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Biocellulose Reduces Body Weight Gain of Rats Fed High-Fat Diet

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Abstract Anti-obesity effect of biocellulose and diet formulation containing it was evaluated using obese rats. Thirty male Sprague-Dawlely (SD) rats were randomly assigned to high-fat diet group (CON), high-fat diet group containing 5% biocellulose (BIO), and high-fat diet group containing 5% dietary formulation (DF). After 10 weeks, body weight gains of BIO and DF groups were reduced by 15.1 and 6%, respectively, as compared with CON group. Epididymus, parirenal, and visceral fat pads of BIO and DF groups were lower than those of CON group. Weight of interscapular brown adipose tissues increased slightly in BIO group and significantly in DF group. Size of adipocyte in rats decreased in BIO and DF groups. Results indicate biocellulose-containing diet has anti-obesity effect by reducing body weight gain and white adipocytes depots in rats fed high-fat diet.

Keywords: biocellulose, obesity, body weight, high-fat diet, rat

Introduction

Recent westernization of the food intake pattern of Koreans has brought about increasing intake of fat, resulting in an increase in the obese population (1). According to the National Nutrition Survey Report, the population with a body fat index over 25 was 17.1% in 1991, 23% in 1998, and is rapidly increasing every year (2-4). The increased incidences of coronary arterial diseases, diabetes, hyperlipidemia, hypertension, etc. appear to be associated with the prevalence of obesity (1). The prevention of obesity, therefore, has become an important social issue (5-7). For the treatment of obesity, various therapies including diet, exercise, behavior modification, and drug have been attempted (8), among which diet therapy may be the best treatment to reduce body weight. Alternative treatments also include food supplementation. Some natural components such as hydroxycitric acid (HCA) (9), conjugated linoleic acid (CLA) (10-12), chitosan (13, 14), and catechin family contained in green tea (15) have been evaluated for anti-obesity effect through both animal and human studies (16).

In this study we evaluated the effects of two diet formulations including biocellulose, which contains high concentration of pure cellulose collected from a fermented broth of acetic acid microorganism (*Acetobacter* bacterium) in coconut water (17). The other diet formulation was a mixture of phaseolamine and consumption of a morning meal containing HCA grape seed and kidney bean extracts as the major components (140 kcal/sachet).

To assess the effect of biocellulose-containing diet on the prevention of obesity, Sprague-Dawlely (SD) rats fed high-fat diet were treated with diet containing biocellulose or diet formulation for 10 weeks, and their body weight gain, distribution of body fat, serum lipid level, and other factors related to energy metabolism were examined.

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Materials and Methods

Preparation of biocellulose To prepare the biocellulose, appropriate amount of apple juice was diluted with distilled water to form 35%(v/v) mixture, and 400 mL medium containing 1% acetic acid (v/v) adjusted to pH 3.5 was poured into a 1-L Erlenmeyer flask. *Acetobacter xylium* strain was inoculated thereto, and stirred for 3 days at 150 rpm and 30°C. The resulting seed culture (5%, v/v) was inoculated into 10 1-L beakers, each containing 400 mL new medium comprised of 1% acetic acid (v/v) and 10% sucrose (v/v) adjusted to pH 3, and subjected to standing cultivation for 7 days at 30°C. Translucent gel-type biocellulose with a width of 6.5 mm was formed. The harvest was soaked in water for 2 days to remove the remaining acetic acid, washed two times with water, and heated at 100°C for 40 min to inactivate the contaminated microorganism. The purified biocellulose was subjected to compression to prepare a wet bio-cellulose sheet with 0.6 mm thickness and 90% water content. The wet sheet was soaked in 6.5% fructose solution to recover its width to 2 to 4 mm. The recovered biocellulose was subjected to compression again to prepare a wet biocellulose sheet having 0.6 mm thickness. The wet sheet was dried for 24 hr at 75°C in an incubator and crushed into powder. The crushed cellulose was passed through a 40-mesh screen to obtain similarly sized powder.

