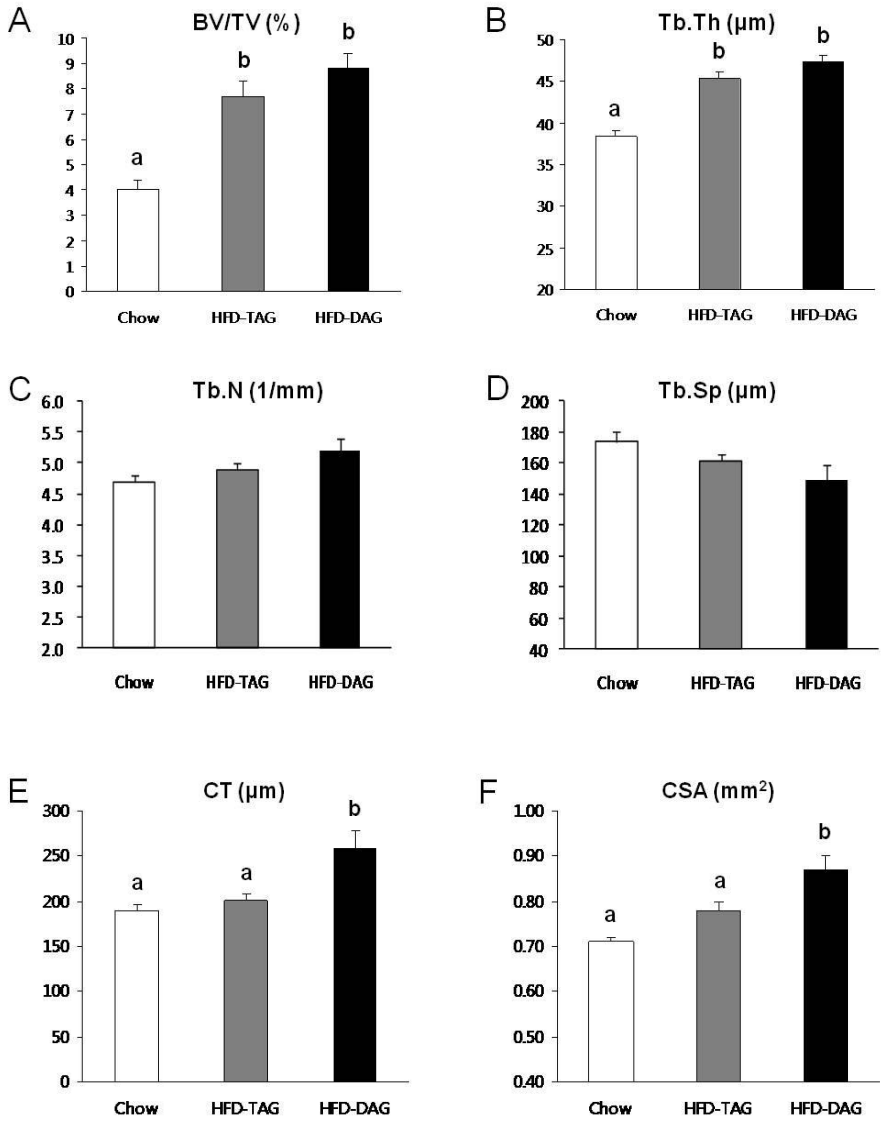


Figure 3. Microstructure parameters of femoral bones in mice. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; BV, bone volume; TV, tissue volume; Tb.Th, trabecular

thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Ct, cortical thickness; CSa, cross-sectional area. Bars labeled with different letters are significantly different (P <0.05) (“a” versus “b” indicates a statistically significant difference, “a” versus “a” or “b” versus “b” means no significant difference).

