Properties and Cholesterol Lowering Effect of Cholesterol-reduced Milk Supplemented with Evening Primrose Oil

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ABSTRACT : This study was carried out to investigate the changes of chemical and sensory properties and cholesterol lowering effect of milk treated with β-cyclodextrin to reduce cholesterol and supplemented with evening primrose oil (EPO). The average cholesterol removal rate was 93.5%. The thiobarbituric acid (TBA) absorbance increased proportionally to storage period and amount of EPO addition. TBA absorbance was significantly different in the EPO-added groups from that in unadded groups in all periods. The production of short-chain free fatty acids (FFA) increased with longer period of storage. From 6 days of storage, the amounts of short-chain FFA in 6 and 10% EPO-added groups were significantly different from other groups. The scores for all sensory characteristics indicated that sensory quality decreased with both storage time and increasing amount of EPO. Oxidative off-flavor and off-taste were more intense with higher amounts of EPO addition and longer storage period. Also, the higher the amount of EPO addition, the lower overall scores throughout the 15 day storage. Feeding 10% EPO-supplemented cholesterol-reduced milk increased high density lipoprotein (HDL) in male Sprague-Dawley rats by 76%, which is significantly different from the control (27%). ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 1041-1047)***

Key Words : Evening Primrose Oil, Blood Cholesterol, β-Cyclodextrin, Milk

INTRODUCTION

Death from coronary heart disease is a major cause of mortality in many economically affluent societies like the USA, Australia and the UK (Charnock, 2000). With all its limitations, total plasma cholesterol remains the best single predictor of coronary heart disease risk in a population, especially if a time lag is introduced into the calculation (Horrobin and Manku, 1983). The predictive value of the total cholesterol may be improved slightly by consideration of the ratio between low density lipoproteins (LDL) cholesterol and high density lipoproteins (HDL) cholesterol.

When the importance of cholesterol was first appreciated, a number of studies were carried out on the effects of various diets on blood cholesterol levels. To produce clinically important changes in plasma cholesterol, large changes in dietary patterns must be achieved. The magnitude of the required changes has led to bitter arguments among experts concerning the practicality of achieving them. Nevertheless, most organizations concerned with cardiovascular health have felt confident enough to recommend substantial increases in polyunsaturated fatty acids (PUFA) intake and decreases in saturated fat intake. The arguments for this have been admirably summarized in the most recent diet heart statement from the American Heart Association (Expert panel on detection, 2001; Grundy et al., 1982). The sustained campaign has led to large increases in PUFA intake in some countries, such as the USA, and such increases may in part be responsible for the recent fall in coronary disease mortality (Horrobin and Manku, 1983). Given the importance of the issue and the number of investigators involved, little attention has been paid to the question of how PUFA lower plasma cholesterol.

Evening primrose (*Oenothera spp.*, particualtly *Oenothera* biennis) is of special interest because its seed contains an oil characterized by its content of γ-linolenic acid (all cis-6:9:12-octadecatrienoic acid) (Hudson, 1984). Particular interest attaches to the recent observation that γ-linolenic acid is present in human milk fat (Hudson, 1984). Indeed, this acid is probably more widely distributed than is at present realized since simple methods have only recently become generally available to identify and differentiate it from α-linolenic acid.

At present, evening primrose oil is the most important source of γ-linolenic acid, which is in growing demand for its clinical and pharmaceutical applications as a very active essential fatty acid, and the precursor of prostaglandin E1 and its derivatives (Hudson, 1984). Although the evening primrose plant does not produce a high yield of seeds compared with the well-known commercial oilseeds, it is preferred to other sources of γ-linolenic acid because it is easy to produce and because it dose not contain any α-linolenic acid.

Despite numerous demonstrations of the cholesterol-lowering property of PUFA, the precise mechanism of their abilities is still not fully understood (Jackson et al., 1978;

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Paul et al., 1980; Haung et al., 1984; Sugano et al., 1986; Ihara-Watanabe et al., 1999). Fairly large amounts of PUFA are required to produce a substantial and meaningful reduction of plasma cholesterol. A modest increase in PUFA intake, however, may in part be responsible for the recent fall in coronary disease mortality (Samuel et al., 1983; Stallones, 1983) and the incidence and severity of cardiac dysfunction (McLennan et al., 1989; Charnock et al., 1991).

