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Effect of Onion Powder Supplementation on Lipid Metabolism in High Fat-cholesterol Fed SD Rats

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Abstract

This study was performed to examine the effects of onion powder supplementation on lipid metabolism in male SD rats fed a high fat and high cholesterol diet. Experimental groups were control (C), high fat fed group (HF), high fat+onion powder intake (OP), high fat+quercetin intake (Q). The HF diet contained 1% cholesterol, 4% lard, 0.3% Na-taurocholate, and quercetin supplementation level was 0.1 g/kg diet. The OP group showed lower body weight gains compared to the control, while there was no significant difference in food efficiency ratio efficiency. When the proportion of fecal bile acids per total lipids was calculated, there was a significant decrease in the HF group compared to the control group, while the levels of the control group was same as that of the OP group. There was no significant difference between the HF and Q groups in bile acid/total lipid in feces. The amount of total cholesterol in liver increased significantly in HF group compared to the control group, while total cholesterol decreased significantly in the OP group compared to the HF group. There was a significant decrease in GOT (glutamic oxaloacetic transaminase) activity in OP and Q groups compared to the HF group. In conclusion, feeding onion powder to hyperlipidemic rates appeared to control weight gain, significantly lower the level of total cholesterol in the liver, and recover GOT activity. We also demonstrated that onion powder intake was more effective than quercetin intake.

Key words: onion, quercetin, lipid metabolism, hyperlipidemic

INTRODUCTION

Cardiovascular diseases are the leading cause of death in Korea, as well as many of the Western countries. Apart from smoking, hypertension and diabetes, high LDL (low density lipoprotein) cholesterol levels are the primary cause of atherosclerosis. It has been shown that the onset and death rates for cardiovascular diseases can be reduced if the risk factor for coronary artery disease is controlled properly (1,2).

Onion (*Allium cepa* L.) is one of the major sources of dietary flavonoids in many countries (3,4). Many studies have found that quercetin is the most active of the flavonoids contained in many plants and exhibits strong free radical scavenging activity, and that its interaction with a variety of human proteins leads to a high level of biological activity which enhances dietary effects. The anti-arteriosclerotic properties of quercetin include a wide range of effects, including antioxidant, anti-inflammatory, anti-tumour and anti-clotting activity (5).

Recent epidemiological studies have reported that dietary fiber plays important physiological and metabolic roles in preventing chronic and degenerative diseases (6). Fruits and vegetables contain a wide variety of antioxidants including dietary fiber, antioxidant vitamins, flavonoid pigments, phenolic groups and aromatic amines, and there have been many studies on their physiological activity (7). In particular, onions are widely used in almost every type of food worldwide. World onion production has increased by at least 25% over the past 10 years with current production being around 44 million tons making it the second most important horticultural crop after tomatoes (5).

Onion has many powerful flavonoid pigment-containing compounds, such as quercetin and rutin, that are potent antioxidants. Also, onions contain allyl propyl disulfide, diallyl disulfide and others that help lower the level of lipids (8). Many studies have suggested that onions contain heavy metal detoxification, antibiotic, blood sugar reduction, cardiovascular disease prevention, xanthine oxidase inhibition, antioxidant, and anticancer components (9-11).

This study was performed to examine the effects of onion powder and quercetin supplementation on blood

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lipid profiles and bile acid in the liver in male SD rats fed a high fat and cholesterol diet.

MATERIALS AND METHODS

Diet and animals

Experimental groups were labeled as control (C), high fat fed group (HF), high fat+onion powder intake (OP), high fat+quercetin intake (Q) (Table 1). For this study, onion samples were collected from Changnyeong area and prepared to produce a fine powder that could pass through a #40 mesh.

Quercetin was isolated form the freeze-dried onion powder by using a solution containing 80% methanol. The dehydrated quercetin was analyzed using HPLC and showed that 100 g of onion powder contain 277.28 mg of quercetin. The onion powder composition was 50 g/kg diet of diet and the standard quercetin level was 0.01% (0.1 g of quercetin per 1 kg diet).