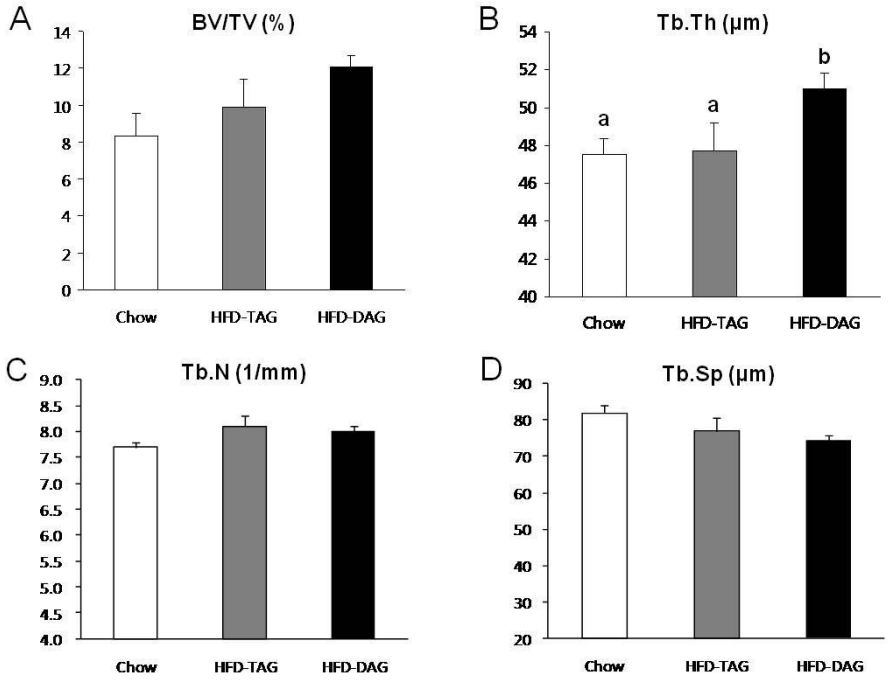


Figure 4. Microstructure parameters of the trabecular bone of the vertebrae in mice. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; BV, bone volume; TV, tissue volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation. Bars labeled with different letters are significantly different (P <0.05) (“a” versus “b” indicates a statistically significant difference, “a” versus “a” means no significant difference).

3. Expression of differentiation marker genes

Bone marrow cells were isolated from mice tibiae (n = 5) to determine the effect of diet on the cell differentiation. The gene expression levels of transcription factors for osteogenic and adipogenic lineage in bone marrow cells are presented in Figure 5. RT-PCR and quantitative real time RT-PCR results show that Runx2 and osterix, the transcription factors for osteoblastic differentiation, were significantly up-regulated in mice fed the HFD-DAG compared with those fed the HFD-TAG. In contrast, the adipocyte-specific transcription factor, PPARγ, was up-regulated in mice fed the HFD-TAG.

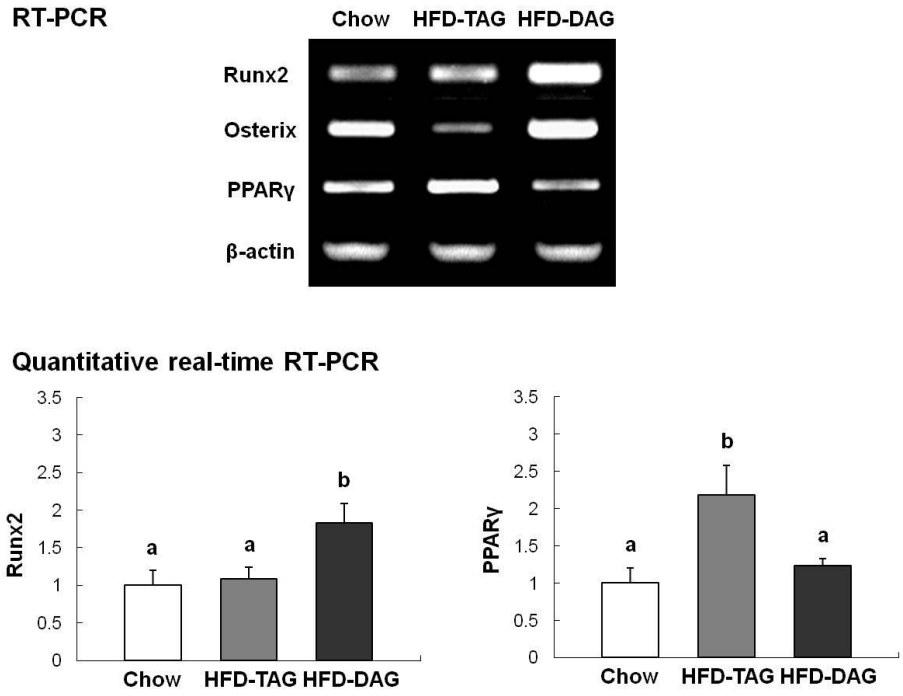


Figure 5. Gene expression levels in bone marrow cells isolated from mice tibiae. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; RT-PCR, reverse transcription-polymerase chain reaction. Bars labeled with different letters are significantly different (P <0.05) (“a” versus “b” indicates a statistically significant difference, “a” versus “a” means no significant difference).

