J. Exp. Biomed. Sci. 2012, 18(3): 237~243
pISSN : 1738-3226

Original Article

The Effect of Fat Diet on Inflammatory Markers and Blood Coagulation System in Rats

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This study was undertaken to know the effect of fat diet (for eight weeks) on changes of inflammatory markers [tumor necrosis factor (TNF-α) and prostaglandin E_2 (PGE_2)] and blood coagulation system [platelet aggregation function (PAF), prothrombin time (PT), activated partial thromboplastin time (aPTT)] in rats. Serum TNF-α, PGE_2, biochemical markers, PAF, PT, aPTT, and body weight were measured and compared between the control (normal diet-rats) and the fat group (fat diet-rats). The weights in the fat group were higher than those of the control group. TNF-α, PGE_2, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels were greater in the fat group compared with the control group. The degree of platelet aggregation was lower, whereas PT and aPTT levels were longer in the fat group than in the control group. These findings have shown that fat diet may cause inflammatory response, diabetes, liver and renal dysfunction, and disturbances of fibrinolysis and coagulation system.

Key Words: Fat diet, TNF-α, PGE_2, Platelet aggregation, Biochemical marker

INTRODUCTION

The development of a worldwide obesity pandemic has gained wide recognition. Approximately 30% of Korean is either overweight or obese. Obesity is a chronic disease affecting over a billion adults in a whole world. It is predicted that its prevalence will have doubled by the year 2030 and that the obesity epidemic is going to become the most serious health problem of our century (Gnacińska et al., 2009). Looking back on the time when the human beings had to spent awfully efforts to collect enough food for survival, there is no doubt that modern industrialization has succeeded at making many choices of calorie-abundant food easily available with little physical efforts. These circumstances may contribute to obesity and/or metabolic disorders. The metabolic syndrome clusters various metabolic abnormalities, including intra-abdominal obesity, impaired glucose tolerance, dyslipidemia, and hypertension. The ultimate importance of this cluster is to identify individuals at high risk of both type 2 diabetes and cardiovascular disease (Ritchie and Conell, 2007). With increasing numbers of patients suffering from obesity, the prevalence of complications resulting from the excess of adipose tissue is growing (Pudel and Ellrott, 2005; Reincke, 2006). Adipose tissue is an endocrine organ producing and secreting biologically active factors such as proinflammatory cytokines and chemokines (Olszannecka-Glinianowicz et al., 2011).

We speculate that fat diet induces obesity and can attribute to the formation of excessive adipose tissue, thereby may overexpress tumor necrosis factor (TNF-α) and lipid metabolites, prostaglandin E_2 (PGE_2). The present study was undertaken to know the effects of fat diet on TNF-α, PGE_2,

*Received: April 3, 2012 / Revised: July 1, 2012
Accepted: July 4, 2012
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biochemical indices, and coagulation system.

MATERIALS AND METHODS

Experimental animal and grouping

The Male Sprague-Dawley rats purchased from Joong-Ang Animal Company (Seoul, Korea) were six weeks of age. The rats were housed under pathogen-free conditions in enclosed filter-topped cages. Clean food and water were provided ad liditum. All of the rats were kept on a 12:12-hrs light/dark cycle at a temperature of 25℃ and humidity of 60%. After adaptation for one week, rats were divided into two groups: the control group (n=8) which was a normal diet and the fat diet group (n=9) which was a fat diet (containing 40% fat component). Each diet feed was each group for eight weeks. This study was approved by the Animal Ethics Committee of Catholic University of Pusan (No. Cup AEC 2011-01).

Sacrifice and blood collection

After eight weeks, all of rats were fasted for 24 hours and were anesthetized by ether and fixed on the Rat Operating Table (Dong Sew Science, Seoul, Korea). The abdominal cava was exposed by lower abdominal incision. 8 mL of blood was collected directly from the abdominal cava.