Based on above information, we proposed that the effect of PUFA on lowering plasma cholesterol level could be more effective when added in cholesterol-reduced food products. Several studies, including our laboratory, have indicated that the cholesterol in food, including milk, cream and cheese, was effectively reduced by β-cyclodextrin (β-CD) (Oakenfull and Sidhu, 1991; Makoto et al., 1992; Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2001). Because β-CD is nontoxic, edible, non-hygroscopic, chemically stable and easy to separate (Nagamoto, 1985), it has positive attributes when used for cholesterol removal from foods. Therefore, this study was designed to examine the chemical and sensory properties and blood cholesterol lowering effect of cholesterol-reduced milk supplemented with evening primrose oil.

MATERIALS AND METHODS

Materials

Commercial milk (3.6% milk fat) was purchased from a retail store as needed, and β-CD (purity 99.1%) was obtained from Nihon Shokuhin Cako Co. Ltd. (Osaka, Japan). Evening primrose oil (7% γ-linolenic acid containing EPO) was obtained from (Il-dong Pharmaceutical Co., Seoul, Korea). Cholesterol and 5α-cholestane were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and all solvents were gas-chromatographic grade.

Manufacture of evening primrose oil-added milk

To manufacture cholesterol-reduced milk supplemented with EPO, first, cholesterol was removed as follows. Fifty grams of milk was placed in a 1,000 ml beaker, and 1% β-CD was added. The mixture was stirred at 800 rpm with a blender (Tops: Misung Co., Seoul, Korea) in a temperature-controlled water bath at 10°C for 10 min. The mixture was centrifuged (HMR-220IV; Hanil Industrial Co., Seoul, Korea) with 166×g for 10 min. Second, different concentrations of EPO (0, 2, 6, and 10%) was added to cholesterol-reduced milk, and homogenized at 1,000 psi at 50°C in a single stage homogenizer (HC 5000, Microfluidics Corp., Newton, MA, USA) (Shim et al., 2004). The milk was stored for 12 h in refrigerator as 0 day sample and all treatments were triplicate.

Extraction and determination of cholesterol

For the extraction of cholesterol from milk, 1 g of the β-CD-treated milk was placed in a screw-capped glass tube (15 mm×180 mm), and 1 ml of 5α-cholestane (1 mg/ml) was added as an internal standard. The sample was saponified at 60°C for 30 min with 5 ml of 2 M ethanolic potassium hydroxide solution (Adams et al., 1986). After cooling to room temperature, cholesterol was extracted with 5 ml of hexane (Adams et al., 1986). The process was repeated four times. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was redissolved in 1 ml of hexane and was stored at -20°C until analysis.

Total cholesterol was determined on a silica fused capillary column (HP-5, 30 m×0.32 mm I.D.×0.25 μm thickness) using Hewlett-Packard 5890A gas chromatograpyh (Palo Alto, CA, USA) equipped with a flame ionization detector. The temperatures of injector and detector were 270 and 300°C, respectively. The oven temperatures were programmed from 200 to 300°C at 10°C /min and held for 20 min. Nitrogen was used as a carrier gas at a flow rate of 2 ml/min with a split ratio of 1/50. Quantitation of cholesterol was done by comparing the peak areas with a response of an internal standard.

The percentage of cholesterol reduction was calculated as follows: cholesterol reduction (%) = 100-(amount of cholesterol in β-CD-treated cheese×100/amount of cholesterol in control). Cholesterol determination for control was averaged with each batch of treatment.

Thiobarbituric acid (TBA) test

Lipid oxidation products in cholesterol were measured using a TBA test at 4°C for 15 days (Hegenauer et al., 1979). Oxidation products were analyzed spectrophotometrically. The reagent for the TBA test was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which was neutralized with NaOH and 2 M H_3PO_4/2 M citric acid. Reactions of the TBA test were started by pipetting 5 ml of milk containing EPO into a glass centrifuge tube and mixed thoroughly with 2.5 ml of TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min and cooled on ice. Ten milliliters of cyclohexanone and 1 ml of 4 M ammonium sulfate were added and centrifuged at 2,490×g for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm measured spectrophotometrically in a 1-cm light path. All measurements were run in triplicate.