4. Biochemical markers of bone turnover

Bone turnover markers measured in plasma are presented in Figure 6. Mice fed the HFD-DAG had significantly higher plasma concentrations of osteocalcin, a marker of bone formation, than mice fed the HFD-TAG. The plasma concentration of NTX, a marker of bone resorption, was not significantly different between the groups.

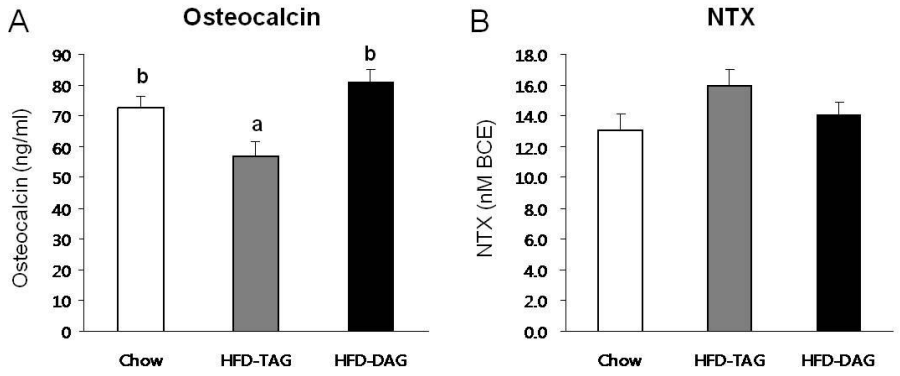


Figure 6. Bone turnover markers measured in the plasma of mice. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; NTX, N-telopeptide. Bars labeled with different letters are significantly different (P <0.05) ("a" versus "b" indicates a statistically significant difference, "b" versus "b" means no significant difference).

5. Intracellular oxidative stress of bone marrow cells

Intracellular oxidative stress in bone marrow cells isolated from mice tibiae was measured using DHE. As shown in the confocal microscopic findings (Figure 7), more cells stained with DHE were found in bone marrow cells from mice fed the HFD-TAG than in mice fed the HFD-DAG or chow diet. Quantitative analysis by flow cytometry also showed that mice fed the HFD-TAG had more bone marrow cells with oxidative stress than those fed the HFD-DAG or chow diet (Figure 8).

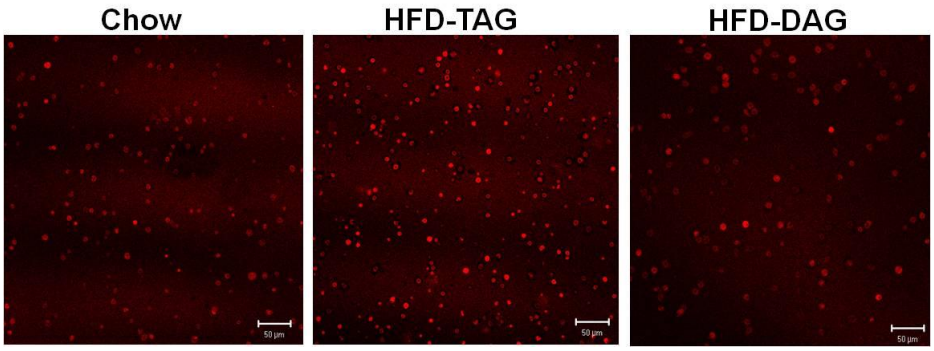


Figure 7. Confocal microscope images of bone marrow cells stained with dihydroethidium. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol.

