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Effects of Soy Isoflavones on Lipid Profiles and Hepatic LDL Receptor mRNA Level in Growing Female Rats

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The present study examined the effect of soy isoflavones on lipid metabolism in growing female rats. Rats were randomly assigned to three different groups and provided experimental diets for 9 weeks. The experimental groups were classified into 1) a control group, 2) a soy protein isolate group: soy (+)) group and 3) a soy protein concentration group: soy (-)) group. Diets contained either casein or one of two soy proteins with (soy (+)) or without isoflavones (soy (-)). Serum triglyceride concentration showed no significant differences among the experimental groups. Serum total cholesterol concentration was significantly lower in both the soy (+) and soy (-) groups than in the control group and LDL-cholesterol concentration was significantly lower in the soy (+). Serum HDL-cholesterol concentration was significantly higher in the control group than in the soy protein groups but the HDL-cholesterol share rate in total cholesterol tended to be lower in the control group than in the soy protein groups, insignificant as it was. Hepatic LDL receptor mRNA level was significantly increased in the soy (+) group when compared to the other two groups to be 20% higher than the control group.
In conclusion, soy protein isolate, soy protein rich with isoflavones reduced serum total cholesterol and LDL-cholesterol concentration and increased hepatic LDL receptor mRNA expression in growing female rats. Therefore, it is considered that the intake of soy isoflvones during puberty can be advantageous in terms of the long-term control of serum lipid.

Key words: Isoflavones, Lipid concentration, LDL receptor mRNA, Growing female rat

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INTRODUCTION

Epidemiological studies suggest that there is a negative relationship between soy isoflavones intake and cardiovascular diseases.[1-3] Clinical intervention studies indicate that some soy components, especially soy isoflavone-containing soy protein isolate, reduced serum total cholesterol and LDL-cholesterol.[4] Zhan and Ho[5] who carried out meta-analysis with the most recent 23 researches also concluded that isoflavone-containing soy protein reduced serum total cholesterol and LDL-cholesterol but increased HDL-cholesterol.

Having a similar structure to that of 17 β-estradiol, isoflavones are limitedly distributed in nature and by this reason, soy and soy foods are the richest and only sources of isoflavones available for dietetic purpose.[6] Isoflavones have been considered to have the effects of estrogen but relatively weakly because they have a significantly low affinity to estrogen receptor-α, a typical estrogen receptor.[7,8] However, they have a higher binding affinity to estrogen receptor-β than to estrogen receptor-α, and so the possibility for the tissue selective effect of isoflavones was suggested, which is that isoflavones would function more selectively on such organs as the thyroid gland, bones and blood vessels where estrogen receptor-β is mostly dispersed.[9] Because of these properties, soy isoflavones are reported to lower serum cholesterol and LDL-cholesterol particularly in postmenopausal women[6] and also reduce the risk of cardiovascular diseases.[10] Some *in vitro* studies have suggested that isoflavones might have estrogenic effects and at the same time, anti-estrogenic effects.[11,12] Because of this theory, there were some worries that isoflavones might not be good for growing and young women, for it would reduce the activation of endogenous estrogen.[13] On the contrary, according to some recent studies, isoflavones

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intake in infancy through soy-based infant formula may have advantages in the long term because of its possible hormone-dependent disease (cancer, osteoporosis, cardiovascular diseases, etc.)-preventive properties, which can be developed in the later part of adulthood.[14] However, disputes over this matter still remain unsettled.

There is a suggestion that the serum total cholesterol reduction effect of isoflavones-rich soy protein accompanies LDL-cholesterol reduction.[15] There is an opinion that the serum LDL-cholesterol reduction effect of soy protein is caused by the elimination of LDL-cholesterol in blood due to the increase of LDL receptor.[16] Reports concerning this include a report that the level of serum LDL-cholesterol depended on the mRNA expression of receptor and estrogen functions as an important regulator in the expression of LDL-cholesterol receptor to increase the expression of LDL receptor mRNA in estrogen treatments[17,18], and another report that genistein, a kind of isoflavones was also responsible for the increase of LDL receptor gene expression.[19] Sirtori *et al.*[20] concluded that soy protein contributed to the up-regulation of LDL receptor in the study where the effect of soy protein was examined with 1,000 persons or more over 20 years. On the other hand, however, he negated the effect of soy isoflavones. In the study carried out by Gardner *et al.*[21] who reported the effect of soy protein on lipid metabolism in postmenopausal women, LDL-cholesterol was lower in soy protein group than in the control group. However, observing no difference according to the diet with or without isoflavones in soy protein, they suggested that such effect in postmenopausal women could be attributable to soy protein rather than to isoflavones.