Male rats were bred in the Animal Unit under controlled conditions (22～24°C, RH 50～60%), and provided food and water *ad libitum*. The weight of each animal was 100 g (6-weeks old) at the beginning of the study and these animals were fed the specified diet for the 8-week study period. Daily feed intake and weekly body weight gain were routinely recorded throughout the experimental period using Computingscale (CAS Co., Korea).

Table 1. Composition of experimental diets (g/kg)

Ingredient	Group[1)]			
	C	HF	OP	Q
Casein	150	150	150	150
Corn starch	576	548	498	548
Sucrose	100	100	100	100
Corn oil	60	60	60	60
Soybean oil	40	40	40	40
Lard	-	40	40	40
Cholesterol	-	10	10	10
Cellulose	25	-	-	-
Mineral mix.[2)]	35	35	35	35
Vitamin mix.[3)]	10	10	10	10
Choline chloride	2	2	2	2
DL-methionine	1.8	1.8	1.8	1.8
Na-taurocholate	-	3	3	3
Onion powder (OP)	-	-	50	-
Quercetin	-			0.1
BHT[4)]	0.01	0.01	0.01	0.01
Total	999.81	999.81	999.81	999.91

[1)]C: control, HF: high fat fed group, OP: high fat+onion powder intake, Q: high fat+quercetin intake.
[2)]AIN-76 mineral mix.
[3)]AIN-76 vitamin mix.
[4)]Dibutylated hydroxytoluene

Collection of blood & fecal samples and rat organ extraction

Five days before the end of the 8-week feeding period, the fecal samples of the rat were collected every day, and were freeze-dried after measuring their weight. The feed container was removed and the rats were not fed 12 hours prior to sacrifice. After they were anesthetized with diethyl ether, blood samples were collected from the abdominal aorta, and the rat's internal organs such as the colon, liver, kidney, heart and spleen were extracted and weighed. The colon and liver were rapidly frozen with liquid nitrogen and stored in the freezer at -80°C. Some blood samples were put in a lithium-heparinic polystyrene tube, the alkaline comet assay on the blood sample was immediately performed within 2 hours and the remaining blood sample was centrifuged at 3000 rpm for 15 minutes. Then, the separated plasma was stored in a freezer at -80°C before measuring the amount of total lipids.

Measurement of lipid profile in plasma, liver and feces

Total cholesterol in blood plasma was measured using an Allain kit (Bioclinical system, Korea) (12). The HDL (high density lipoprotein) concentration was measured by the method same as total cholesterol after isolation of dextran sulfate-$MnCl_2$ precipitation (13). Triglyceride concentration was measured using the BCS kit (Bioclinical system, Korea) based on the lipase-glycerolphosphate oxidase method (14). LDL (low density lipoprotein)-cholesterol was calculated using the method of Friedewald [Total cholesterol－(HDL cholesterol－triglyceride/5)], and LDL-cholesterol levels were calculated using the method of Friedwald. The atherogenetic index was calculated as follows: Atherosclerotic index=(total cholesterol－HDL- cholesterol/HDL- cholesterol).

The amount of total lipids in the liver and fecal samples was determined using the method of Folch et al. (15) and the level of bile acids in the fecal sample was measured using a bile acid kit using the method of Tokunage et al. (16) after the extraction of bile acids from the feces. Blood GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) activities were measured using an automatic blood analyzer using a diagnostic test kit and reagent (need to state name of kit and its manufacturer) to evaluate liver functions.

Statistical analysis

Statistical analysis was performed using the SPSS program. The results were presented as mean±SEM and the differences among experimental groups were analyzed using a one-way analysis of variance (ANOVA) with Duncan's multiple range test at $p<0.05$.