Materials Biocellulose, diet formulation, and phaseolamine were supplied by 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 as the experimental diet. The compositions of the materials used in experiments are listed in Table 1 and 2.

Animals and diets Six-week-old male Sprague-Dawley rats were purchased from Jungang Lab Animal Inc. (Seoul, Korea). The animals were maintained under constant temperature (24±2°C), with a 12 hr light-dark cycle and 40-70% relative humidity, and were allowed to

Table 1. Composition of dried biocellulose* powder

Composition	%
Cellulose	45-60
Fructose	35-40
Water contents	3-6

*Biocellulose is the dried powder containing high concentration of pure cellulose collected from fermenting an acetic acid microorganisms (*Acetobacter* bacteria) in the coconut water.

Table 2. Composition of diet formulation

Materials	%
Psyllium husk powder	30.0
Glucomannan	15.0
Chicory fiber	12.1
Grape tasted powder	18.0
Lactulose50	6.6
Opuntia ficus-indica Ext. powder	3.6
Fructooligosaccharide	3.6
Erythritol	3.6
Biocellulose	2.1
DL-Malic acid	1.8
Garcinia cambogia Ext. powder	1.2
Citric acid	0.6
Kidney bean Ext. powder	0.5
Grape flavored powder	0.45
Aspartame	0.2
Sucrose esters of fatty acid	0.188
Ascorbic acid	0.164
Sucralose	0.1
Pinus densiflora Siebold et zucarini Ext. powder	0.06
Grape seed Ext. powder	0.06
Zinc oxide	0.044
Maltol	0.03
Pyridoxine hydrochloride (vitamin B_6)	0.004

take food and water *ad libitum*. They were adapted to a commercial rodent chow diet (Samyang rodent diet; Samyang Feed, Seoul, Korea) for 1 week. Thirty rats (227.9±14.9 g body weight (B.W.) were randomly divided into three groups with n=10 in each group. All groups were fed high-fat diet, a modified AIN93G diet (Dyets Inc., Bethlehem, PA, USA) with 30% of the energy derived from hydro-genated soybean oil, for 10 weeks. The groups consisted of control group fed only high-fat diet (CON group), that fed high-fat diet with 5% biocellulose (BIO group), and that fed high-fat diet with 5% diet formulation (DF group). Compositions of diets for each group are shown in Table 3. Body weight and food intake were measured weekly for 10 weeks. The food efficiency ratio (FER) was calculated by dividing the increased body weight by the amount of food consumed during the same period. Care of the experimental animals were followed the 116th regulation for the use and care of laboratory animals of the Korea Food & Drug Administration.

Table 3. Composition of experimental diets (g/kg diet)

Ingredients \ Groups*	CON	BIO	DF
Casein	217.20	217.20	217.20
Cornstarch	276.91	226.91	226.91
Dextrose	143.35	143.35	143.35
Sucrose	108.60	108.60	108.60
Hydrogenated soybean oil	144.79	144.79	144.79
Cellulose	54.30	54.30	54.30
TBHQ	0.02	0.02	0.02
Mineral mixture	38.01	38.01	38.01
Vitamin mixture	10.86	10.86	10.86
L-Cysteine	3.26	3.26	3.26
Choline bitartrate	2.71	2.71	2.71
Diet formulation	-	-	31.48
Phaseolamine	-	-	18.52
Biocellulose	-	50	-
Fat % (calories)	30	31.4	31.4

*CON : High-fat diet (AIN-93G diet + 30% hygrogenated soybean oil), BIO : High-fat diet + 5% Biocellulose, DF : High-fat diet + 5% diet formulation.

Blood sampling and biochemical measurements At the final day of animal experiment, animals were fasted for 12 hr and anesthesized with ethyl ether. Their abdomens were opened, and the blood was obtained from the abdominal aorta using a 10-mL syringe. The blood samples were collected in vacutainers, and the plasma was seperated by centrifugation (833×g, 15 min).