As mentioned earlier, it has been reported that soy protein with rich isoflavones has a good effect on serum lipid metabolism and prevention of cardiovascular diseases, but research results vary as to whether the effect comes from isoflavones and moreover, its mechanism has not been verified. Until now, as research on soy isoflavones is focused on the point that isoflavones has the properties of estrogen, most researches have been carried out with pre- and postmenopausal women or ovariectomized animals. However, no research has been carried out with growing female and it is not also clearly verified if soy isoflavones known as phytoestrgen are advantageous to growing period.

Furthermore, recent reports suggested that the development of arterial sclerosis which usually outbreaks in the adulthood could be associated with lipid profiles in the 10's[22] and the elevation of serum cholesterol in the childhood could be used as an important factor to foresee the possible outbreak of cardiovascular diseases in the adulthood.[23] As it is deemed important to manage serum lipid continuously during puberty to lower the risk rate of cardiovascular diseases, it is necessary to carry out research on soy isoflavones effects on lipid metabolism. In this context, the study attempts to examine the effects of soy protein according to soy isoflavone content on serum and hepatic lipid concentration, and LDL receptor gene expression which can be a mechanism of serum cholesterol reduction with growing female rats.

MATERIALS AND METHODS

1. Experimental Animals and Diets

Thirty six Sprague-Dawley female rats (60±5 g) were purchased from KLEC (Korea Life Engineering Co., Seoul, Korea). Rats were fed stock diets (rat chow made by Samyangsa) for a week of adaptation period. Then, they were randomly divided into three experimental dietary groups and provided experimental diets for 9 weeks. The experimental groups were classified into 1) a control group, 2) a soy protein isolate group: soy (+)) group and 3) a soy protein concentration group: soy (-)) group. The diets were basically formulated based on AIN-93G.[24] The composition of experimental diets is shown in Table 1. Protein sources used to determine effects of isoflavones were soy protein isolate, soy protein contained high isoflavones (isoflavones 3.4 mg/g protein) or soy protein concentrate, soy protein containing trace amounts

Table 1. Composition of experimental diets

(g/kg of diet)

Ingredients	Casein	Soy (-)	Soy (+)
Casein[1]	200	-	-
Soy protein concentrate[2]	-	226	-
Soy protein isolate[3]	-	-	202
Corn starch	530	504	528
Suc rose	100	100	100
Soybean oil	70	70	70
Cellulose	50	50	50
Min-mix[4]	35	35	35
Vit-mix[5]	10	10	10
L-cystine	3	3	3
Choline	2.5	2.5	2.5
Tert-butyl hydroquinone	0.014	0.014	0.014

[1] Casein high protein (total protein 85%), Teklad Test Diets, Madison, Wisconsin, USA
[2] Soy protein concentrate (total protein 75%), Protein Technologies International, St. Louis, MO, USA
[3] Soy protein isolate (total protein 84%, total isoflavones 3.4 mg/g protein), Protein Technologies International, St. Louis, MO, USA
[4] AIN-93G-MX, Teklad Test Diets, Medison, Wisconsin, USA
[5] AIN-93G-VM, Teklad Test Diets, Medison, Wisconsin, USA

isoflavones (isoflavones > 0.1 mg/g protein).

All rats were individually housed in stainless steel wire cages in an air-conditioned room with controlled temperature (25±2 ℃) and humidity (63±5%) and automatic lighting (alternation 12-h period of light and dark). The experimental diet and deionized water were provided *ad libitum*.

2. The Analysis of the Experiment

Measurement of food intake and body weight

During the experiment period, food intake was measured every other day and the weight of experimental animals, once a week at a specific time.

Sample preparation

After 9 weeks on the experimental diets, the rats were fasted for 24 hours and then etherized using ether. Blood samples were taken from the main artery and 20 mL of cold saline solution was perfused through the hepatic portal vein to excise liver tissues. The blood samples were left intact for 30 minutes and then centrifuged at 3000 rpm for 20 minutes to take out serum and then kept in a -70 ℃ deep freezer. The excised liver tissues were rinsed twice with 1% diethyl procarbonate (DEPC) solution and then quick-frozen with liquid nitrogen to be kept in -70 ℃ deep freezer until further analysis. All apparatuses used for the sacrifice of the rats were sterilized or rinsed with 1% DEPC solution before use in order to prevent RNAse contamination.