Table 2. Food intake, weight gains and food efficiency ratio of the experimental group

Variables	Group[1)]			
	C	HF	OP	Q
Food intake (g/day)	15.6±1.9[ab2)]	15.7±1.4[ab]	14.7±2.1[a]	16.6±2.3[b]
Body weight gain (g/8 wk)	216.5±18.6[a]	231.3±23.8[ab]	208.1±23.0[a]	251.8±34.0[b]
FER[3)]	0.29±0.02[ns4)]	0.31±0.03	0.30±0.03	0.32±0.04

[1)]Refer to Table 1.
[2)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM of 8 rats for 8 weeks in each group.
[3)]Food efficiency ratio.
[4)]Not significant.

RESULTS AND DISCUSSION

Food intakes, weight changes and organ weight changes

While there was no significant difference in food intake among experimental groups, Q group showed significantly greater intakes of food and higher body weight gains compared to OP group. There was no significant difference in food efficiency ratio(Table 2).

Onions are very rich in fiber (19% in dry weight), and insoluble fiber which helps not only clear carcinogenic or potentially carcinogenic substances (including bile acid) more quickly, but also make these carcinogenic substances be more watery in the colon (5). It is also known that the soluble fiber intake reduces the risk of cardiovascular diseases by lowering cholesterol levels in the blood (17). While there is a significant difference in functions between the two fibers, onions are so rich in both soluble and non-soluble fiber that they can be very effective for weight loss, as well as for prevention against cardiovascular diseases (5,18).

The weights of extracted organs are shown in Table 3. We found a significant increase in the liver weight of the HF group compared to the control group. There was no significant difference in the heart and kidney weights between all experimental groups, However, there was a significant increase in the spleen weight of the HF group compared to the control group.

Table 3. Percent of organ weight of the experimental group (%)

Variables	Group[1)]			
	C	HF	OP	Q
Liver	2.8±0.1[a2)]	5.0±0.1[b]	5.3±0.4[c]	5.3±0.2[c]
Heart	0.4±0.0[ns3)]	0.4±0.0	0.4±0.0	0.3±0.0
Spleen	0.2±0.0[a]	0.3±0.1[b]	0.3±0.0[b]	0.3±0.0[b]
Kidney	0.7±0.0[ns]	0.6±0.0	0.6±0.1	0.6±0.0

[1)]Refer to Table 1.
[2)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM of 8 rats group.
[3)]Not significant.

Fecal lipid profiles

The fecal weight and lipid profile are shown in Table 4. There was a significant decrease in the fecal weight of the HF group compared to the control group, while no significant difference was found between the OP group and the control group. There was a significant decrease in the fecal weight of the Q group compared with the control and OP groups The total lipid content in feces were significantly higher in the HF group compared to other groups, and the Q group was significant higher than in control and OP group. In general we found that onion powder intake has no significant effect on the amount of total lipids in feces, except for the significant increase in fecal triglycerides. On the other hand, the addition of onion powder and quercetin could decrease

Table 4. Bile acid and lipid profiles in the feces of the experimental group

Variables	Groups[1)]			
	C	HF	OP	Q
Fecal weight (g wet wt/d)	0.9±0.1[c2)]	0.7±0.0[b]	0.8±0.1[c]	0.6±0.1[a]
Total lipid (mg/g dry wt)	27.4±4.4[a]	110.9±19.7[d]	68.4±8.5[b]	94.0±14.2[c]
Total cholesterol (mg/g dry wt)	4.2±0.5[a]	53.5±6.8[b]	47.5±3.9[b]	49.7±5.9[b]
Triglycerides (mg/g dry wt)	0.6±0.0[a]	1.2±0.1[a]	1.8±0.1[b]	2.0±0.1[b]
Bile acid (mg/g dry wt)	12.2±2.9[a]	34.0±3.6[b]	30.6±5.1[b]	30.8±5.2[b]
Bile acid/Total lipid (%, wt/wt%)	44.5±8.6[b]	30.6±12.3[a]	44.4±10.0[b]	32.8±10.9[ab]

