Food Sci. Biotechnol. Vol. 18, No. 1, pp. 197 ~ 201 (2009)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Less Weight Gain in Obese Rats by Feeding Biocellulose

Daeseok Han*, Tae-Cheol Song, Dong Woo Kim[1], Chang-Ho Lee, Young-Eon Kim, and In-Ho Kim
Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Korea
[1]Natural F&P Corp., Seoul 138-805, Korea

Abstract This study evaluated the effect of biocellulose and the diet formulation on reducing body weight gain of obese rats induced by high-fat diet for 10 weeks. Thirty male Sprague-Dawley rats were randomly assigned to high-fat diet group (OB-CON), high-fat diet group containing 5% biocellulose (OB-BIO), and high-fat diet group containing 5% dietary formulation (OB-DF). After feeding each diet for additional 10 weeks, body weight gains of OB-BIO and OB-DF groups were lower by 3.3 and 4.8%, respectively as compared with that of OB-CON group. Although not significant, measured values of the perirenal and visceral fat pads of OB-BIO and OB-DF groups were lower than those of the OB-CON group. The weight of interscapular brown adipose tissue did not show significant difference in all groups. The size of adipocyte in rats was lower in both OB-BIO and OB-DF groups. Thus, biocelluose and the diet formulation showed the anti-obesity effect.

Keywords: biocellulose, obesity, high-fat diet, body weight, animal experiment

Introduction

The food intake pattern of Koreans shows a trend to follow the western pattern. This change results in an increase in the obese population. According to the National Nutrition Survey Report (1), the population with a body mass index over 25 was increased from 23.0% in 1998 to 31.8% in 2005, and it is expected to reach 50% in 2020. Obese individuals encounter considerable discrimination at school, at the workplace, when applying for health and life insurance, when seeking a mate, and in a host of other social situations. Not surprisingly, obese individuals spend billions of dollars of disposable income each year in pursuit of a socially acceptable weight. Bad situation is the circumstance that most of them depend on unproved therapies in around the world (2), since it is difficult to find a sound method to treat obesity.

Obesity is a complex condition resulting from a mixture of psychological, environmental, and physiological factors. It is a proven causative agent in the so-called 'diseases of affluence' such as cardiovascular disease and adult onset diabetes. For the treatment or prevention of obesity, various therapies including diet, exercise, behavior modification, and drug have been attempted, among which diet therapy may be the best treatment to reduce body weight (3).

Intake of dietary fiber, one of the diet therapies, has been reported to be beneficial to prevention or/and treatment of chronic degenerative diseases such as obesity, diabetes mellitus, and coronal cardiovascular disorders (4,5). Dietary fibers consist of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated and non-digestible carbohydrates that have beneficial physiological effects in human (5).

In a previous paper (6), we found that biocellulose reduced body weight gain of rats fed high-fat diet, *i.e.*, it could prevent obesity. Based on the results, we expected that the biocellulose would treat obesity. In this study, we evaluated the effect of biocellulose and a diet formulation on weight gain of obese rats induced by high-fat diet for 10 weeks. Biocellulose ia a dietary fiber collected from a fermented broth of acetic acid microorganism (*Acetobacter* bacterium) in coconut water (7). The diet formulation was a mixture of phaseolamine and a substitution of the morning meal which contained hydroxycitric acid (HCA), grape seed extract, and kidney bean extract as major components (140 kcal/sachet). This study used obese animal models, and examined body weight gain, serum lipid level, fat accumulation, and other factors related to energy metabolism in Sprague-Dawley rats.

Materials and Methods

Materials Biocellulose, diet formulation, and phaseolamine were supplied from Natural F&P Co., Ltd. (Seoul, Korea). Phaseolamine was extracted from beans, mixed with the diet formulation at the ratio of 1.7:1, and used for the experimental diet. The compositions of the materials were the same as listed in Table 1 and 2 of the previous paper (6). A high-fat diet (modified AIN93G diet) was purchased from Dyets Inc. (Bethlehem, PA, USA).