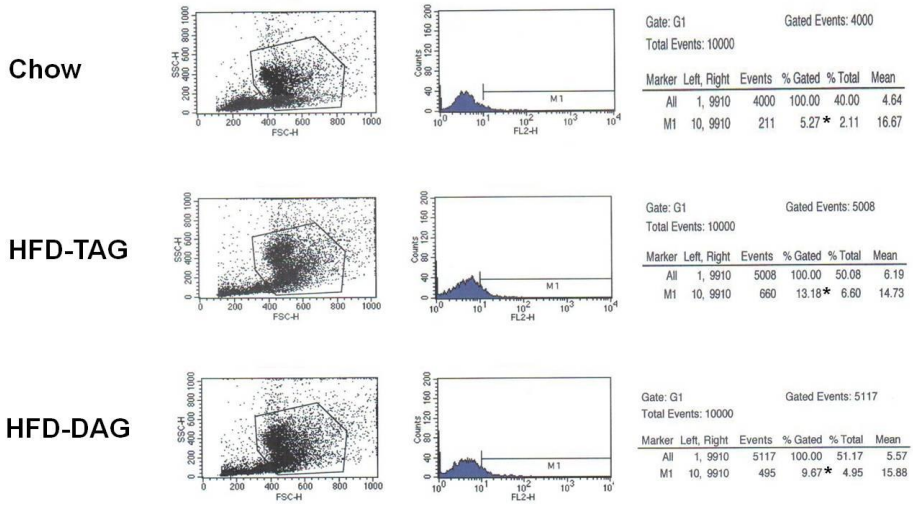


Figure 8. Quantitative analyses by flow cytometry in bone marrow cells stained with dihydroethidium. HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol. Numbers labeled with asterisk indicate percentage of gated cells.

6. Metabolic parameters

Biochemical parameters including fasting plasma glucose, insulin, and lipid profiles are presented in Table 3. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma glucose and insulin concentrations. Mice fed the HFD containing either TAG or DAG had higher fasting glucose, HOMA-IR, total cholesterol, and triglyceride than mice fed the chow diet. Among mice fed the HFD, however, metabolic derangement was more severe in the mice fed the HFD-TAG than in those fed the HFD-DAG.

Table 3. Comparison of metabolic parameters measured in the plasma of mice

	Chow diet	HFD-TAG	HFD-DAG	*P* value
Glucose (mg/dL)	81.5 ± 2.0^{a}	169.7 ± 4.7^{c}	106.2 ± 4.3^{b}	<0.001
Insulin (ng/mL)	0.6 ± 0.1^{a}	1.4 ± 0.2^{b}	0.9 ± 0.1^{a}	<0.001
HOMA-IR	2.8 ± 0.5^{a}	13.8 ± 2.0^{b}	5.4 ± 0.6^{a}	<0.001
Triglyceride (mg/dL)	77.3 ± 2.5^{a}	102.2 ± 4.0^{c}	91.1 ± 2.7^{b}	<0.001
Cholesterol (mg/dL)	113.5 ± 4.3^{a}	168.9 ± 5.3^{c}	130.0 ± 6.3^{b}	<0.001
HDL-cholesterol (mg/dL)	67.6 ± 2.5	61.9 ± 1.7	65.3 ± 1.7	0.149

HFD-TAG, high fat diet containing triacylglycerol; HFD-DAG, high fat diet containing diacylglycerol; HOMA-IR, homeostatic model assessment of insulin resistance. Means with different superscript letters within a row are significantly different (P <0.05) ("a" versus "b" or "a" versus "c" or "b" versus "c" indicates a statistically significant difference, "a" versus "a" means no significant difference).

IV. DISCUSSION

The results of this study show that mice fed a HFD-DAG had lower body weight, lower fat mass, and higher lean mass than mice fed a HFD-TAG, which is consistent with previous studies evaluating the metabolic effects of DAG. Biochemical tests from this study indicate that the HFD-DAG group had lower levels of plasma glucose, insulin, HOMA-IR, total cholesterol, and triglyceride than the HFD-TAG group. This study revealed that mice fed the HFD-DAG had greater BMD and superior bone microstructure compared to those fed the HFD-TAG. These findings suggest that the beneficial effects of DAG on bone might result from increased differentiation of bone marrow cells into an osteogenic rather than adipogenic lineage. The results also suggest that the difference between DAG and TAG on bone metabolism might be due to less oxidative stress and lipotoxicity in mice fed the HFD-DAG compared to those fed the HFD-TAG. However, mice fed the HFD-DAG also had greater BMD and superior bone microstructure compared to those fed the chow diet despite higher oxidative stress and lipotoxicity. Although there is no clear explanation for it, I speculate that higher body weight might have a positive effect on the bone metabolism in mice fed the HFD-DAG compared to those fed the chow diet.

