Effect of Chicory Extract on Triglyceride Metabolism in Rats

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We investigated the effect of chicory (*Chicorium intybus*) extract on triglyceride concentration and microsomal triglyceride transfer protein (MTP) activity in rats. The effect of water-soluble extract of chicory fed at the 2.0% and 4.0% (w/w) levels for 2 weeks on the concentration of serum triglyceride and the activity of hepatic microsomal triglyceride transfer protein (MTP) was investigated in male Sprague-Dawley rats. The triglyceride concentrations in serum of the chicory extract fed groups were significantly lower than in the control group. MTP activity, known to be essential for the assembly/secretion of apolipoprotein B-containing lipoproteins, was also significantly lower in the chicory extract groups than in the control group. The concentrations of other lipids in serum and liver and the activity of phosphatidate phosphohydrolase, the rate-limiting enzyme in triglyceride synthesis, showed no significant differences among in the chicory fed groups. These results indicate that dietary chicory extract decrease hepatic MTP activity and serum triglyceride concentration, and therefore reduces hepatic lipoprotein assembly and secretion.

Key words – Chicory extracts, Microsomal triglyceride transfer protein, Triglyceride, Phosphatidate phosphohydrolase

The liver plays a central role in lipid synthesis and in the lipoprotein metabolism. Triglyceride is synthesized in the smooth endoplasmic reticulum through the glycerol-3-phosphate metabolic pathway and that is catalyzed by glycerol-3-phosphate acylransferase, phosphatidate phosphohydrolase (PAP) and diglyceride acyltansferase[18]. Recent reports suggest that PAP is involved in the rate-limiting step of triglyceride synthesis[3,11]. This is based on the observation that changes in the activity of microsomal PAP under various physiological conditions are much greater than those of other enzymes involved in triglyceride synthesis[2,3,11]. Triglyceride, along with apolipoprotein, is assembled into very-low-density lipoprotein (VLDL) and secreted from the liver into the blood circulation by a process that MTP[19]. MTP exists in the lumen of the endoplasmic reticulum as a heterodimer with protein-disulfide isomerase and is involved in the transfer of triglyceride and cholesterol ester to newly synthesized apolipoprotein B-containing lipoproteins[13]. MTP is necessary for hepatocyte assembly and secretion of apolipoprotein B-100 containing lipoproteins. Abetalipoproteinemia is a genetic disease, a defect in the assembly and secretion of hepatic apo B containing lipoprotein, particular in VLDL[31]. Recent studies using hepatic specific MTP overexpressing[26] and knock-out mice[5,24] have demonstrated that MTP is rate-limiting for VLDL apolipoprotein-B secretion.

Recently, the action mechanisms of new compounds derived from natural products on lipid metabolism has been intensively investigated in experimental animals or in human volunteers[4,15]. Chicory (*Chicorium intybus*), which contains inulin and oligosaccharides, is recognized as a natural food ingredient in most European countries, Japan, and Korea [20,21]. Rats fed 1~5% chicory extracts had significantly lower serum apolipoprotein B concentration and apolipoprotein B/A-1 ration compared with the fiber-free diet feeding rats[14]. It has been reported that chicory extracts decrease the concentration of triglyceride in the serum of rats after one week, however, the mechanism underlying this effect has not been determined[23]. We have reported that both roasted and unroasted chicory extracts at the levels of 5% (w/w) reduces serum triglyceride concentration in rats and this effect was more pronounced in the unroasted chicory feeding rats compared with the roasted chicory feeding rats[8]. Therefore, in this study we investigated the effect for lower supplemented concentration of chicory extracts on both serum triglyceride concentration and hepatocyte MTP activity. Our results show a definite correlation between the activity of MTP in liver and the concentration of triglyceride in serum.

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Materials and Methods

Materials

Phosphatidic acid, phosphatidylcholine, trioleic acid and cardiolipin were purchased from Wako Pure Co. (Osaka, Japan). [^{14}C]Triglyceride (specific activity: 55 mCi/mM) was purchased from Amersham International (Amersham, UK). Water-soluble extract prepared from chicory were provided by KT&G (Daejon, Korea). All other chemicals and reagents were of the best commercial grade available.

Diets and animal experiments

Male Sprague-Dawley rats aged four weeks were purchased from Kyudo Experimental Animals Co. (Tosu, Japan) and housed individually in suspended wire-mesh stainless cages in a temperature controlled animal room (21~24℃) with a 12 hr light/dark cycle (07:00~19:00). Before the experimental started, rats were allowed free access to a basal semi-synthetic control diet (the control group) without chicory extract for 5 days. Rats were then divided into two experimental dietary groups and a control group of six rats apiece. The experimental diets received the basal semi-purified diet containing roasted chicory extract 2.5% (w/w) and 5.0% at the expense of sucrose (Table 1). Food and water were provided *ad libitum* for 2 weeks because the triglyceride-lowering effect induced by dietary oligofructose in rats was observed after one week[14]. Body weight and food intake were recorded every other day and everyday, respectively. At the end of the treatment period, the animals were killed by withdrawing blood from the abdominal aorta under light diethylether anesthesia after 12 hr starvation. Tissues were removed, weighed, and kept frozen at -80℃ until required for analysis.