Animal and diets Six-week male Sprague-Dawley rats imported by Jungang Lab Animal Inc.(Seoul, Korea) from Japan SLC, Inc.(Shizuoka prefecture, Japan) were adapted to Samyang rodent diet (Samyang Feed, Seoul, Korea) for 1 week prior to the beginning of the experiment. The animals were housed in cages (20×32×14 cm) under automatically controlled conditions of temperature (22±1 °C), humidity (about 60%), and lighting (light from 07:00 to 19:00). After the adaptation, they were induced obese by high-fat diet for 10 weeks. Thirty obese rats weighing average 397.6 g were randomly divided into 3 groups with 10 rats/group. The obese control group (OB-CON) was fed

---

*Corresponding author: Tel: +82-31-780-9246; Fax: +82-31-780-9226
E-mail: imissu@kfri.re.kr

Received July 10, 2008; Revised August 19, 2008;
Accepted August 24, 2008

a modified AIN93G diet in which 30% of the energy was derived from lipids. The second group (OB-BIO) was fed a further modified AIN93G diet in which biocellulose by 5% of the whole diet was substituted for corn starch. The third group (OB-DF) was fed another further modified AIN93G diet in which diet formulation by 5% of the whole diet was substituted for corn starch. Compositions of 3 experimental diets were listed in a previous paper (6). For an additional 10 weeks, diet consumption and body weight were recorded weekly. Animals took diet and water *ad libitum*. The food efficiency ratio (FER) was calculated by dividing the increased body weight by the amount of food consumption during the same period.

Blood sampling and biochemical analyses At the final day of animal experiment, animals were fasted for 12 hr, anesthetized with ethyl ether, opened the abdomen, and then, the blood was obtained from the abdominal aorta with a 10-mL syringe. The blood samples were collected in vacutainer tube, and serum was separated by centrifugation (833×g, 15 min). Analyses of biochemical parameters such as serum triglyceride, total and high density lipoprotein (HDL)-cholesterols, free fatty acids, and C-peptide followed the same methods and procedures as described previously (6).

Preparation of organs and tissues Immediately after the collection of the blood, liver, kidney, epididymal fat, perirenal fat, visceral fat, and interscapular brown adipose tissue (IBAT) were excised and rinsed with 0.1 M phosphate buffer (pH 7.4). After weighing, the visceral fat was rapidly frozen in liquid nitrogen and stored at −70°C.

Measurement of morphology, size, and number of adipocytes A portion of visceral fat was dissected to 0.1 mm^3 in 0.1 M phosphate buffer in a tissue culture dish, and fixed for 24 hr in 4% *para*-formaldehyde containing glutaraldehyde (1%). The prepared adipose tissue slides were developed by a light microscope (Axioplan 2; Zeiss, Goettingen, Germany), and the area of the tissue sections was calculated by the Discovery series Quantity One (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The procedure was described in detail in a previous paper (6).

Statistical analysis All analytical data were expressed as the mean of experimental groups and standard deviation, and were subjected to one-way ANOVA using SAS (SAS Institute, Cary, NC, USA) at the $p<0.05$ significant level. The statistical significance among groups was accessed by Duncan's multiple-range test.

Results and Discussion

Body weight gain and FER After adaptation, rats were fed high-fat diet to induce obesity, and their body weights were measured every week. After 10 weeks, average body weight of rats fed high-fat diet was significantly higher by 10% than that fed normal AIN93G diet. The obese rats were randomly divided to 3 groups such as OB-CON, OB-BIO, and OB-DF for the treatment. The following results were obtained after an additional 10-week experiment. Food intake, body weight gain, and FER are shown in Table 1. In all experimental groups, food intake was in the range of 24.4-26.9 g, and FER was in the range of 0.10-0.13. There was no significance among the 3 groups. After the experimental period for 10 weeks, the body weight gain was significantly lower in OB-BIO (181.2±46.3 g) and OB-DF groups (171.8±69.7 g) than in OB-CON group (236.1±33.4 g) ($p<0.05$). The body weight changes for 10 weeks are shown in Fig. 1. Until the third week, the body weights of OB-BIO and OB-DF groups were not significantly different from those of OB-CON group. From the 4th week, however, it began to be lower in comparison with OB-CON group. At the 10th week, body weights of treatment groups were significantly lower by 3.3 and 4.8%, respectively, as compared with OB-CON group ($p<0.05$).