The serum triglyceride concentration was measured by glycerophosphate oxidase (GPO) Trinder w/o serum blank (non-colorimetric method), and analyzed using Triglyceride reagents kits (Bayer, Tarrytown, NY, USA) and Chemistry Autoanalyzer (ADVIA 1650, Bayer, Tokyo, Japan). The serum total cholesterol concentration was measured by enzymatic method, and analyzed using Direct HDL-cholesterol II reagent kits (Bayer) and Chemistry Autoanalyzer (ADVIA 1650, Bayer, Tokyo, Japan). The serum LDL-cholesterol concentration was calculated by applying Friedewald formula (18).

The concentration of C-peptide was measured by Double Antibody C-peptide kit EURO DPC (Diagnotic Products Corp., Los Angeles, CA, USA) based on the radio-isotope analysis method, and analyzed using the r-counter COBRA 5010 series Quantum (Packard, Chicago, IL, USA). The serum-free fatty acid concentration was assessed by enzymatic method using Sicdia Nefazyme kit (EIKEN, Tokyo, Japan) and Hitachi 7150 (Hitachi, Tokyo, Japan)

Tissue collection 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 into 0.1 mm^3 pieces in 0.1 M phosphate buffer in a tissue culture dish (100 mm in diameter), and fixed for 24 hr in 4 % paraformaldehyde containing glutaraldehyde (1%). The dissected tissues were washed with 0.1 M phosphate buffer three times for 10 min, and fixed again with 1% osmium tetraoxide for 30 min. The tissues were immersed sequentially in 70, 80, 90, and 95% alcohol solutions for 10 min to remove water. After the removal of water with 100% alcohol for 1 hr twice, the tissues were incubated in Epon solution for 24 hr. The formation of air bubbles in the newly prepared Epon solution was blocked using a syringe, and, after embedding, the sections were made into slides. The prepared adipose tissue slides were developed using a light microscope (Axioplan 2, Zeiss, Goettingen, Germany) attached with a digital camera. Magnifications of the eye piece and objective lens were 2.5 and 10 times, respectively. The area of the tissue sections was measured by the Discovery series Quantity One (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the number of cells per unit area was calculated as follows:

Adipocyte size = adipose tissue area/adipocyte number in the area

GOT and GPT The glutamic-oxaloacetic Transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities were analyzed using commercial analytical kits. Serum GOT and GPT activities were analyzed according to the IFCC method using an AST reagent kit (Bayer) and ADVIA 1650 (Bayer).

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

Results and Discussion

Body weight gain reduction of biocellulose and diet formulation Food intake, body weight gain, and food efficiency ratio are shown in Table 4. In all experiemental groups, food intake was in the range of 22.5-22.8 g. During the experimental period, the body weight gain of BIO group (113.9±47.4 g) was significantly lower than that of CON group (174.8±53.9 g) ($p<0.05$). Body weight gain of DF group (150.2±58.8 g) was also lower than that of CON group, but not at the significance level. The food efficiency ratio was in the range of 0.11-0.17, with no significant differences observed among the three groups. The body weight changes during the 10-week period are shown in Fig. 1. Body weights of BIO group were similar

Table 4. Food intake, body weight gain, and food efficiency ratio

Group	Food intake (g/day)	Body weight gain (g/10 weeks)	Food efficiency ratio
CON	22.8±1.3	174.8±53.9^{a*}	0.11±0.03
BIO	22.5±2.9	113.9±47.4^{b}	0.07±0.03
DF	22.7±1.5	150.2±58.8^{ab}	0.10±0.04