Table 1. Composition of experimental diets (%)

Ingredients	Control	Chicory (%)	
		2.0	4.0
Casein	20.0	20.0	20.0
α-Corn starch	15.0	15.0	15.0
Palm oil	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0
AIN-93 mineral mixture	4.0	4.0	4.0
AIN-93 vitamin mixture	1.0	1.0	1.0
L-Methionine	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2
Chicory extract	-	2.0	4.0
Sucrose	to make 100		

Lipid analysis

The serum was separated by centrifuging blood at 3,000 rpm for 15 min. Total cholesterol (Cholesterol C-test), high-density lipoprotein (HDL)-cholesterol (HDL-cholesterol E-test), triglyceride (Triglyceride E-test) and phospholipid (Phospholipid C-test) in serum were measured enzymatically by using commercial kits (Wako Pure Chemical Ind., Osaka, Japan). Free fatty acid in serum was measured enzymatically using commercial kits from NEFA zaimu-S kit (Eiken Ind., Tokyo, Japan). Liver lipids were extracted and purified by the method of Folch *et al.*[10] The concentrations of triglyceride, cholesterol, and phospholipid in lipid extracts were measured by methods of Fletcher[9], Sperry and Webb[25], and Bartlett[1], respectively.

Preparation of hepatic subcellular fractions and assay of protein concentration

Portions of the fresh livers from individual rats were homogenized in ice-cold 0.25 M sucrose solution containing 10 mM Tris-HCl buffer (pH 7.4), 1 mM ethylenediamine tetraacetate (EDTA) and 0.2 mM dithiothreitol. Microsomal and cytosolic fractions were prepared as described previously [3], and stored at -80℃ until required for enzyme activity assay. Proteins were measured by the method of Lowry *et al.*[22] using bovine serum albumin as a standard.

Assay of PAP and MTP activity

Phosphatidate phosphohydrolase (PAP; EC 3. 1. 3. 4) was assayed using a minor modification of the method described by Walton and Possmayer[29]. MTP activity was determined by measuring the transfer of radiolabeled triglyceride between two populations of unilamellar vesicles by using the method by Wetterau *et al.*, with minor modification[31]. Radioactivity was measured with a liquid scintillation counter (Wallac system 1410, Pharmacia, Finland).

Statistical anlysis

All values are presented as means±SE. Data were analyzed using one way analysis of variance (ANOVA), and differences were analyzed using Duncan's new multiple-range test[7]. Statistical significance was accepted when $p<0.05$.

Results

Food intakes, body weight and tissue weight

Food intakes, body weights and tissue weights are shown in Table 2. No significant difference was found among the

experimental groups in terms of body weight, food intake, and tissue weight.

Lipid concentrations of Serum and liver

The concentrations of serum lipids are shown in Table 3. The concentrations of triglyceride in the chicory groups were significantly lower than in the control group. This triglyceride-lowering effect of chicory extracts was more pronounced in the high consumption chicory-feeding group. The concentrations of total cholesterol, HDL-cholesterol, phospholipid and non-esterified fatty acid in serum were comparable in the chicory groups and the control group. The concentrations of triglyceride, cholesterol and phospholipid in liver are shown in Table 4. Liver lipids did not differ significantly between the control group and the chicory groups.

PAP and MTP activities

Chicory extracts had no influence on the activity of phosphatidate phosphohydrolase (Fig. 1). Liver MTP activity was significantly lower in both chicory extract groups than in the control group (Fig. 2).

Discussion

Chicory extracts had no influence on the body weight, food intake, and tissue weight in the present study condition. Because the supplementation of dietary chicory extracts in

Table 2. Effect of dietary chicory extracts on body weight, food intake, and tissue weight in rats

Ingredient	Control	Chicory (%)	
		2.0	4.0
Initial body weight(g)	97.24±2.68	98.11±2.11	68.01±2.14
Final body weight(g)	186.8±2.42	187.50±3.55	191.77±5.19
Food intake(g/day)	15.35±0.34	17.83±0.82	17.09±0.94
Tissue weight(g)			
Liver	10.74±0.18	10.10±1.65	10.62±0.99
Visceral adipose tissue	2.17±0.11	2.23±0.18	2.26±0.24

Rats were fed a semi-purified diet containing chicory extract at the 2.0% and 4.0%(w/w) levels for 2 weeks.
Values are means±SE of six rats per group.