Analysis

Measurement of platelet aggregation. 4.5 mL of blood was infused into 3.2% sodium citrate-bottle. Platelet-rich plasma was centrifuged at 125 ×*g* for 10 min to remove the red blood cells, and the platelets were washed twice with washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 5.5 mM glucose, and 1 mM MEDTA, pH 6.9). The washed platelets were then re-suspended in suspension buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 0.49 mM $MgCl_2$, 5.5 mM glucose, 0.25% gelatin, pH 7.4) to a final concentration of 5 × 10^8/mL. All of the above procedures were carried out at 25℃ to avoid platelet aggregation on cooling. Washed platelets (10^8/mL) were preincubated for 3 min at 37℃ in the presence of 2 mM exogenous $CaCl_2$ and them stimulated with collagen (10 μL/mL) or thrombin (10 μl/mL) for 5 min. Aggregation was monitored using an aggregometer (Chrono-Log Corp., Havertown, PA, U.S.A.) at a constant stirring speed of 1,000 rpm. Each aggregation rate was evaluated as an increase in light transmission. The suspension buffer was used as the reference.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT)

PT and APTT in the plasma with 3.8% sodium citrate were measured using Human Clot Duo Plus (Human GmbH, Berlin, Germany).

Biochemical markers

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, glucose, and creatinine levels were measured in all rats. Biochemical markers in serum were analyzed by Autohumalyzer 9500 (Human Lab., Berlin, Germany).

Tumor necrosis factor-α (TNF-α)

ELISA method was applied for measuring TNF-α concentrations in the serum. Biotrak II microplate reader (Biochrom Ltd., Vienna, Austria) and Thermo Scientific rat TNF-α ELISA kit (Pierce Biotechnology, LA, USA) were used.

Prostaglandin E_2 (PGE_2)

The serum PGE_2 concentration was measured using an ELISA kit according to the manufacturer's instructions. The PGE_2 ELISA kit was purchased from R&D system (Minneapolis, MN, USA).

Data analysis and statistics

For analysis of all variables, unpaired *t*-test was applied for comparing any differences between the control and the fat group. Statistical significance was accepted with $P \leq 0.05$. All data were expressed as the mean ± standard deviation (SD).

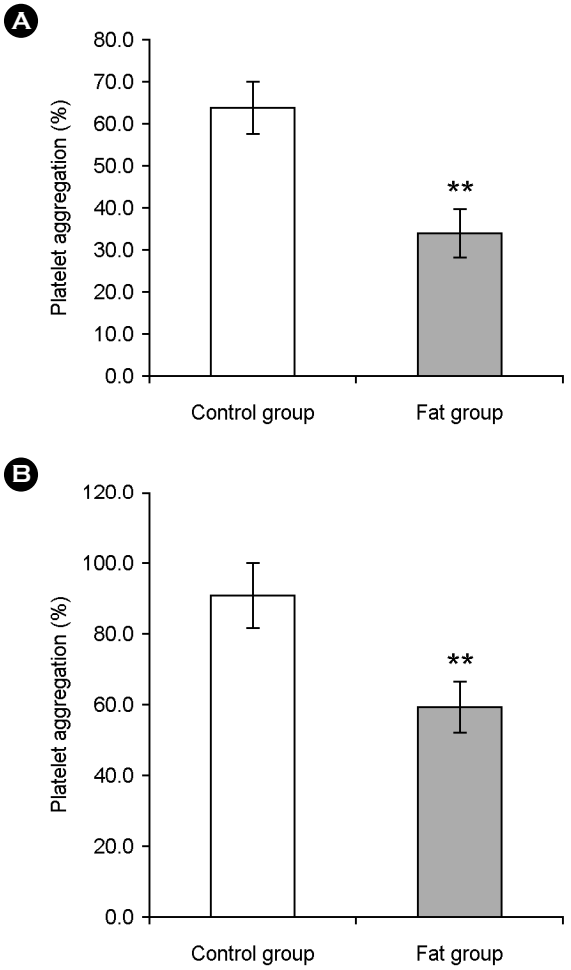

Fig. 1. Platelet aggregation with collagen (A) and thrombin (B) between the control and fat groups. The platelet aggregation in the fat group was lower than that of the control group (**, $P<0.01$).

RESULTS

Weights

At the point of purchase, there was significantly not different in the weights between the control (198.75 ± 8.37 g) and the fat group (184.67 ± 8.20 g). After 8 weeks of the experiment, the weights in the fat group (513.25 ± 12.70 g) were higher than those of the control group (432.75 ± 13.52 g) (P=0.02, Table 1).