Analysis of short-chain free fatty acid

The treated milk sample (1 ml) was taken periodically, stored for 0, 3, 6, 9, 12 and 15 days, and extracted with diethylether and hexane for 2 h, and eluted through a 10

Table 1. Composition of 40% beef tallow modified AIN-76A purified rodent diet with 5% cholesterol and 0.5% cholic acid

Ingredient	g/kg
Casein, high nitrogen	200
Corn starch	150
Beef tallow	400
Sucrose	95
Cholesterol	50
Cellulose	50
Mineral mix[1]	35
Vitamin mix[2]	10
Cholic acid	5
DL-methionine	3
Choline bitartrate	2

[1] AIN-76 mineral mix (g/kg): $CaHPO_4$ 500, NaCl 74, K citrate monohydrate 220, K_2SO_4 52, MgO, Mn carbohydrate 3.5, Fe citrate 6.0, Zn carbonate 1.6, Cu carbonate 0.3, KIO_3 0.01, $Na_2SeO_4 \cdot H_2O$ 0.01, $CrK(SO_4) \cdot 12H_2O$ 0.55, Sucrose 118.

[2] AIN-76 vitamin mix (g/kg): thiamin·HCl 0.6, riboflavin 0.6, phydoxine·HCl 0.7, nicotinic acid 3, D-calcium pantothenate 1.6, folic acid 0.2, D-biotin 0.02, cyanocobalamin 0.001, retinyl palmitate 0.8, DL-α-tocopheryl acetate 20, cholecalciferol 0.00025, menaquinone 0.005.

mm i.d. glass column containing neutral alumina as described by Kwak et al. (1990). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used. The quantitation of FFA was achieved using a 15 m× 0.53 mm i.d. Nukol fused-silica capillary column (Supleco Inc., Bellefonte, PA, USA). The GC was operated with helium carrier gas at 2 ml/min, hydrogen gas 37 ml/min, and air at 300 ml/min. The column oven was programmed as an initial holding for 1 min at 110°C and first level holding to 180°C at 5°C/min for 10 min and holding for 20 min. Both temperatures for injector and detector were 250°C. All quantitative analyses were done by relating each peak area of individual FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of standard.

Sensory analysis

Seven trained sensory panelists evaluated randomly coded milks. Oxidative off-flavor, off-taste, and bitterness were evaluated on a 7-point scale (1 = very slight and 7 = very strong). Overall quality was scored on a 7-point scale (1 = dislike extremely and 7 = like extremely).

Animals and diets

Male Sprague-Dawley rats obtained from the Jung-Ang Lab. Animal, Inc. (Seoul, Korea) weighing 60 to 75 g were placed individually in stainless-steel wire cages in a windowless room and were subjected to a light cycle with the light period from 1500 to 0300 and the dark period from 0300 to 1500. The rats were acclimatized for 1 week and fed a commercial rat chow during this period. All diets were formulated as recommended by the American Institute of Nutrition (Tables 1 and 2). All animals were fed a 40% beef tallow modified rodent diet with 5% cholesterol and 0.5% cholic acid for 5 weeks, and normal rodent diet containing 2 different milks for 6 weeks *ad libitum*. Animals were given free access to tap water via a stainless steel delivery system.

Table 2. Composition of fat free AIN-76A purified diet

Ingredient (%)	g/kg
Casein	200
Corn starch	150
Sucrose	550
Cellulose	50
Salt mix[1]	35
Vitamin mix[2]	10
DL-methionine	3
Choline bitartrate	2

[1] AIN-76 mineral mix (g/kg): $CaHPO_4$ 500, NaCl 74, K citrate monohydrate 220, K_2SO_4 52, MgO, Mn carbohydrate 3.5, Fe citrate 6.0, Zn carbonate 1.6, Cu carbonate 0.3, KIO_3 0.01, $Na_2SeO_4 \cdot H_2O$ 0.01, $CrK(SO_4) \cdot 12H_2O$ 0.55, Sucrose 118.

[2] AIN-76 vitamin mix (g/kg): thiamin·HCl 0.6, riboflavin 0.6, phydoxine·HCl 0.7, nicotinic acid 3, D-calcium pantothenate 1.6, folic acid 0.2, D-biotin 0.02, cyanocobalamin 0.001, retinyl palmitate 0.8, DL-α-tocopheryl acetate 20, cholecalciferol 0.00025, menaquinone 0.005.