Table 3. Effect of dietary chicory extracts on the concentration of serum lipids in rats

Ingredient	Control	Chicory (%)	
		2.0	4.0
		(mg/dL)	
Triglyceride	183.77±14.25[a]	110.12±11.84[b]	81.09±7.48[b]
Phospholipid	188.47±8.20	181.69±10.21	187.55±8.97
Total Cholesterol	96.65±5.23	86.74±6.89	86.17±9.25
HDL-Cholesterol	69.92±4.74	65.31±4.32	72.92±4.38
Non esterified fatty acid (μEq/L)	473.38±39.44	525.99±37.21	492.21±23.66

Rats were fed a semi-purified diet containing chicory extract at the 2.0% and 4.0% (w/w) levels for 2 weeks.
Values are means±SE of six rats per group.
Between the groups, values with different letters are significantly different at $p<0.05$.

Table 4. Effect of chicory extracts on the concentrations of liver lipids in rats

Ingredient	Control	Chicory (%)	
		2.0	4.0
		(mg/g liver)	
Triglyceride	16.86±2.34	18.55±3.92	18.47±1.98
Phospholipid	19.12±1.04	22.81±2.89	19.74±1.55
Total cholesterol	2.56±0.21	2.58±0.22	2.44±0.23

Rats were fed a semi-purified diet containing chicory extract at the 2.0% and 4.0%(w/w) levels for 2 weeks.
Values are means±SE of six rats per group.

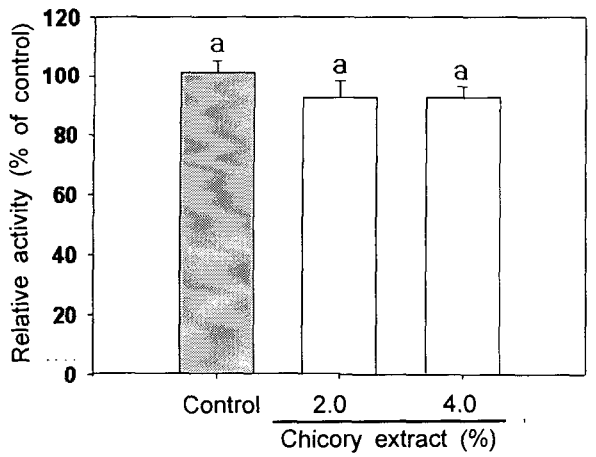

Fig. 1. Effect of chicory extract on the activity of microsomal phosphatidate phosphohydrolase (PAP) in rat liver. Rats were fed a semi-purified diet containing chicory at the 2.0% and 4.0% (w/w) levels for 2 weeks. Values with different letters are significantly different at $p < 0.05$. (mean±S.E., n=6).

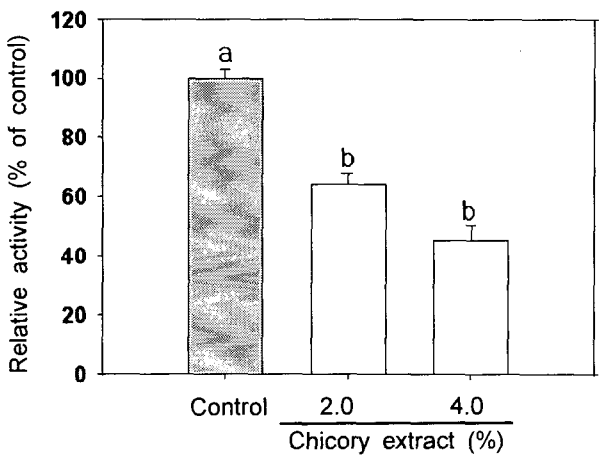

Fig. 2. Effect of chicory extract on the activity of microsomal triglyceride transfer protein (MTP) in rat liver. Rats were fed a semi-purified diet containing chicory at the 2.0% and 4.0% (w/w) levels for 2 weeks. Values with different letters are significantly different at $p < 0.05$. (mean±S.E., n=6).

diet had no influence on the growth, appearance or behavior of these rats, suggesting that chicory extracts have no toxic effect at the experimental condition used.

The concentrations of triglyceride in the roasted-chicory groups were significantly lower than in the control group. This triglyceride-lowering effect of chicory extracts was more pronounced in a dose-dependent manner. Our previous study also reported that roasted chicory extracts at the levels of 5% reduces serum triglyceride concentration[23]. It has been reported that the triglyceride-lowering effect in serum appears 1 week after placing male rats on a diet containing 10% chicory fructooligosaccharides, but reductions in cholesterolemia requires treatment for 10 weeks[8]. This early hypotriglyceridemic effect is exclusively due to a reduction in the VLDL fraction of the lipoproteins in serum. It has also been demonstrated that the oligofructose-like synthetic fructooligosaccharides called Neosugar similarly reduce triglyceridemia by 30% in rats fed for 6 weeks with a supplemented (10% or 20%) diet[15]. The findings of the study also agree with previous observation that one of the chicory components, inuline, which is known as an effective non-digestible carbohydrates, reduces the concentration of triglyceride in serum and liver[27,28].