Biochemical markers

Glucose, AST, ALT, and creatinine levels were higher in the fat group compared with the control group ($P<0.05$, Table 1). However, there was significantly not different in total cholesterol levels between the two groups ($P>0.05$, Table 1).

Table 1. Comparison of body weights and biochemical markers between the two groups

Variable	Group	
	Control	Fat
BE-BW (g, %)	198.75 ± 8.37 (100%)	184.67 ± 8.20 (100%)
AE-BW (g, %)	432.75 ± 13.52 (218.40%)	513.25 ± 12.70* (277.39%)
T-cholesterol (mg/dL)	53.80 ± 9.92	50.27 ± 10.40
Glucose (mg/dL)	121.85 ± 11.25	148.59 ± 9.36*
AST (IU/L)	58.00 ± 8.80	68.10 ± 10.43*
ALT (IU/L)	38.04 ± 3.04	45.81 ± 5.15*
Creatinine (mg/dL)	0.28 ± 0.01	0.33 ± 0.01*

Data were expressed as the mean ± SD.
*, $P<0.05$ (compared with the control group).
Abbreviation: BE, before experiment; BW, body weight; ED, experimental diet; AE, after experiment; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Platelet aggregation

Platelet aggregations by collagen and thrombin were lower in the fat group (58.29 ± 7.28% and 33.52 ± 5.60%, respectively) than in the control group (89.73 ± 9.41% and 63.60 ± 6.03%, respectively) (P=0.003 and P=0.002, respectively, Fig. 1A and B).

PT and aPTT

Both PT (29.07 ± 2.74 sec vs. 27.34 sec, P=0.04) and aPTT (59.45 ± 4.02 sec vs. 34.27 ± 1.60 sec, P=0.001) levels were longer in the fat group than in the control group (Fig. 2A and B).

TNF-α and PGE_2

Both TNF-α (49.08 ± 8.78 pg/mL vs. 149.92 ± 11.46 pg/mL, P=0.0001) and PGE_2 (584.19 ± 29.81 vs. 904.43 ± 8.13, P=0.001) levels were higher in the fat group than in the control group (Fig. 3 and 4).

DISCUSSION

In the present study, we confirmed that 8 weeks-fat diet can induce obesity as evidenced by increased body weight

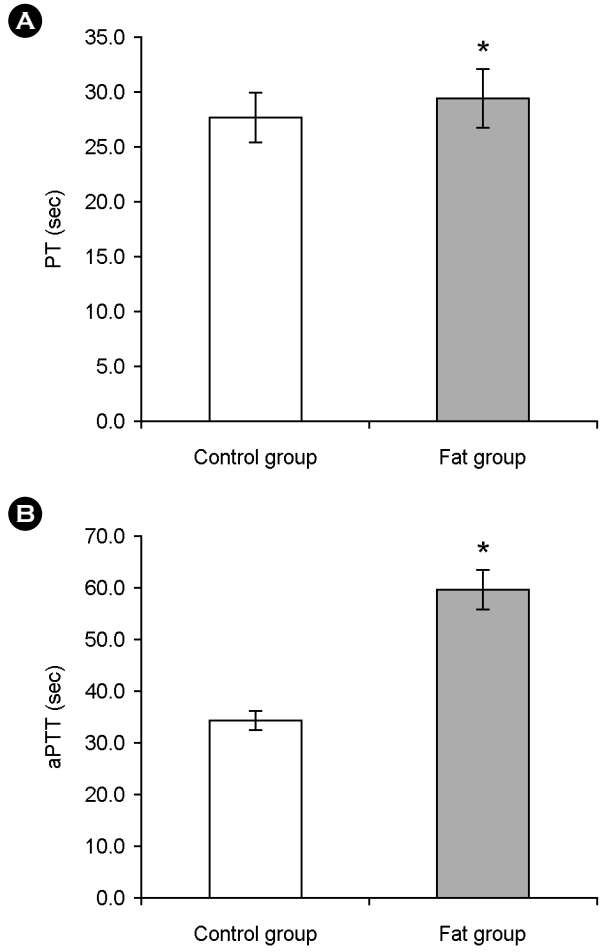

Fig. 2. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) between the control and fat groups. The PT and aPTT in the fat group were significantly longer than those of the control group (*, $P<0.05$).