Two different milk groups were as follows: 1) control, fed a commercial milk containing no EPO and 2) EPO-added group (EPO), fed a cholesterol-reduced milk supplemented with 10% EPO. To examine blood analysis, animals were fasted for 12 h and 1.5 ml blood sample was withdrawn from a tail and centrifuged at 3,000 rpm for 10 min, and stored at -20°C until analysis. Total blood cholesterol, triglyceride, and HDL were measured by kit from Fuji Photo Film Co., LTD. (Kanagawa-ken, Japan).

Statistical analysis

Data from the determination of optimum conditions of milks, one-way ANOVA (1985) was used. The significance of the results was analyzed by the least significant difference (LSD) test. Difference of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Cholesterol removal rate

The cholesterol content of control milk was 13.4 mg/100 g and the cholesterol reduction reached 93.5% with 1% β-CD treatment. This result is consistent with our previous study, indicating 95% cholesterol reduction rate in milk (Lee et al., 1999). Efficient removal of cholesterol removal, over 90%, using β-CD was demonstrated in our laboratory (Ahn and Kwak, 1999; Lee et al., 1999; Kwak et al., 2001).

TBA test

Polyunsaturated fatty acids like EPO are susceptible to oxidation, resulting in oxidative off-flavor with the

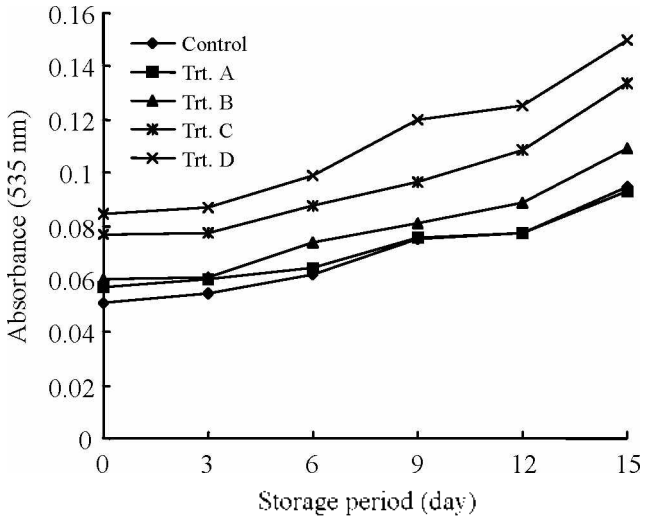

Figure 1. Changes of thiobarbituric acid values (TBA) for evening primrose oil-added and cholesterol-reduced milk stored at 4°C for 15 days. Control, not treated and added; Trt.A, milk was treated with 1% β-CD; Trt B-D, 2, 6, and 10% EPO was added in cholesterol-reduced milk, respectively.

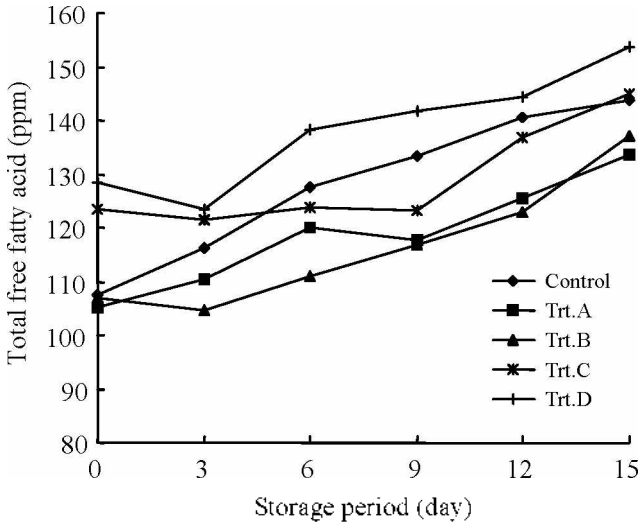

Figure 2. Production of total free fatty acid in evening primrose oil-added and cholesterol-reduced milk stored at 4°C for 15 days. Control, not treated and added; Trt.A, milk was treated with 1% β-CD; Trt B-D, 2, 6, and 10% EPO was added in cholesterol-reduced milk, respectively.

development of an unpleasant odor and flavor. Therefore, lipid oxidation levels associated with EPO supplementation of milk was measured via the TBA test during 15 days of storage, and the results are shown in Figure 1. The treatments were divided into five different groups as follows: (1) Control: commercial milk without β-CD treatment, (2) Trt A: milk with 1% β-CD treatment and 0% EPO addition, (3) Trt B: milk with 1% β-CD treatment and 2% EPO addition, (4) Trt C: milk with 1% β-CD treatment and 6% EPO addition, and (5) Trt D: milk with 1% β-CD treatment and 10% EPO addition.