Triglyceride biosynthesis in the liver occurs via the glycerol-3-phosphate pathway and is catalyzed by three enzymes; glycerol-3-phosphate acyltransferase, phosphatidate phosphohydrolase and diacylglycerol acyltransferase[18]. Recent findings demonstrated that phosphatidate phosphohydrolase is a rate-limiting enzyme in triglyceride synthesis[2,3,11,19]. Therefore, in order to elucidate the mechanism by which chicory extracts reduces the concentration of triglyceride in serum, the activity of phosphatidate phosphohydrolase was measured. Chicory extracts had no influence on the activity of phosphatidate phosphohydrolase (Fig. 1), suggesting that they had no inhibitory effect on triglyceride biosynthesis in liver under our experimental conditions. However, several studies have found that hepatocytes isolated from the liver of rats fed with chicory fructooligosaccharides reduced the incorporation of [^{14}C]-labeled acetate or palmitate in both their intercellular triglycerides and extracellular VLDL compared to control liver cells[14,16]. Furthermore, all activities of liver enzymes related to lipogenesis, including, glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthase, ATP-citrate lyase and acetyl CoA carboxylase, which catalyses a key step in the synthesis of fatty acids, were also significantly reduced[16]. This required chronic feeding (at least 5~6 weeks or more) of an oligosaccharide supplement to diet. Therefore, it remains another precise mechanism on early triglyceride-lowering effect in rats fed with a diet containing chicory extracts.

MTP catalyzes the transport of triglyceride between membranes and is necessary for the secretion and assembly of apo B containing lipoproteins[12,31]. Recently, it was reported that the activity of MTP and its mRNA level are acutely regulated by fatty acids, garlic and cycloalliin, as dietary components in animals, and in human hepatoma

HepG2 cells and intestinal carcinoma Caco-2 cells[6,17,19,30]. The hypotriglyceridemic effect of dietary components was paralleled by the inhibition of MTP in liver, which has been recently reported[6,17,19,30]. The activity of MTP was measured in order to elucidate the mechanism of the triglyceride-lowering effect of chicory extracts. Liver MTP activity was significantly lower in both chicory extract groups than in the control group (Fig. 2). A positive correlation (r=0.86) was also observed between liver MTP activity and serum triglyceride concentration, which suggested a mechanism for hypotriglyceridemic effect of chicory extract. Our results indicate that the decrease in liver MTP activity induced by chicory extract is associated with a impairment in the assembly and secretion of lipoprotein. The serum apolipoprotein B/A-1 ration in rats fed diet containing 1 or 5% chicory extracts for 4 weeks was significantly lowered due to significant reduction in apolipoprotein B concentration [14]. The decreased hepatic MTP activity and in serum triglyceride concentrations after dietary chicory extract administration observed in the present study may be important in the prevention and treatment of hypertriglyceridemia. However, the detailed mechanism by which chicory extract inhibits the activity of MTP in the liver remains to be clarified.

In conclusion, the present study indicates that dietary roasted-chicory reduces hepatic MTP activity, and thus, serum triglyceride concentration in rats.

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초록 : 흰쥐의 중성지질 대사에 미치는 치커리 추출물의 영향

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치커리 수용성 추출물을 2% 및 4% 수준으로 첨가한 식이를 Sprague-Dawle계 흰쥐에 2주간 투여하여 중성지질 대사에 미치는 영향을 검토하였다. 혈청 중성지질 농도는 대조군에 비해 치커리 추출물 투여군에서 현저히 감소하였다. Apolipoprotein B-함유 lipoprotein의 합성 및 분비에 필수적인 간장 microsomal triglyceride transfer protein (MTP) 활성도 치커리 투여군에서 현저히 감소하였다. 간 조직에서 중성지질 합성의 중요 조절효소로 알려진 phosphatidate phosphohydrolase (PAP) 활성은 각 실험군간에 큰 차이는 없었다. 또한 혈청 및 간 조직의 콜레스테롤, 인지질, 유리지방산 농도도 각 실험군간에 유의적인 차이는 없었다. 이상의 실험결과에서 치커리 수용성 추출물 2% 및 4% 첨가는 간 조직에서 혈중으로 분비하는데 중요한 역할을 하는 MTP 활성의 감소가 혈청 중성지질 농도의 감소에 영향을 미치는 중요한 요인으로 사료되었다.