Dietary fat intake can affect bone metabolism in several ways. Excess body fat accumulation resulting from a HFD has both positive and negative effects

on bone. The mechanical loading of accumulated fat mass can stimulate osteogenesis and estrogen produced in adipose tissue can also inhibit bone resorption by osteoclasts[27]. In contrast, adipocytes may have lipotoxic effects on osteoblasts, as shown in an experiment where adipocytes induced an inhibition of osteoblast proliferation in cocultures *in vitro*[28]. Adipocytokines secreted by adipose tissue can also affect bone metabolism in both positive and negative ways. For example, leptin inhibits bone formation through a central mechanism that involves the sympathetic nervous system[29,30]. However, peripheral administration of leptin increases cortical bone growth and differentiation of mesenchymal stem cell into osteoblasts rather than adipocytes[31,32], and reduces ovariectomy-induced bone loss in rats[33]. Adiponectin also has both positive and negative impacts on bone metabolism[34]. The overall effect of adipocytokines on bone remains unclear.

Recently, oxidative stress was suggested as an important mediator of HFD-induced bone loss[7]. It was shown that excess fat intake increases oxidative stress by overproducing reactive oxygen species (ROS) and decreasing antioxidant enzyme activity[35,36]. Oxidative stress has been suggested as a responsible factor for the development of osteoporosis. In previous clinical studies, oxidative stress had an inverse association with bone mass in humans[11,12,37,38]. *In vitro* and animal studies showed that oxidative stress can inhibit the differentiation of osteoblasts[39,40], while stimulating the formation and activation of osteoclasts[41,42]. Parhami et al. also suggested that oxidized

lipids play a role in bone metabolism[10,43-45]. They suggested that lipoproteins and lipids accumulate in bone and undergo oxidation, after which these oxidized lipids can inhibit the differentiation of preosteoblastic cells and marrow stromal cells (MSCs), as well as inducing the RANKL-dependent osteoclastic differentiation of marrow preosteoclasts. Parhami et al. speculated that the effects of oxidized lipids on bone cells may be mediated through direct interactions via receptor-mediated responses and/or through the generation of other inflammatory cytokines such as MCP-1, M-CSF and IL-6.

Although DAG is almost identical to TAG in terms of digestibility and caloric value, this study and previous studies found that DAG may be metabolically beneficial in terms of preventing hyperlipidemia and excess body fat accumulation[16-25]. While not fully understood, the underlying mechanism for this physiological difference between TAG and DAG is likely due to the difference in absorption and metabolism[14,15]. It appears that the position of the fatty acid on the glycerol skeleton is responsible for the metabolic difference of DAG and TAG[14,15]. After TAG is ingested in the form of dietary oil, it is hydrolyzed by lipase to produce free fatty acids and 2-monoacylglycerol in the small intestinal lumen, and these molecules are absorbed into intestinal cells. In intestinal cells, they are reconstituted to form TAG via the 2-monoacylglycerol pathway through the action of monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT). TAG is then incorporated into chylomicrons, which are released into

the intestinal lymph and poured into the bloodstream. The absorption pathway of 1,3-DAG in intestinal cells is different from that of TAG. In the small intestine, dietary DAG oil mainly in the form of 1,3-DAG is hydrolyzed to 1-monoacylglycerol and free fatty acids, which may be less readily resynthesized to TAG via the 2-monoacylglycerol pathway in the intestinal cells because 1-monoacylglycerol cannot be the substrate for both MGAT and DGAT. Instead, TAG is synthesized by the glycerol-3-phosphate pathway, which is less active than the 2-monoacylglycerol pathway. Moreover, compared with TAG oils, larger amounts of fatty acids may be released from digested DAG into the portal circulation rather than being incorporated into chylomicrons, and this hepatic exposure to fatty acids leads to greater β-oxidation by the liver. This metabolic difference of DAG from TAG likely leads to improvement in glucose and lipid metabolism, as shown in this study. Furthermore, the metabolically positive effect of DAG results in less lipotoxicity and/or oxidative stress in the body, which is probably beneficial for skeletal health.

This study has some limitations. First, bone histomorphometry was not conducted. Although bone formation and resorption markers were measured in the plasma, it is not clear how a HFD-DAG affects bone remodeling differently than a HFD-TAG or chow diet. Dynamic histomorphometry study might have provided a more definitive explanation. In addition, plasma concentrations of adipocytokines such as leptin and adiponectin were not

measured, and these adipocytokines might play a role in mediating a HFD and bone metabolism.