Sample analysis

Lipid analysis

Serum lipid profiles were enzymatically assayed by commercial kit (YD Diagnostics. Co., Korea). TG kit (BC118), cholesterol E kit (BC108-E) and HDL-cholesterol kit (BC 308-HDL) were used for the analysis of serum triglyceride, serum total cholesterol and HDL-cholesterol, respectively. LDL-cholesterol was calculated by the method of Friedewald formula[26] as follows:

LDL-cholesterol=(total cholesterol)-(HDL-cholesterol) -(triglyceride/5)

Atherogenic index, a variable to estimate the risk rate of arterial sclerosis was calculated as follows:

Atherogenic index={(total cholesterol-HDL cholesterol) /HDL cholesterol}

Lipids in the liver were extracted by the method of Folch *et al.*.[27] Triglyceride and total cholesterol in liver tissues were enzymatically assayed by commercial kit (YD Diagnostics. Co., Korea) in the same way as described for serum.

Analysis of hepatic LDL-receptor mRNA by using RT-PCR

First, total RNA was extracted from the liver tissues. After homogenizing 20 mg of the excised liver tissues, total RNA was extracted by using RNeasy Mini Kit (QIAGEN Cat. No. 74104). Second, after a transcriptase (QIAGEN)-added master mix was made, it was put into tubes by 18 mL and then added with 2 mL of the extracted RNA solution before reverse transcription for 60 minutes at 37 ℃ and then treated for 5 minutes at 93 ℃ to inactivate the reverse transcriptase. Third, after cDNA was amplified with template in polymerase chain reaction (PCR), the expression was checked through electrophoresis. The PCR reaction of LDL-receptor was repeatedly carried out 35 cycles at 94 ℃ for 15 seconds, at 60 ℃ for 15 seconds and at 72 ℃ for 15 seconds and then pre-denaturation at 94 ℃ for 3 minutes and post extension at 72 ℃ for 5 minutes before and after the reaction. For the controlled GAPDH (glyceraldehyde-3-phosphate dehydrogenases), it was carried out repeatedly 25 cycles at 95 ℃ for 30 seconds, at 56 ℃ for 30 seconds and at 74 ℃ for 45 seconds and then pre-denaturation at 95 ℃ for 2 minutes and post-extention at 72 ℃ for 7 minutes before and after the reaction. Products of Neurotics Inc. (5 U/mL) were used for Taq. DNA polymerase. Oligonucleotide primers was designed by reference to the sequence of LDL-receptor mRNA and the sequence of GAPDH mRNA expressed in the liver of Sprague-Dawley rats and Table 2 shows the sequence of oligonucleotides. Designed oligonucleotide primers used after request synthesis to Bioneer Co. (Daejeon, Korea).

After mixing 10 mL of the product finished of PCR reaction with 1.5 mL of dye, it underwent electrophoresis in 1.2% agarose gel. The electrophoretic gel was soaked in 0.5 μg/mL of ethidium bromide (EtBr) solution for dying and then put on the UV transilluminator to check DNA band and take a picture with a polaroid to preserve the finished product. In order to check the dye degree, the image of scanned DNA band was analyzed with the image analyzer (NIH image 1.61). The dye degree was

Table 2. Oligonucleotide primers used in the reverse transcription-polymerase chain reaction (RT-PCR)

Name	Direction	Sequence	Base number	GC%	Tm (℃)
LDL receptor	sense	5'-CAA-GAC-GTC-CTC-CCT -GGA-TGA -GTT-CC-3'	26 mer	58	66.5
	anti -sense	5'-CCA-GTC-TTC-GTC-ACA -CAC-AAA-CTG-3'	24 mer	50	63.6
GAPDH	sense	5'-ATC-AAA-TGG-GGT-GAT -GCT-GGT -GCT-G-3'	25 mer	48	66.4
	anti -sense	5'-CAG-GTT-TCT-CCA-GGC -GGC-ATG -TCA-G-3'	25 mer	60	68.2

expressed with the degree of GAPDH expression against the degree of LDL receptor expression.

3. Statistical Analysis

Based on the data attained in the present study, averages and standard deviations were calculated by SAS package for each experimental group. Comparisons between the experimental groups were made by One Way ANOVA analysis and matters of significance between the experimental groups were verified through Duncan's multiple range test.