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

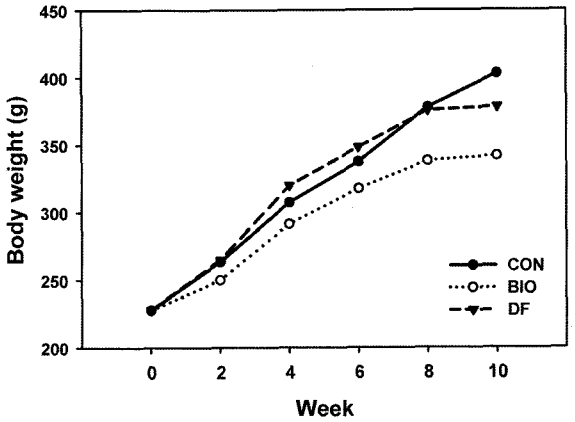


Fig. 1. Body weight change of rats fed high-fat diet (CON), high-fat + biocellulose diet (BIO), and high fat + diet formulation diet (DF) for 10 weeks (n=10).

to those of CON group during the first week. However, from the second week, when the experimental diet was fed, the weight began to decrease, and, at the last 10th week, significantly decreased by 15.1% in comparison with the CON group ($p<0.05$). In contrast, the body weight of DF group increased compared to other groups up until 7 weeks after feeding the diet. But the body weight of DF group decreased from 8 weeks in comparison with the CON group, and was 6% lower on the last day of the experiment period.

These results showed that SD rats fed biocellulose and diet formulation had less body weight gain compared with the CON group fed a high-fat diet. Biocellulose is a bacterial fiber separated from the fermentation broth of coconut water by *Acetobacter*. Study of these bacterial fibers on the digestive tract and lipid metabolism revealed that bacterial fibers have the same function on the animals as the other celluloses and pectins (19). Most dietary fibers bring about the reduction of body weight; in particular, soluble fibers such as guar gum, alginate, polysaccharides, and synthetic polydextrose, etc. showed greater effect than insoluble fibers (20). Chai *et al.* (21) reported that the increased excrement amount and the reduced gastrointestinal transit time in the groups fed dietary fiber could reduce the absortion time of glycolipid; therefore, dietary fiber can affect the glycolipid metabolism of rats fed high cholesterol diets. This is consistent with the report of Kelsay *et al.* (22) that there was a remarkable reduction in the gastrointestinal tansit time when 5% cellulose and guar gum were added to diets. Stephen and Cumming (23) reported that the matrix of the dietary fiber remains intact

Table 5. Serum triglyceride(TG), total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations

Group	Triglyceride (mg/dL)	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)
CON	158.2±40.6	66.4±8.6	28.0±4.9[a*]	6.8±5.4
BIO	175.4±64.7	65.2±7.1	24.3±2.9[b]	9.0±5.5
DF	179.4±78.8	61.4±8.7	23.7±3.4[b]	9.9±6.7

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

within the large intestine, such that the weight and amount of excrement can increase effectively, indicating that biocellulose could reduce the body weight of rats fed high-fat diets.

Composition of serum lipid The concentrations of triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol are shown in Table 5. The HDL-cholesterol level in the CON group was 28.0±4.9 mg/dL, which was higher than those of BIO (24.3±2.9 mg/dL) and DF (23.7±3.4 mg/dL) groups ($p<0.05$). No differences were observed in serum triglyceride, total cholesterol, and LDL-cholesterol levels of all groups, suggesting that biocellulose and diet formulation diets did not affect the serum lipids. This was different from the results of other studies that diet fibers lowered the serum cholesterol level (24, 25, 26). Based on our results, the effect of biocellulose, or diet formulation containing it, on the improvement of the composition of serum lipid could not be detected. Serum triglyceride concentration strongly correlates to the body fat mass, whereas the cholesterol level showed weak; thus, the cholesterol concentration has been used as an index assessing the collateral effect of lipid metabolism improvement *in vivo*. In our study, although biocellulose did not improve the serum lipid level, it showed good effects on the other indexes. Therefore, biocellulose and its products as diet formulations may be used for functional foods that are effective in preventing obesity.