Table 1. Food intake, body weight gain, and food efficiency ratio

Group$^{1)}$	Food intake (g/day)	Body weight gain (g/10 weeks)	Food efficiency ratio
OB-CON	26.9±1.3	$236.1\pm33.4^{a2)}$	0.13±0.02
OB-BIO	24.4±2.4	181.2 ± 46.3^{b}	0.11±0.02
OB-DF	24.9±2.4	171.8 ± 69.7^{b}	0.10±0.03

1)OB-CON, high-fat diet; OB-BIO, high-fat+biocellulose; OB-DF, high-fat+formulation diet.

2)Values with different alphabet within the column are significantly different at $p<0.05$ by Duncan's multiple-range test.

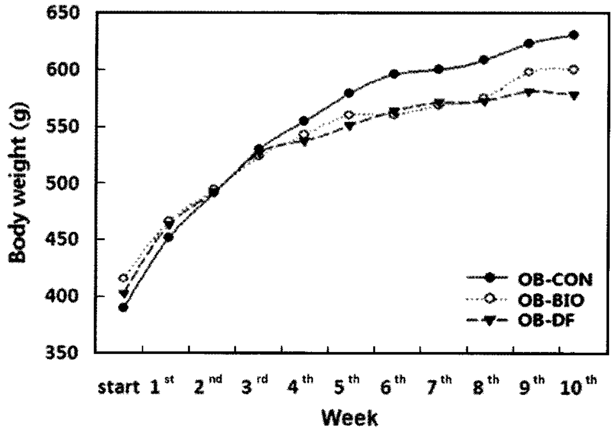

Fig. 1. Body weight change of rats fed high-fat diet (OB-CON), high-fat+biocellulose diet (OB-BIO), and high-fat+diet formulation diet (OB-DF) for 10 weeks (*n*=10).

Heaton (8) reported that fiber acts as a physiologic obstacle to energy intake by at least 3 mechanisms; 1) fiber displaces available calories and nutrients from the diet; 2) fiber increases chewing, which limits intake by promoting the secretion of saliva and gastric juice, resulting in an expansion of the stomach and increased satiety; and 3) fiber decreases the absorption efficiency of the small intestine. Jenkins *et al.* (9) has shown that fiber-rich meal is processed more slowly, and nutrient absorption occurs over a longer period. Further, a diet of foods that provide adequate fiber is usually less energy dense and larger in volume than a low-fiber diet. This may limit spontaneous intake of energy (10). Restricted intake of energy by fiber may be the major reason why fiber can reduce body weight gain.

Table 2. Serum triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations

Group	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
OB-CON	95.4±34.2	57.6±17.0	24.3±5.9	33.3±9.8
OB-BIO	75.1±26.6	68.8±17.2	25.4±5.3	43.4±6.4
OB-DF	68.0±18.4	64.3±11.2	24.4±4.6	39.9±7.0

As dietary fiber can affect the glycolipid metabolism of rats fed high cholesterol diets, most dietary fibers bring about the reduction of body weight, etc (11). Many reports supported that biocellulose, a kind of fibers, could reduce the body weight gain of obese rats induced by high-fat diet. Study of this bacterial fiber on the digestive tract and lipid metabolism revealed that this fiber had the same function on the animals like the other celluloses and pectins (12). Our results showed that obese rats fed biocellulose or diet formulation containing it had less body weight gain as compared with OB-CON group. From a point of view described above, biocellulose might play a similar role like the other fibers (12).

Concentration of serum lipid The concentrations of triglyceride, total cholesterol, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol are listed in Table 2. Serum triglyceride in OB-CON group was 95.4±34.2 mg/dL, which was higher than those of OB-BIO (75.1±26.6 mg/dL) and OB-DF (68.0±18.4 mg/dL) groups. According to the data book 'Japan SLC experimental animals' published by Japan SLC, Inc. (13), average serum triglyceride of 30-week old male SD rats were 128.0±44.6 mg/dL. This shows that triglyceride levels of OB-BIO and OB-DF are lower than SD rats fed normal diet. This result means that biocellulose and diet formulation containing it have a beneficial effect on lipid metabolism in obese rats. In addition, there was no significant difference in serum total cholesterol, HDL-cholesterol and LDL-cholesterol concentrations among the all groups. This was different from the result of the other studies reported that dietary fibers lowered serum cholesterol (14,15). In those studies, however, not the obese rats but the normal rats were used. Since triglyceride levels of OB-BIO and OB-DF groups were lower than those of OB-CON, however, it could be considered that biocellulose and diet formulation containing it had a high potential for the improvement of lipid metabolism *in vivo*.