RESULTS AND DISCUSSION

1. Weight, Food Intake and Food Intake Efficiency Ratio

The increase of weight, the mean food intake and food intake efficiency ratio (FER) observed for 9 weeks are shown in Table 3. The weight gains and mean food intake were not significantly different among the experimental groups. The weight gains and the mean food intake did not vary according to protein sources and soy isoflavone content. When looking at previous research on the effect of isoflavone addition on weight and food intake, Arjmani *et al.*[28] who supplied genistin, a separated soy isoflavone to postmenopausal models reported that there was no difference in weight gains between the ovariectomized group and sham group according to the genistin concentration. Kim[29] who added soy isoflavones to ovariectomized rats for 6 weeks also reported that there was no difference in mean food intake when varying the amount of isoflavone addition. In the present study which examined growing rats, there was also no difference in weight and the mean food intake according to soy protein and soy isoflavones.

FER was significantly higher in the control group than in the soy (+) group and soy (-) group ($p<0.05$), which indicates that there is no difference in FER between the soy protein intake groups but FER varies according to protein sources. The reason why there was no significant difference in weight between the two soy protein intake groups in spite of their low FER seems because the mean food intake of the soy protein groups was a little higher than that of the control group. When compared with the findings in previous research, the result that the FER of soy protein was lower than that of casein is identical with the result of Choi[30] who reported that the FER of soy protein was lower than that of casein in growing male rats.

Table 3. Effect of soy protein with isoflavones on weight gains, mean food intake and food intake efficiency ratio (FER) in growing female rats

Group	Weight gains (g)	Mean food intake (g/day)	FER[3]
Casein	160.8±12.31[1]	13.75±0.82	0.17±0.01[a2]
Soy (-)	158.9±13.92	14.62±1.35	0.16±0.01[b]
Soy (+)	160.6± 9.71	14.20±1.72	0.16±0.01[b]

[1] Mean±SD
[2] Values with different superscripts within the column are significantly different at $p < 0.05$ by Duncan's multiple range test
[3] Food intake efficiency ratio (FER)=weight gains (g)/total food intake (g)

2. Serum Lipid Concentration

Serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, HDL-cholesterol to total cholesterol content ratio (HTR) and atherogenic index are shown in Table 4. There was not significant difference between the experimental groups in serum triglyceride concentration. Serum total cholesterol concentration was significantly lower in the soy (+) and soy (-) groups which took soy protein than in the control group ($p<0.05$). The soy (+) group (83.93 mg/dl) which took soy protein isolate rich in isoflavones was lower in serum total cholesterol concentration than the soy (-) group (90.75 mg/dl) which took soy protein concentrate containing few isoflavones but the difference was not significant. In previous research, intake of soy protein with isoflavone reduced serum LDL-cholesterol in postmenopausal women, and serum LDL-cholesterol concentration was the lowest in groups highest in isoflavone content.[31] Zhou *et al.*[32] have verified that isoflavones exerted the cholesterol reduction effect on soy protein independently. He reported that serum LDL-cholesterol concentration was significantly reduced in groups taking soy protein isolate high in isoflavone

Table 4. Effect of soy protein with isoflavones on serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, HDL/total cholesterol (HTR) and atherogenic index in growing female rats

Group	Casein	Soy (-)	Soy (+)
TG (mg/dl)	53.11±10.60[1]	46.66± 6.45	50.33±9.94
Total cholesterol (mg/dl)	112.10±28.40[a2]	90.75±14.03[b]	83.93±9.59[b]
HDL-cholesterol (mg/dl)	86.77±15.08[a]	70.77±10.25[b]	73.17±5.56[b]
LDL-cholesterol (mg/dl)[3]	19.73± 7.28[a]	14.75± 5.62[ab]	9.07±4.70[b]
HTR[4]	0.68± 0.19	0.74± 0.18	0.74±0.17
Atherogenic index[5]	0.28± 0.14	0.18± 0.07	0.19±0.08

[1] Mean±SD
[2] Values with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test
[3] LDL-cholesterol=(total cholesterol)-(HDL-cholesterol)-(triglyceride/5)
[4] HTR=HDL-cholesterol / total cholesterol
[5] Atherogenic index={(total cholesterol-HDL cholesterol) / HDL cholesterol}

content than in groups taking soy protein isolate low in isoflavone content, and both in normocholesterolemic and hypercholesterolemic conditions, serum LDL-cholesterol concentration was significantly reduced in high isoflavone intake groups than in low isoflavone intake groups.[32] In the present study as well, soy protein isolate rich in isoflavones showed more outstanding LDL-cholesterol reduction effect than soy protein without isoflavones, which is similar with the findings in the previous research.[32] In addition, it is considered that the finding that soy protein isolate intake is responsible for the significant reduction both of serum total cholesterol and LDL-cholesterol supports the finding in the previous research,[16] which is that the serum total cholesterol reduction effect of soy protein accompanies LDL-cholesterol reduction.