In all groups, TBA absorbance increased proportionally to storage period and amount of EPO addition. In Trt D (10% EPO-added β-CD-treated milk), TBA absorbance increased dramatically from 0.083 to 0.15 from 0 to 15 days. TBA values for all EPO groups in all periods were significantly different than for Control and Trt A. Even though 10% EPO was added, the TBA value reached at most 0.15 during 15 day storage. Therefore, this result indicated that chemical lipid oxidation proceeded more highly in milk with EPO-added than in milk without EPO, regardless of β-CD treatment. However, that value may not show a significantly adverse changes in chemical and sensory characteristics.

Production of short-chain free fatty acids (FFA)

The production of short-chain FFA in control and experimental milks stored for 15 days at 5°C is shown in Table 3. The amounts of individual short-chain FFA, especially C_4 and C_6 were higher in milks in Trt C and D, which had 6 and 10% EPO added, respectively, than in other groups ($p<0.05$). Higher amounts of EPO (above 2%) was also associated with more short-chain FFA.

Short-chain FFA production increased with longer period of storage. During the 15 day storage, the total release of short-chain FFA from the high amount of EPO-added milks (Trt C and D) was not much different from those of other groups at 0 and 3 day storages. However, from 6 day, the total amount of short-chain FFA in Trt C and D was significantly different. In control, the amount of total short-chain FFA increased from 7.6 to 25.0 ppm during 15 days. In 6 and 10% EPO-added milks, 5.9 and 7.7 ppm of total short-chain FFA were found at 0 day, and 33.2 to 36.7 ppm were produced at 15 day, respectively. The present results indicated that EPO-added milks, especially the high amount above 6%, produced more short-chain FFAs than without EPO regardless of β-CD treatment.

Figure 2 shows the amount of total FFA from C_4 to C_{18} (including C18:0, 18:1, 18:2, and 18:3) during 15 day storage at 5°C. The trend of total amount of FFA including long-chain FFA was different from that of short-chain FFA. The amount of total FFA was affected by β-CD treatment. When Control, Trt A and B (0 and 2% EPO added milks with β-CD treatment) were compared, the release of total FFA was significantly lower in Trt A than that of Control. Another aspect was that even in Control and Trt A, the total FFA increased during storage, which indicated that the production of FFA may be enhanced during storage regardless of amount of EPO addition.

Sensory evaluation

The sensory attributes of the EPO-added milk are

Table 3. Concentrations of short-chain free fatty acids in evening primrose oil added cholesterol-reduced milk stored at 4°C for 15 days[1]