Weight of organs Table 3 shows the weights of liver and kidney. Both weights of liver and kidney of treatment groups were not significantly different from that of OB-CON group. Their weights were in a normal range (13). This result means that biocellulose and diet formulation containing it did not have negative effect on the 2 organs. Normal weights of liver and kidney might be one of the evidences that consumption of biocellulose is safe.

Table 3. Liver and kidney weights

Group	Liver (g/100 g BW)	Kidney (g/100 g BW)
OB-CON	2.3±0.2	0.55±0.09
OB-BIO	2.2±0.2	0.53±0.07
OB-DF	2.2±0.1	0.58±0.06

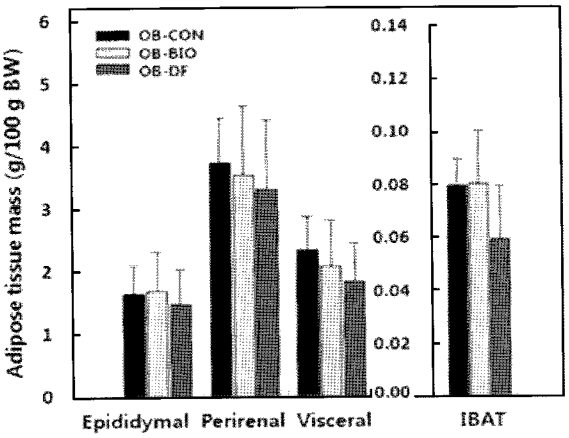

Fig. 2. Adipose tissue mass of rats fed high-fat diet (OB-CON), high-fat+biocellulose diet (OB-BIO), and high-fat+diet formulation diet (OB-DF) for 10 weeks (n=10). *IBAT, interscapular brown adipose tissue.

Weight and size of white adipose tissue (WAT) and interscapular brown adipose tissue (IBAT) Adipose tissue is located primarily under the skin, in the mesenteries and omentum, and behind the peritoneum. Although it is a fat tissue, adipose tissue is also composed of small amounts of protein and water (16). WAT serves as a repository for triglycerides, a cushion to protect abdominal organs, and an insulator to preserve body heat. It looks yellow because of carotene. To examine the adipose tissue accumulation in the body of obese rats induced by high-fat diets, the wet weights of epididymal, perirenal, and visceral fat pads were measured. Their weights were converted to the tissue weight/100 g BW, and are shown in Fig. 2. It has been known that visceral fat strongly correlates to diabetes, hypertension, and cardiovascular diseases (17,18). This means that reduction of visceral fat could bring about not only improvement of obesity but also prevention of disease of adult people. The wet weight of epididymal fat pad showed a tendency of decreasing in the OB-DF group (1.49±0.30 g) than in the OB-CON group (1.65±0.31 g), though it was not a significant difference in all groups. In case of perirenal and visceral fat pads, they also showed a similar result like epididymal fat pad.

Klaus (18) reported that the IBAT appears to be brown in color, and is different from WAT. It is functioned in the production of energy *in vivo*. Brown adipose tissue (BAT), seen much in infants but very small in adults, occurs primarily in the scapular and subscapular areas. The brown color is due to extensive vascularization. It has been studied most extensively in animals, where it appears to be involved in heat production as a means of adapting to cold and possibly of dissipating excess energy (19). It has been known that the size of the adipose tissue stores increases in periods of positive energy balance and decreases when energy expenditure is in excess of intake. IBAT masses of

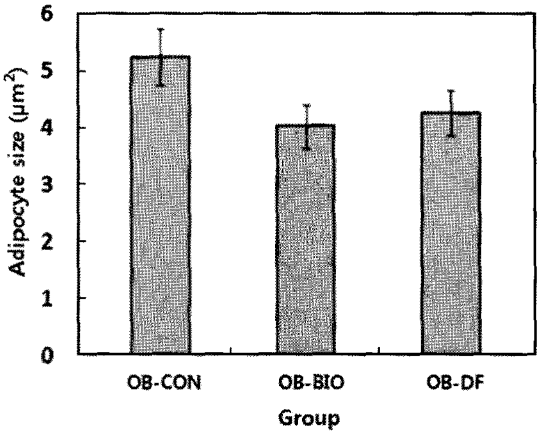

Fig. 3. The size of adipocyte from isolated visceral fat of rats fed high-fat diet (OB-CON), high-fat+biocellulose diet (OB-BIO), and high-fat+diet formulation diet (OB-DF) for 10 weeks (*n*=10). Adipocyte size = adipose tissue area/adipocyte number in the area