Weight of organs The weights of liver and kidney are shown in Table 6. The wet liver weight was significantly lower in the BIO (2.3±0.2 g/100 g B.W.) and DF groups (2.5±0.4 g/100 g B.W.) than the CON group (3.0±0.2 g/100 g B.W.) ($p<0.05$), whereas no significant differences were observed in the wet kidney weights of all groups. These results suggest that the liver weight of the CON group was higher due to the accumulation of fat in the liver caused by high-fat diet. On the other hand, in BIO and DF groups, the fat accumulation in livers decreased due to the treatment of biocellulose or diet formulation.

Table 6. Liver and kidney weights

Group	Liver (g/100 g B.W.)	Kidney (g/100 g B.W.)
CON	3.0±0.2[a*]	0.75±0.12
BIO	2.3±0.2[b]	0.81±0.12
DF	2.5±0.4[b]	0.75±0.10

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

Biocellulose and diet formulation reduced white adipose tissue (WAT) and increased IBAT To examine fat accumulation in the body of SD rats, the wet weights of epididymal, perirenal, and visceral fat pads were measured, converted into tissue weight per 100 g body weight, and compared (Fig. 2). The wet weights of epididymal fat pads of the BIO group (0.92±0.27 g, $p<0.05$) and the DF group (1.05±0.27 g) were lower than those of the CON group (1.26±0.22 g). The wet weight of perirenal fat pad was also lower in the BIO and DF groups (1.04±0.47 and 1.24±0.42 g, respectively, $p<0.05$) as compared to the CON group (1.75±0.51 g). The wet weights of visceral fat of the BIO and DF groups (0.52±0.08 and 0.59±0.14 g, $p<0.05$) were also lower than that of the CON group (0.79±0.21 g). In comparison with WAT, the wet weight of IBAT was enhanced significantly in the DF group (0.066±0.016 g, $p<0.05$) than the CON group (0.047±0.008 g). BIO group showed a slightly increasing tendency, although not at a significant level.

To assess the size of adipocytes, visceral fat tissues of SD rats were stained, and their sizes were examined by the image analyzer (Fig. 3) and the results are shown in Fig. 4. The sizes of adipocytes in CON, BIO, and DF groups were 2.75, 2.06, and 2.20 μm^2, respectively, showing that the CON group had the largest adipocyte. Size reduction of the adipocytes in BIO and DF groups was also confirmed through light micrographs of adipocytes from visceral fat.

Biocellulose and diet formulation feeding were effective in reducing body fat mass. In particular, viceral fat is strongly correlated with diabetes, hypertension, and cardiovascular diseases (16, 27). In our study, the visceral

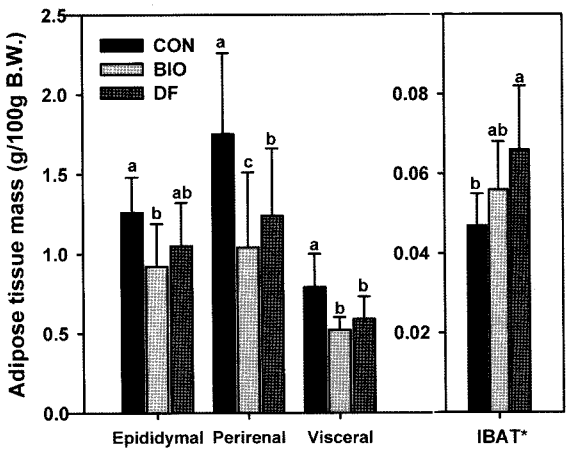


Fig. 2. Adipose tissue mass of rats fed high-fat diet (CON), high-fat + biocellulose diet (BIO), and high-fat + diet formulation diet (DF) for 10 weeks (n=10). *IBAT : Interscapular brown adipose tissue.