Storage period (day)	Treatments	SFFA concentration (ppm)				
		C_4	C_6	C_8	C_{10}	Total
0	Control	2.7^{a}	1.7^{a}	0.9^{a}	2.3^{ab}	3.6^{b}
	Trt.A	1.8^{ab}	1.3^{a}	0.5^{ab}	2.0^{b}	5.6^{ab}
	Trt.B	1.3^{b}	1.4^{a}	0.2^{b}	2.2^{b}	5.1^{ab}
	Trt.C	1.7^{ab}	1.5^{a}	0.4^{b}	2.3^{ab}	5.9^{ab}
	Trt.D	2.0^{ab}	1.7^{a}	1.1^{a}	2.9^{a}	7.7^{a}
3	Control	3.0^{a}	2.0^{b}	1.1^{ab}	3.7^{a}	9.8^{a}
	Trt.A	2.3^{ab}	1.8^{b}	0.5^{b}	2.4^{b}	7.0^{b}
	Trt.B	2.4^{ab}	1.9^{b}	1.0^{ab}	2.8^{b}	8.1^{ab}
	Trt.C	1.9^{b}	2.0^{b}	1.0^{ab}	2.5^{b}	7.4^{b}
	Trt.D	2.3^{ab}	3.0^{a}	1.2^{a}	2.7^{b}	9.2^{a}
6	Control	3.7^{a}	3.6^{a}	1.9^{b}	3.8^{ab}	13.0^{ab}
	Trt.A	2.0^{b}	3.3^{a}	1.2^{c}	3.7^{b}	10.2^{b}
	Trt.B	2.5^{b}	3.0^{ab}	0.7^{c}	3.5^{b}	9.7^{b}
	Trt.C	2.7^{ab}	2.6^{b}	1.2^{c}	3.2^{b}	9.7^{b}
	Trt.D	3.9^{a}	3.1^{ab}	3.0^{a}	4.3^{a}	14.3^{a}
9	Control	3.9^{c}	4.5^{c}	2.4^{b}	4.0^{ab}	14.8^{b}
	Trt.A	3.0^{c}	3.9^{c}	1.4^{c}	4.0^{ab}	12.3^{b}
	Trt.B	3.2^{c}	3.6^{c}	1.0^{c}	3.8^{b}	11.6^{b}
	Trt.C	3.5^{c}	3.8^{c}	1.4^{c}	3.2^{b}	11.9^{b}
	Trt.D	7.7^{a}	7.9^{a}	4.1^{a}	4.9^{a}	24.6^{a}
12	Control	5.5^{b}	5.1^{b}	3.3^{ab}	6.0^{a}	19.9^{ab}
	Trt.A	5.1^{b}	4.5^{b}	3.4^{ab}	5.6^{a}	18.6^{ab}
	Trt.B	5.1^{b}	3.6^{b}	2.6^{b}	4.0^{b}	15.3^{b}
	Trt.C	7.6^{a}	8.9^{a}	2.2^{b}	4.1^{b}	22.8^{a}
	Trt.D	8.5^{a}	8.0^{a}	4.4^{a}	5.6^{a}	26.5^{a}
15	Control	7.2^{b}	6.0^{b}	4.1^{ab}	7.7^{a}	25.0^{b}
	Trt.A	6.7^{b}	5.2^{b}	3.3^{b}	7.1^{a}	22.3^{b}
	Trt.B	7.8^{b}	5.7^{b}	3.3^{b}	7.7^{a}	24.5^{b}
	Trt.C	11.6^{a}	9.0^{ab}	4.8^{a}	7.8^{a}	33.2^{a}
	Trt.D	12.5^{a}	10.9^{a}	5.3^{a}	8.0^{a}	36.7^{a}

[1] Means within column by the same letter are not significantly different ($p<0.05$). Control: no added.
Trt A: milk was treated with 1% β-CD. Trt B, Trt C, and TrtD: 2, 6, 10% EPO added cholesterol-reduced milk, respectively.

shown in Table 4. The scores of all characteristics increased with an increase of EPO amount added and storage periods. The difference of oxidative off-flavor score between Trt A and other treatments was significant even in 0 day storage. In particular, Trt D, the 10% EPO-added group, showed the significantly higher scores in every period. The scores of off-taste were similar to that of oxidation. The significant high scores of these two aspects probably resulted from the EPO itself or derived from the fat oxidation. Oxidative off-flavor and off-taste would be more intense with higher amounts of EPO addition and longer storage period.

Compared with above aspects, the difference of bitterness score between Trt A and other groups was little, indicating that EPO may not affect significantly to bitterness scores. However, the overall quality was highly affected by EPO addition. Only 2% EPO added group (Trt B) showed a significant decrease in overall score, compared with Trt A in the early stage of storage (0 day). The higher the amount of EPO addition, the lower overall scores throughout the 15 day storage.

Animal study

After 5 weeks of 40% beef tallow and 5% cholesterol containing diet, the average food intake was 22.9 g/day in both groups during next 8 weeks. Interestingly, body weight gain was significantly lower in 10% EPO added group (76.6 g) than that in Control (100.2 g) for 8 week period (Table 5).

In blood analysis, after 5 weeks of high cholesterol and high fat diet feeding, the average total serum cholesterol was 120.8 and 122.4 mg/dl in Control and EPO-added group, respectively (Table 6). During 8 weeks of experimental milk feeding period, the blood cholesterol of rats fed 10% EPO-added milk increased significantly from 166.0 to 197.0 mg/dl, while in the Control the change was from 120.8 to 166.0 mg/dl. The blood high density lipoprotein (HDL) increased dramatically in EPO-added group (76%), which was significantly different from that in Control (27%). Blood triacylglycerol (TG) did not increase profoundly from the initial to final week.