[1)]Refer to Table 1.
[2)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM of 8 rats.

the amount of total lipids in feces compared to that of HF group, while fecal triglyceride levels was significant increase in Q group compared to the HF group, being similar to the OP group

When fecal excretion of total sterols was analyzed (19), neutral sterol was shown to account for 55% of the total sterols in feces, and bile acid accounted for 45%. The increases in the excretion of bile acids and sterols might be one of the mechanisms for lowering cholesterol (20). The results of this study of onion powder supplementation showed that onion or quercetin intake had little or no effect on the fecal bile acid contents in the HF group, although the fecal bile acid contents of the HF group and the control group were significantly different. When the proportion of fecal bile acids in total lipids was calculated, we found that there was a significant decrease in the HF group compared to the control group, that the proportion of the control group was same as that of the OP and Q groups.

Lipid profiles in the liver and plasma

The lipid profiles in liver are shown in Table 5. The HF group showed a significant increase in the amount of total lipids in the liver compared to the control group. When the onion powder was added to their diets, there was no significant difference in the amount of total lipids in liver. There were no significant differences in triglyceride concentration among the groups. The amount of total cholesterol significantly increased in the HF group compared to the control group, while it decreased significantly in the OP group.

The effects of onion powder supplementation on lipid profiles in plasma are shown in Table 6. The plasma triglyceride levels significantly decreased in the OP and HF groups compared to the control group. However, there was no significant difference in the effects of onion powder intake between the HF group and other diet groups. In addition, no significant differences were found in both the plasma HDL-cholesterol/total cholesterol proportion and atherosclerotic index (AI) among HF, OP and Q group.

It has been known that Na-taurocholate causes cholesterol accumulation in the liver (21). The group fed high fat-cholesterol diets with 0.3% Na-taurocholate showed significantly larger increases in the amount of total lipids in the liver compared to the control group. When onion powder was added, there was no significant change in the amount of total lipids. No significant difference was found in the triglyceride among groups. The total cholesterol concentration in liver increased significantly in the H group compared to the control group, while it decreased significantly in the OP group as compared to H group. The total cholesterol concentration of the quercetin group decreased slightly but was not significantly different from the H group. Quercetin in onions occurs

Table 5. Lipid profiles in the liver of the experimental group (mg/g wet wt)

Variables	Group[1)]			
	C	HF	OP	Q
Total lipids	31.4±10.1[a2)]	229.8±29.4[b]	200.1±24.1[b]	230.3±37.5[b]
Triglyceride	17.3±2.2[ns3)]	18.4±9.9	16.3±2.6	21.2±2.0
Total cholesterol	69.9±12.0[a]	90.3±3.9[c]	80.9±7.0[b]	82.4±5.3[bc]

[1)]Refer to Table 1.
[2)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM of 8 rats.
[3)]Not significant.

Table 6. Lipid profiles in plasma of the experimental group (mg/dL)

Variables	Group[1)]			
	C	HF	OP	Q
Triglyceride	65.1±2.6[b2)]	47.1±3.1[a]	39.75±1.1[a]	80.9±4.6[c]
Total cholesterol	49.0±3.8[a]	99.3±7.6[b]	101.88±6.2[b]	111.3±3.1[b]
HDL-Cholesterol	31.6±2.2[b]	17.8±1.7[a]	16.81±1.3[a]	15.9±0.8[a]
LDL-Cholesterol	4.4±3.4[a]	72.1±8.6[b]	77.11±6.1[b]	79.1±3.4[b]
HDL-C/TC[3)]	0.7±0.2[a]	0.2±0.1[b]	0.2±0.0[b]	0.1±0.0[b]
AI[4)]	0.59±0.33[a]	5.11±2.4[b]	5.33±2.0[b]	6.06±0.8[b]