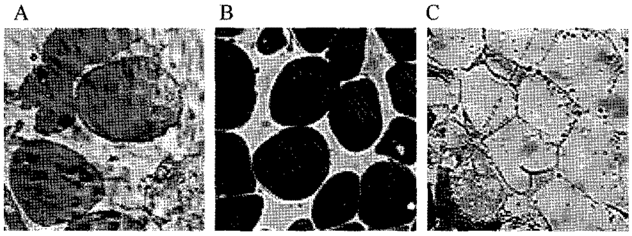

Fig. 4. Micrography of adipocytes isolated from visceral fat of rats fed high-fat diet (OB-CON), high-fat+biocellulose diet (OB-BIO), and high-fat+diet formulation diet (OB-DF) for 10 weeks.

control and treatment groups were not significantly different. This means that biocellulose and a diet formulation did not affect thermogenic capacity in obese rats.

To assess the size of adipocytes, visceral fat tissue of SD rats was stained, and their sizes were examined with the image analyzer. The results are shown in Fig. 3. Adipose cells are derived from pluripotent stem cells, and the process of adipogenesis involves a complex communication network between various transcription factors, some of which are sensors for nutrients, metabolites and hormones (20,21). The sizes of adipocytes of rats in OB-BIO and OB-DF groups were 4.17 and 4.43 μm^2, respectively. Their sizes were smaller than that of rats in OB-CON group (5.41 μm^2). Less body weight gains of OB-BIO and OB-DF groups might be resulted from smaller adipocytes. It seemed to be that feeding of biocellulose or the diet formulation containing it could suppress the hypertrophy of adipocytes, and thus it may be very useful for the reduction of body weight gain of obese rats.

Biochemical analyses of serum factors Serum biochemical factors such as blood glucose level, C-peptide, and free fatty acid data are shown in Table 4. According to the guidebook of Japan SLC experimental animal, serum glucose concentration of 30-week old male SD rats were 138.0±10.9 mg/dL. In our study, glucose concentrations in the all groups were in 146.3-148.4 mg/dL. They were considered to be normal. During the experimental period, obesity had not spread to a complication such as diabetes. C-peptide has been used as the marker that well reflects the secretion capacity of beta cells in the pancreas *in vivo* (22). C-peptide is partially removed by the liver, but is completely eliminated in the kidney. Thus the C-peptide concentration is accepted as representing the true evaluation of the insulin secretion capacity in human. Serum C-peptide levels were not significantly different among the all groups (Table 4). It was also considered from serum glucose and C-peptide concentrations that experimental animal had not spread to diabetes.

Table 4. Analysis of biochemical parameters of blood

Group	Glucose (mg/dL)	C-peptide (ng/mL)	Free fatty acid (μEq/L)
OB-CON	148.3±11.7	0.10±0.00	336.6±99.0
OB-BIO	146.3±17.7	0.11±0.02	327.1±99.7
OB-DF	148.4±23.5	0.10±0.00	377.4±84.8

Serum free fatty acid is generated and released from adipocytes during the lipid degradation process, in which triglyceride is degraded to free fatty acids and glycerol (23). In this study, there was no significant difference in serum concentrations of free fatty acid among the all groups. Although these were not significantly different, dietary formula used in this study were considered that they may reduce accumulation of triglyceride concentration in experimental animal and has function of effectively turnover from triglyceride to free fatty acid in adipocytes during the lipid degradation process.