Serum HDL-cholesterol concentration was significantly low in the soy (+) and soy (-) groups which were significantly low in total cholesterol concentration ($p<0.05$). However, when looking at the HDL-cholesterol to total cholesterol ratio (HRT), the control group was 0.68, while both soy (+) and soy (-) groups were 0.74. Insignificant as it was, atherogenic index was lower in both soy (+) and soy (-) groups which were soy protein intake groups than in the control group. In case of isoflavone-containing soy protein intake, the reduction of serum total cholesterol, LDL-cholesterol and triglyceride appeared very strongly only with a short-term intervention but it is reported that the increase of HDL-cholesterol was observed only in researches which lasted for 12 weeks or more.[5] Teixeira *et al.*[33] observed that serum cholesterol and LDL-cholesterol were reduced at 6 weeks after changing 20 g out of 50 g of the total protein supply from casein to soy protein but serum total cholesterol was reduced, while HDL-cholesterol and triglyceride concentration remained the same at 3 weeks after replacing 40 g out of 50 g of soy protein supply with soy protein. It was reported that the cholesterol reduction effect of isoflavones-containing soy protein is associated with the level and duration of intake, sex, the initial serum lipid concentration of subjects.[5,33] The reduction of total cholesterol and LDL-cholesterol was larger in men than in women and much larger in hypercholesterolemia.[5] Therefore, it is considered that when the experiment period is extended or subjects are changed in the following research, different HTR values can be obtained.

In the meantime, serum total cholesterol was reduced only with soy protein intake. As previous research suggested, a soybean component responsible for serum cholesterol reduction was the compositional difference of amino acids contained in soy protein, for instance, sulfur-containing amino acids content, lysine-to-arginine rate[34-36] other than soy fiber,[37] phytic acid,[38] soy saponin[39] and isoflavones[40]. In fact, however, soy protein used in the present study are mostly removed of fiber, saponin and phytic acid in soy processing. Because the soy protein used in the present experiment contains more arginine than casein by 2.3 times, it can be considered that the difference in amino acid composition could be the cause but it is difficult to jump to conclusions only with the findings in the present study. Recently, it is predominantly accepted that such cholesterol reduction effect of soy protein may result not from a specific component or an action but from various components working separately or reciprocally. Because it was also observed in the present study that serum cholesterol was reduced in the soy (-) group which took soy protein containing few isoflavones, the cholesterol reduction effect of soy protein cannot be entirely attributable to isoflavones. It is considered that other factors such as amino acid composition as mentioned earlier could be partially responsible for the effect. The possibility for unknown components cannot be also left out.

3. Hepatic Lipid Concentration

Hepatic triglyceride and total cholesterol concentration are shown in Table 5. The hepatic triglyceride showed no significant difference among the three groups, which implicates that protein sources and isoflavone content do not influence on hepatic triglyceride concentration in growing female rats. There was also no difference in hepatic total cholesterol according to protein sources or isoflavones content. Kim[29] carried out an experiment in which separated soy isoflavones were added to ovariectomized models, and reported that serum cholesterol reduction effect was observed but there was no change in hepatic lipid concentration, which is identical to the findings of the present study. Soy protein containing isoflavones significantly reduced serum total cholesterol and LDL-cholesterol but it depended on the level and duration of intake, sex and initial serum lipid concentrations of subjects.[5] It is considered that quite different results may come out if the experiment period is extended or there

Table 5. Effect of soy protein with isoflavones on hepatic triglyceride and total cholesterol in growing female rats

Group	Casein	Soy (-)	Soy (+)
TG (mg/g liver)	38.63±6.65[1)]	39.55±6.98	39.29±8.54
Total cholesterol (mg/g liver)	37.75±2.44	45.94±3.54	38.93±2.67

[1)] Mean±SD

is change in sex, or in hypercholesterolemic conditions.