[1)]Refer to Table 1.
[2)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM of 8 rats for 8 weeks in each group.
[3)]HDL-C/TC = HDL-cholesterol/Total cholesterol
[4)]AI (Atherosclerotic index) = (Total cholesterol − HDL-cholesterol)/HDL-cholesterol

Table 7. GOT and GPT activities in plasma of the experimental group (U/L)

Variables	Groups[1)]			
	C	HF	OP	Q
GOT[2)]	75.8±8.9[a4)]	162.6±45.0[c]	121.0±29.0[b]	142.3±35.9b
GPT[3)]	30.5±5.8[a]	72.8±28.0[b]	75.6±21.4[b]	61.3±22.8[b]

[1)]Refer to Table 1.
[2)]GOT: Glutamic oxaloacetic transaminase.
[3)]GPT: Glutamic pyruvic transaminase.
[4)]Values with the different letter within the same row are significantly different by Duncan's multiple range at $p<0.05$. Values are the mean±SEM for 8 rats.

mainly as a mixture of the 4'-monoglucoside and 3,4'-diglucoside and a number of studies indicate that these have a much greater bioavailability than quercetin from other food sources containing different sugar moieties (22,23).

When blood lipids were examined in this study, we found a significant decrease in plasma triglyceride levels in the OP and HF groups after the intake of 1% cholesterol, 4% lard and 0.3% Na-taurocholate. We also found that there was no significant difference in the effects of onion intake between the HF group and other experimental groups. In addition, no difference was found in both the HDL-cholesterol/total cholesterol proportion and atherosclerotic index (AI).

Some epidemiological studies (24,25) have reported that high intake of dietary flavonoids is associated with the reduction in the risk of cardiovascular diseases, and the studies carried out with two animal models reported that quercetin demonstrates anti-arteriosclerotic activity and blood lipid reduction activity. Ajay et al. (26) indicated that quercetin acutely improved vascular responsiveness in blood vessels from diabetic rats, and that these effects were mediated by enhanced endothelial nitric oxide bioavailability. It has been known that 7α-hydroxylase activity depending on cytochrome P450 is closely associated with cholesterol metabolism. Juzwiak et al. (27) reported that quercetin helps increase the amount of cytochrome P450 in the liver. It is possible, therefore, that adding the quercetin compound helps change cholesterol to bile acid by activating the microsomal 7α-hydroxylation in the liver.

Yugarani et al. (28) reported, however, when rats with dietary arteriosclerosis were fed quercetin for 10 weeks, the level of HDL-cholesterol increased but the level of LDL-cholesterol decreased, without any change in triglyceride and total cholesterol. Because the CETP (cholesteryl ester transfer protein activity) in the liver varies according to animal species, there is also a difference in the effects of flavonoids on plasma concentration of HDL and LDL. This experiment was performed with rats during an 8-week period and it seems, therefore, that the study period was not long enough to demonstrate the blood lipid level lowering activity of quercetin. Juzwiak et al. (27) reported that when rabbits with hyperlipidemia and arteriosclerosis were fed a high fat-cholesterol diet and evaluated after the addition of quercetin to their diet, no significant effect was found during the first 4-week period, but quercetin was effective in reducing triglycerides and cholesterol level elevated by high fat diet, after 12 weeks of the experiment.

GOT and GPT activities in plasma

The GOT and GPT activities in plasma are shown in Table 7. Increased activities of GOT and GPT have been shown in the liver injury (29). The GOT and GPT activities were significantly higher in the HF group compared to the control group, while GOT activity significantly decreased in the OP group and Q group compared to HF group, without any effect on GPT.

Based our findings, onion powder fed to hyperlipidemic rats controlled weight gain, significantly lowered the level of total cholesterol in the liver, and recovered GOT activity. Onions rich in polyphenol and fiber were more effective in triggering these effects compared to purified quercetin.

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