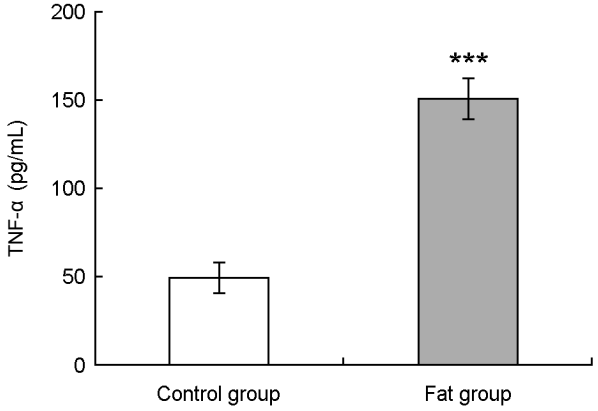

Fig. 3. Serum tumor necrosis factor-α (TNF-α) levels between the control and fat groups. The serum TNF-α level in the fat group was significantly higher than that of the control group (***, $P=0.0001$).

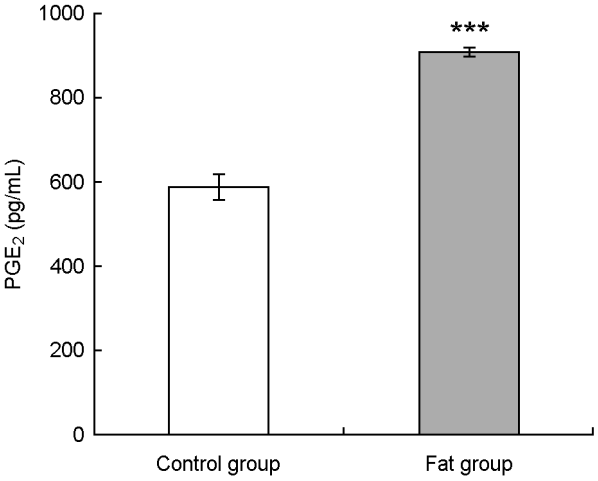

Fig. 4. Serum prostaglandin E_2 (PGE_2) levels between the control and fat groups. The serum PGE_2 level in the fat group was significantly higher than that of the control group (***, $P=0.001$).

in the fat group. An elevated body weight may lead to obesity, which can be implicated in the pathogenesis of insulin resistance, the excess of visceral adipose tissue and increased production of adipokines (Gnacińska et al., 2009). Obesity may contribute to metabolic syndrome such as hypertension, diabetes, dyslipidemia, hyperglycemia, hyperuricemia, and proinflammatory status. Furthermore, other consequences of obesity include heart failure, endocrine disorders, obstructive sleep apnea, restrictive ventilation disorder, fatty liver, and malignant tumors (Gnacińska et al., 2009). However, we didn't fat stain in this study, and thus further study should be need. The biochemical results in this study showed that 8 weeks-fat diet caused hyperglycemia, and the elevation of AST, ALT and creatinine levels although there was no difference in total cholesterol level between the two groups, suggesting that fat diet-induced obesity can be risk factors of diabetes, liver dysfunction, and renal disorder.

In obesity-related type 2 diabetes TNF-α levels are increased in adipose tissue (Hotamisligil et al., 1993; Xu et al., 2002). Previous studies have reported that the proinflammatory cytokine, TNF-α was overexpressed in obesity (Hotamisligil et al., 1993). TNF-α is multi-functional cytokine that can regulate many cellular and biological processes such as immune function, cell differentiation,

proliferation, apoptosis and energy metabolism. It is synthesized as a 26-kDa transmembrane monomer (mTNF-α) (Krigler et al., 1988) that undergoes proteolytic cleavage by the TNF-α converting enzyme to yield a 17-kDa soluble TNF-α molecule (sTNF-α) (Black, 1997). The role of TNF-α in the development of insulin resistance has been demonstrated in various animal obesity models (Śledziewski et al., 2003). The expression of TNF-α mRNA in adipose tissue increases in obesity and hyperinsulemia (Hotamisligil et al., 1995).