In summary, this study demonstrated that compared to TAG, DAG has a positive effect on bone metabolism in mice. The results of this study suggest that the beneficial effect of DAG on bone is probably due to less oxidative stress and lipotoxicity, which might increase the differentiation of bone marrow mesenchymal cells into osteogenic rather than adipogenic lineage. Further research is needed to elucidate the mechanism behind the beneficial effects of DAG on bone.

V. CONCLUSION

In this study, I have demonstrated that

1. Mice fed the HFD-DAG had lower body weight, lower fat mass, and higher lean mass than mice fed the HFD-TAG.

2. Mice fed the HFD-DAG had greater BMD and superior bone microstructure compared to those fed the HFD-TAG.

3. Mice fed the HFD-DAG had less oxidative stress in bone marrow cells than the mice fed the HFD-TAG.

4. Mice fed the HFD-DAG had increased expression of osteogenic differentiation marker genes in bone marrow cells compared to mice fed the HFD-TAG.

5. Mice fed the HFD-DAG had higher osteocalcin levels in plasma than mice fed the HFD-TAG.

6. Mice fed the HFD-DAG had lower levels of plasma glucose, insulin, HOMA-IR, total cholesterol, and triglyceride than mice fed the HFD-TAG group.

The results of this study indicated that compared to TAG, DAG has a positive effect on bone and bone metabolism in mice. Therefore, consuming a diet rich in DAG instead of TAG may be beneficial for preventing bone loss and managing osteoporotics taking western style high fat diet.

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ABSTRACT (IN KOREAN)

Diacylglycerol oil이 C57BL/6J 쥐의 골대사에 미치는 효과

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고지방식이의 섭취는 역학 연구 및 동물 실험에서 골밀도 감소 및 골다공증성 골절의 증가와 관련이 있다고 보고되어 왔다. 한편 고지방식이의 성분으로서 triacylglycerol (TAG) 대신 diacylglycerol (DAG)를 포함한 고지방식이의 섭취는 체중 및 복부 지방 감소 등 대사적으로 유익한 효과가 있는 것으로 보고되고 있다. 본 연구자는 DAG를 포함한 고지방식이의 섭취가 쥐에서 산화스트레스 및 골대사에 미치는 효과에 대해서 연구하고자 하였다.

생후 4주 된 수컷 C5BL/6J 쥐 39 마리를 식이의 차이에 따라서

세 군, 즉 정상 식이 군, TAG을 포함한 고지방식이 군 및 DAG을 포함한 고지방식이 군으로 나누었다. 식이 처치 후 주기적으로 이중 에너지 흡수법을 이용하여 체구성 및 골밀도를 측정하였고, 20주 후 미세 컴퓨터 단층촬영을 이용하여 뼈의 미세구조를 분석하였다. 쥐의 골수에서 채취한 세포를 대상으로 dihydroethidium 염색을 하여 골수 세포의 산화스트레스를 측정하였다. 또한 reverse transcription-polymerase chain reaction (RT-PCR)과 quantitative real-time RT-PCR를 이용하여 골수 세포에서 골 세포 또는 지방 세포로의 분화와 관련된 전사 인자의 표현을 분석하였다.

본 연구 결과 TAG을 포함한 고지방식이에 비하여 DAG을 포함한 고지방식이를 섭취한 쥐는 체중 감소뿐 아니라 골밀도 증가 및 골 미세구조의 개선을 보여주었다. 또한 TAG을 포함한 고지방식이에 비하여 DAG을 포함한 고지방식이를 섭취한 쥐의 골수 세포에서 산화스트레스는 더 낮았으며, Runx2의 발현은 증가되어 있었고 PPARγ의 발현은 감소되어 있었다. 혈청 osteocalcin의 농도는 TAG을 포함한 고지방식이에 비하여 DAG을 포함한 고지방식이를 투여한 쥐에서 더 높게 나타났다.

결론적으로 C57BL/6J 쥐에 DAG을 포함한 고지방식이를 투여한 경우 TAG을 포함한 고지방식이를 투여한 경우에 비하여 쥐 골수 세포의 산화스트레스는 더 낮았으며 골밀도, 골의 미세 구조 및 골

대사에는 더 유익한 효과를 보여주었다.

핵심되는 말: 고지방식이, diacylglycerol oil, 산화스트레스, 골대사