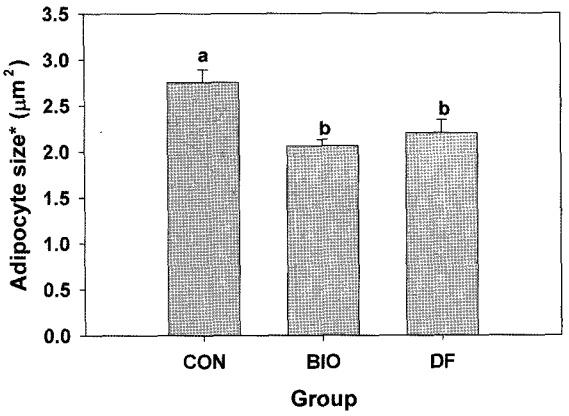


Fig. 3. The size of adipocyte from isolated visceral fat of rats fed high-fat diet (CON), high-fat + biocellulose diet (BIO), and high-fat + diet formulation diet (DF) for 10 weeks (n=10). *Adipocyte size = adipose tissue area/adipocyte number in the area.

fat mass was lower in BIO and DF groups than CON group. The size of adipocyte from visceral fat decreased in rats fed biocellulose and diet formulation. In other words, previous measurements of the weight of various adipose tissues showed that the weight decreased, particularly in regard to visceral fat. As obesity progresses, the size of adipocytes is enlarged due to the accumulation of fat. On the other hand, as the body weight decreases, the size of adipocytes also decreases due to the reduction of fat. Therefore, the reduction of the weight of adipose tissue in BIO and DF groups may be due to the decrease of the size of adipocytes, suggesting that biocellulose and the diet formulation containing biocellulose could suppress the hypertrophy of adipocytes, and thus may be very useful for the control of obesity. In contrast, IBAT increased significantly in DF and BIO groups. IBAT appears to be brown in color, and different from WAT which is white. It functions in the production of energy *in vivo* (27). The presence of uncoupling protein I (UCPI) in brown adipose tissues has been established. Uncoupling protein (UCP), which is present in the inner membrane of mitochondria, is known to be a thermogenic protein. Various UCP, UCP1, UCP2 (various tissues), and UCP3 (primarily in skeletal muscle) have been reported, and the mutation of UCP gene may result in obesity because of problems with energy generation (28). If the expression of UCP in obese rat is increased by genetic manipulation, the body weight decreases and the size of white adipocyte also decreases. In other words, because the feeding of biocellulose and the diet formulation containing it increased the IBAT in SD rats, it may have thermogenic capacity of reducing the body weight gain and WAT.

Biochemical analysis of serum factors Serum biochemical factors, blood glucose level, C-peptide, free fatty acid, GOT, and GPT data are shown in Table 7. Blood glucose level was lower in BIO (97.3±10.9 mg/dL) and DF groups (105.6±9.2 mg/dL) than CON group (118.5± 15.7 mg/dL) ($p<0.05$). C-Peptide value was higher in BIO (0.20±0.06 ng/mL) and DF groups (0.25±0.10 ng/mL) than CON (0.14±0.06 ng/mL) ($p<0.05$). There was no difference in serum concentrations and GOT activities of free fatty acids. The serum GPT activity was higher in BIO (99.7±31.2 U/L) and DF (101.2±36.8 U/L) groups than in CON group (43.5±11.5 U/L) ($p<0.05$).