Our data also show fat diet-induced elevation of body weight, thereby glucose and TNF-α levels were increased. These findings indicate a close link between obesity, TNF-α, and hyperglycemia. Moreover, PGE_2, product of prostanoids, levels were elevated in the fat group of this study. PGE_2, which is formed from arachidonic acid by the prostaglandin synthesizing cyclooxygenase (COX) enzymes and prostaglandin synthase (Simmon et al., 2004), is involved in many diseases. During an inflammatory response, the level of PGE_2 production can change dramatically. While PGE_2 levels are generally very low in uninflamed tissues, they increase immediately in acute inflammation prior to the recruitment of leukocyte (Tilley et al., 2001). PGE_2 exhibits biphasic effects on bone formation, stimulating bone formation at low concentrations, but inhibiting it at high concentration (Raisz, 1999). COX-2 is highly expressed in osteoarthritis (OA) cartilage and is induced by various cytokines that are involved in destructive processes in OA cartilage, for example, IL-1 and TNF-α (Pelletier et al., 2001; Nieminen et al., 2005). PGE_2 mediates inflammation, tissue destruction, and pain in OA (Simmon et al., 2004). PGE_2 has been implicated in human obesity, in which increased circulating levels of PGE_2 have been observed (Fain et al., 2002). PGE_2 is a lipid mediator with effects in the central nerve system including activation of the hypothalamic-pituitary-adrenal (HPA) axis (Derijk and Berkenbosch, 1991) and febrile (Ushikubi, 1998). PGE_2 has also been shown to inhibit lipolysis in white adipose tissue and stimulate the secretion of leptin, suggesting that PGE_2 signaling is important for body weight homeostasis (Fain et al., 2000).

Finally, 8 weeks-fat diet in this study induces obesity, and thus fat diet can cause increase of TNF-α and PGE_2 levels. Fat diet-induced obesity is considered to be the cause of a joint elevation in TNF-α and PGE_2 levels that may contribute to the potential of the development of several diseases. On the other hand, lower platelet aggregation percent and longer PT and aPTT levels were observed in the fat group of this study, suggesting that fat diet-induced obesity adversely affects platelet function and coagulation system. We had been confused for these findings because obesity-induced elevation of TNF-α may lead to thrombogenesis, which can cause cerebral and cardiovascular disorders. Previous studies have shown that the proinflammatory mediators, especially TNF-α, can induce a procoagulant state by eliciting tissue factor production on the surface of vascular endothelium and monocyte, downregulating the protein C anticoagulant pathway and stimulating thrombin and fibrin formation (Esmon, 1999). However, some studies have suggested that TNF-α is a pleiotropic cytokine that exerts a large variety of biological effects on multiple cell types (Vassalli, 1992). TNF can interact with two distinct surface receptors with molecular weights of 55 kDa and 75 kDa, respectively (Tartaglia and Goeddel, 1992). The domains of these receptors are p55 (type I) and p75 (type II), respectively. van der Poll et al. (1996) demonstrated that p55 TNF receptor mediates TNF-induced stimulation of coagulation, fibrinolysis, neutrophil degranulation, and release of secretory phospholiphase A_2. van Hinsbergh et al. (1990) reported that TNF and other cytokines induce urokinase-type plasminogen activator (u-PA) production by human endothelial cells, that TNF can increases the degree of u-PA activation, and that a concomitant induction of u-PA and plasminogen activator inhibitor-1 (PAI-1) might represent an additional aspect of altered fibrinolytic properties of endothelial cells during inflammation. Cambien et al. (2003) also revealed that TNF-α decreases platelet activation and inhibits thrombin formation. PGE_2 also shows a biphasic, concentration-dependent effect on platelet aggregation. Even though low concentrations enhances platelet aggregation, higher concentrations inhibits it (Gresele et al., 1988; Thierauch and Prior, 1991; Vezza et al., 1993; Philipose et al., 2010). Ultimately, Fat diet-induced obesity lead to excessive production of TNF-α and PGE_2 levels, there by

cause decreased platelet aggregation and prolonged PT and aPTT levels.

In conclusion, Our data suggest that fat diet can induce obesity, metabolic syndrome, and cytokine overexpression, which may disturb coagulation and fibrinolysis systems.

Acknowledgements

This study was supported by the research funds from Catholic University of Pusan.

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