4. Hepatic LDL Receptor mRNA

The level of hepatic LDL receptor mRNA measured with RT-PCR is shown in Fig. 1. The values represent the LDL receptor expression against the GAHDH expression. Table 6 shows relative rates of isoflavones-added groups on the condition that the value of the control group is 1. The hepatic LDL receptor mRNA level was significantly higher in the soy (+) group than in control group and soy (-) group ($p<0.05$). The hepatic LDL receptor mRNA level was higher in the soy (+) group than in the control group by 20%. The increase or decrease of LDL receptor can be regulated initially by the changes in the transcription stage or changes in the interpretation stage.[41,42] In the present study, as the hepatic LDL receptor mRNA level was directly measured, it is thought that soy protein rich in isoflavones which substantially increased the level of hepatic LDL receptor mRNA influenced on the hepatic LDL receptor transcription process in growing female rats. Of course, the possibility for down-regulation cannot be left out if LDL and LDL receptors combine to go into each cell and take apart very fast.

A few theses reported that the elevation of serum LDL-cholesterol was caused by the reduction of LDL receptor gene expression.[43,44] A number of theses[21,43,45-47] published recently observed that the intake of soy protein rich in isoflavones reduced the level of serum cholesterol in postmenopausal women and concluded that it resulted from the reduction of LDL-cholesterol. Considering that estrogen functions as an important regulator of LDL

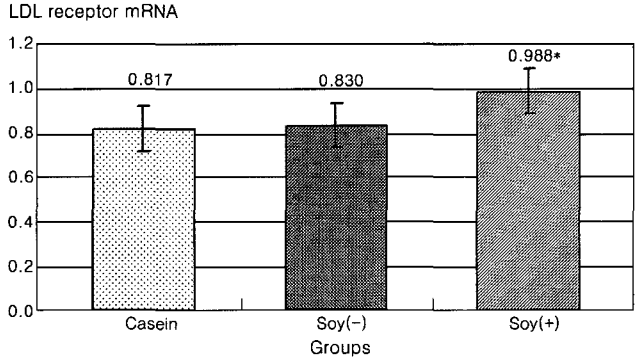

Fig. 1 Effect of soy protein with isoflavones on hepatic LDL receptor mRNA level in growing female rats

*: significant difference at $p < 0.05$.

Table 6. Effect of soy protein with isoflavones on relative percent of hepatic LDL receptor mRNA level

Group	Casein	Soy (-)	Soy (+)
relative %	100	101.6	120.9

receptor expression[17] and LDL receptor mRNA expression increases in postmenopausal women during hormone treatment,[48] it is suggested that there is a possibility that isoflavones weakly having the properties of estrogen can increase LDL receptor mRNA to reduce the level of serum LDL-cholesterol. In fact, however, research on the direct effect of isoflavones on LDL receptor mRNA expression can be found rarely.

When looking at recent previous researches, Lovati *et al.*[49] attributed the serum LDL-cholesterol reduction which was considered to have been caused by soy protein intake to the increase of LDL receptor activity. Potter[50] reported that in animals and men, the LDL receptor activity was increased after soy protein or soy extract intake.

Baum *et al.*[16] reported that in the experiment where soy protein was supplied to postmenopausal women for 6 months, serum non-HDL-cholesterol concentration was reduced compared to the control group and LDL receptor mRNA level in blood mononuclear cells was also increased. Kirk *et al.*[51] observed that isoflavones intake did not show serum cholesterol reduction in rats lack in LDL receptor, and isoflavones did not prevent the development of arterial sclerosis. But they reported that when LDL receptor was in normal condition, isoflavones intake reduced serum cholesterol and the risk of arterial sclerosis. They suggested that soy isoflavones reduced cholesterol by increasing the LDL receptor activity and the cholesterol reduction in turn worked against arterial sclerosis.[51]

The soy (+) group was the highest in the level of hepatic LDL receptor mRNA in the present study, which was similar with the findings in the previous research which observed that soy isoflavone intake reduced LDL-cholesterol by increasing hepatic LDL receptor mRNA.

In the present study, hepatic LDL receptor mRNA was increased significantly only in the soy protein with rich isoflavones group but in case of soy protein without isoflavones, or isoflavones addition as in the previous research,[52] it was increased insignificantly. Therefore, it is difficult to conclude that isoflavone-containing soy protein is entirely responsible for the effect but it can be presumed that soy protein could have worked in association with some other factors inside it.

SUMMARY AND CONCLUSIONS

In conclusion, soy protein isolate reduced serum total cholesterol and LDL-cholesterol concentration in growing female rats. Particularly, soy protein isolate rich in isoflavones

increased LDL receptor mRNA expression in the liver. Therefore, it is considered that the intake of soy isoflvones during puberty can be advantageous in terms of the long-term improvement of serum lipid. In addition, it is considered that the cholesterol reduction effect of soy protein cannot be entirely attributable to isoflavones but some other factors should have played a role partially in association with isoflavones.

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