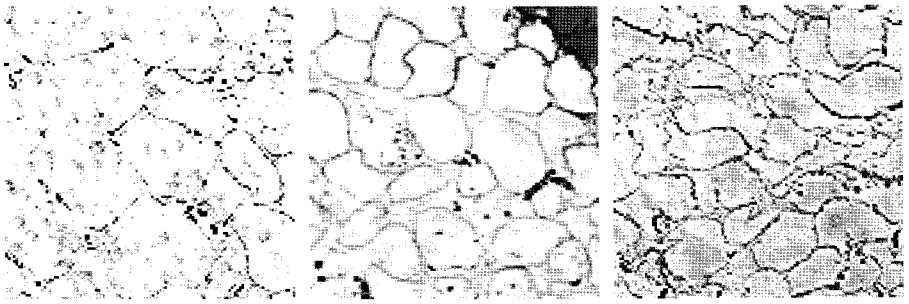
In obese cases, as the sensitivity of insulin receptor in the body decreases, blood glucose level increases, resulting in the induction of diabetes (29, 30). The blood glucose level decreased in BIO and DF groups. These results demonstrate that biocellulose and diet formulation feeding were beneficial to the high blood glucose level in obese rats. Serum C-peptide has been used as the marker that well reflects the secretion capacity of beta cells in the pancreas *in vivo* (31). Human C-peptide is a polypeptide with a molecular weight of 3,617 daltons. In human body, proinsulin is converted into insulin, and, at the time of insulin secretion by the pancreas, it is secreted to the blood at the same concentration (32). Before entering the systemic circulation, insulin and C-peptide pass through the hepatic portal vein, at which time 50-60% portal insulin is removed (33). 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 humans. In other words, in comparison with SD rats fed high-fat diet, the C-peptide value was enhanced in the groups fed biocellulose or the diet formulation. This suggests that biocellulose and the diet formulation increased the insulin secretion *in vivo* in SD rats, and it could be speculated that the serum glucose concentration decreased due to insulin. Serum-free fatty acid is generated and released from adipocytes during the lipid degradation process, in which triglyceride is degraded into free fatty acid and glycerol. It has also been reported that, as the body weight is increased, the free fatty acid concentration increases (33). In this study, however, no differences were observed among all groups.

GOT and GPT are the enzymes that synthesize amino acids in the liver and other organs. They are maintained in the blood at a constant concentration due to the normal destruction of cells. If many cells are destroyed due to the injury of the liver and other specific organs, these enzymes are released to the outside of cells, which results in the elevated level of enzyme activities (34). Because GOT is present in a large quantity in the liver, cardiac muscles,

Table 7. Analysis of biochemical parameters of blood

Group	Glucose (mg/dL)	C-peptide (ng/mL)	Free fatty acid (iEq/L)	GOT (U/L)	GPT (U/L)
CON	118.5±15.7^{a*}	0.14±0.06^{b}	499.6±100.6	90.7±25.3	43.5±11.5^{b}
BIO	97.3±10.9^{b}	0.20±0.06^{ab}	561.7±127.0	110.0±30.4	99.7±31.2^{a}
DF	105.6±9.2^{b}	0.25±0.10^{a}	500.4±87.9	93.2±14.7	101.2±36.8^{a}

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



A. CON group **B. BIO group** **C. DF group**

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

skeletal muscles, and red blood cells, it is used to diagnose myocardiac infarction and hemolysis. In addition, GPT distribution primarily in the liver makes it an important marker for liver diseases (34). In general, GPT tends to increase in liver diseases, and GOT primarily in cardiac diseases. In this study, GOT activities were within the normal range (approximately 92 U/L in 20-week-old normal SD rat), and there was no difference among all groups. GPT activity, however, was higher in BIO and DF groups than CON group as compared with the fact that the serum GPT activity of 20-week-old SD rats is approximately 50 U/L. Previous study has shown that addition of 2% bacterial fibers produced in coconut water maintained the GOT and GPT activities within the normal range (19).

According to the report of Korea Health Industry Development Institute (2003), the most important criteria to evaluate the effectiveness of diet products are body weight and body fat mass. Serum components, triglyceride, HDL-cholesterol, and total cholesterol are also important; however, they are more useful for the interpretation of the experimental results. Reduction of the body weight and body fat mass can be considered to be effective for anti-obesity. In addition, it can be determined to be harmless to the body if the changes in the weights of liver and other organs and that of serum components are not significantly different (35). According to the USA evaluation guideline for the effectiveness of therapeutics for obesity, body weight reduction is the most important index, followed by body fat and distribution rate of body fat. In human experiments, the weight reduction effect is considered to be significant at weight reduction rate over 5% in comparison with the placebo group at the 12th month of weight reduction (35, 36). Thus, the results of this study strongly support that the two diet formulations prevent obesity.

Acknowledgments

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