References

1. Blundell JE, Stubbs RJ, Golding C, Croden F, Alam R, Whybrow S, Le Noury J, Lawton CL. Resistance and susceptibility to weight gain: Individual variability in response to a high-fat diet. Physiol. Behav. 86: 614-622 (2005)
2. Gortmaker SL. Social and economic consequences of obesity. New Engl. J. Med. 348: 2082-2090 (1993)
3. Kang KJ, Lim SJ, Jeong JH, Han HK, Choi SS, Kim MH, Kwon SY. Effects of wax guard on weight, triglyceride, leptin, and fat cell size in rats fed on a high fat diet. Korean J. Nutr. 36: 446-451 (2003)
4. Lee SH, Park HJ, Cho SY, Jung IK, Cho YS, Kim TY, Hwang HG, Lee YS. Supplementary effect of the high dietary fiber rice on blood glucose in diabetic mice. Korean J. Nutr. 37: 75-80 (2004)
5. Slavin JL. Dietary fiber and body weight. Nutrition 21: 411-418 (2005)
6. Han DS, Kwon EK, Kim DW, Kim YE, Lee CH. Kim IH. Biocellulose reduces body weight gain of rats fed high-fat diet. Food Sci. Biotechnol. 15: 70-76 (2006)
7. Raghavendra SN, Rastogi NK, Raghavarao KSMS, Tharanathan RN. Dietary fiber from coconut residue: Effects of different treatments and particle size on the hydration properties. Eur. Food Res. Technol. 218: 563-567 (2004)
8. Heaton KW. Food fiber as an obstacle to energy intake. Lancet 22: 1418-1421 (1973)
9. Jenkins D, Wolever T, Rao AV, Hegele RA, Mitchell SJ, Ranson T, Boctor DL, Spadafora PJ, Jenkins AL, Mehling C, Relle LK, Connelly PW, Story JA, Furumoto EJ, Corey P, Wursch P. Effect of blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. New Engl. J. Med. 329: 21-26 (1993)
10. Saris WH. Glycemic carbohydrate and body weight regulation. Nutr. Rev. 61: S10-S16 (2003)

11. Kelsay JL, Behall KM, Prather ES. Effect of fiber from fruits and vegetables on metabolic responses of human subjects. Am. J. Clin. Nutr. 31: 1149-1156 (1978)
12. Cho SH, Lee JY, Choi YS, Choi KH. Dietary effects of fiber produced from gluconoacetobacter hansenii on digestive tract and lipid metabolism in rats. J. Korean Soc. Food Sci. Nutr. 31: 802-807 (1997)
13. SLC. Experimental Animals. Japan SLC, Inc., Shizuoka Prefecture, Japan. pp. 1-2 (2005)
14. Lee BH, Cho KD. Effects of herb and fiber-rich dietary supplement on body weight, body fat, blood lipid fractions, and bowel habits in collegians. J. Korean Soc. Food Sci. Nutr. 34: 644-651 (2005)
15. Yook HS, Kim JO, Choi JM, Kim DH, Cho SK, Byun MW. Changes of nutritional characteristics and serum cholesterol in rats by the intake of dietary fiber isolated from ascidian (*Halocynthia roretzi*) tunic. J. Korean Soc. Food Sci. Nutr. 32: 474-478 (2003)
16. Jensen MD, Research techniques for body composition assessment. J. Am. Diet. Assoc. 92: 454-460 (1992)
17. Mermel VL. Old paths new directions: The use of functional foods in the treatment of obesity. Trends Food Sci. Technol. 15: 532-540 (2004)
18. Klaus S. Adipose tissue as a regulator of energy balance. Curr. Drug Targets 5: 241-250 (2004)
19. Kathleen ML, Sylvia Escott-Stump MA. Kreause's Food, Nutrition & Diet Therapy. 11th ed. Saunders Press, St. Louis, MO, USA. p. 560 (2000)
20. Cornelius P, MacDougald OA, Lane MD. Regulation of adipocyte development. Annu. Rev. Nutr. 14: 99-129 (1994)
21. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol. Rev. 78: 783-809 (1998)
22. Lee GE, Lyu JS, Kim YT, Song YK, Kim KS, Lee MH. Clinical characteristics of Korean diabetic patients classified by fasting plasma C-peptide level and degree of obesity. Korean J. Intern. Med. 42: 315-321 (1992)
23. Elam MB, Wilcox HG, Cagen LM, Deng X, Raghow R, Kumar P, Heimberg M, Russell JC. Increased hepatic VLDL secretion, lipogenesis, and SREBP-1 expression in the corpulent JCRLA-cp rat. J. Lipid Res. 42: 2039-2048 (2001)