Unexpectedly, plasma total cholesterol in EPO group increased significantly more than in Control in the present study. The difference between initial and final was 45 mg/dl

Table 4. Sensory characteristics for evening primrose oil added and cholesterol-reduced milk stored at 4℃ for 15days[1]

Storage period (day)	Treatments	Sensory description			
		Oxidative off-flavor	Off-taste	Bitterness	Overall[2]
0	Trt.A	3.9^{b}	4.1^{c}	4.0^{a}	7.0^{a}
	Trt.B	4.9^{a}	4.4^{bc}	4.1^{a}	6.2^{ab}
	Trt.C	5.0^{a}	4.9^{b}	4.2^{a}	5.6^{b}
	Trt.D	5.4^{a}	5.6^{a}	4.3^{a}	3.3^{c}
3	Trt.A	4.0^{c}	4.1^{c}	4.0^{b}	7.0^{a}
	Trt.B	4.9^{a}	4.8^{b}	4.4^{ab}	5.9^{b}
	Trt.C	5.4^{ab}	4.9^{b}	4.2^{ab}	4.1^{b}
	Trt.D	5.7^{a}	5.7^{a}	4.8^{a}	3.0^{c}
6	Trt.A	4.0^{c}	4.2^{c}	4.0^{b}	6.7^{a}
	Trt.B	4.2^{c}	4.8^{bc}	4.3^{b}	5.8^{ab}
	Trt.C	5.1^{b}	5.0^{b}	4.4^{b}	4.0^{b}
	Trt.D	5.9^{a}	5.8^{a}	5.2^{a}	2.9^{c}
9	Trt.A	4.1^{c}	4.1^{c}	4.0^{b}	6.1^{a}
	Trt.B	4.4^{bc}	4.9^{b}	4.4^{ab}	5.3^{ab}
	Trt.C	5.3^{a}	5.3^{b}	4.6^{ab}	3.6^{b}
	Trt.D	5.7^{a}	6.0^{a}	5.1^{a}	2.2^{c}
12	Trt.A	4.1^{c}	4.1^{c}	4.0^{c}	5.9^{a}
	Trt.B	4.6^{bc}	4.8^{b}	4.6^{bc}	5.0^{ab}
	Trt.C	5.4^{a}	5.6^{a}	4.8^{b}	3.6^{c}
	Trt.D	5.7^{a}	6.1^{a}	5.4^{a}	1.9^{d}
15	Trt.A	4.2^{c}	4.2^{c}	4.0^{c}	5.7^{a}
	Trt.B	4.8^{bc}	5.0^{b}	4.6^{bc}	4.2^{b}
	Trt.C	5.5^{ab}	5.8^{a}	5.0^{ab}	3.6^{bc}
	Trt.D	6.0^{a}	6.3^{a}	5.4^{a}	1.2^{d}

[1] Means within column by the same letter are not significantly different ($p<0.05$).
The scale of sensory score: 1 = very slight, 2 = slight, 3 = slight-moderate, 4 = moderate, 5 = moderate-strong, 6 = strong, 7 = very strong.
[2] The scale of overall score: 1 = dislike extremely, 4 = neither like nor dislike, 7 = like extremely.
[3] Trt.A: milk was treated with 1% β-CD.
Trt.B, Trt.C and Trt.D: 2, 6, 10% EPO added cholesterol-reduced milk, respectively.

in Control and 74.6 mg/dl in EPO group, respectively. Interestingly, the difference of plasma HDL cholesterol between Control and EPO group was 11.5 and 27.6 mg/dl, respectively. Recently, the predictive value of the total cholesterol may be improved slightly by consideration of the ratio between low density lipoprotein (LDL) cholesterol or total cholesterol and high density lipoproteins (HDL) cholesterol. Therefore, even though total cholesterol and triglyceride increased in EPO group, the ratio of total cholesterol and HDL was similar in both groups.

Several studies have investigated the effect of γ-linolenic acid on lowering cholesterol in human and animal. Sugano et al. (1986) have indicated a significant hypocholesterolemic efficacy of γ-linolenic acid in rats. Horrobin and Manku (1983) found that human γ-linolenic acid, as EPO has an approximately 170 times greater cholesterol-lowering ability than linoleic acid, indicating that linoleic acid is converted to GLA to exert its hypocholesterolemic effects. Huang et al. (1984) fed high-

Table 5. Effects of experimental diets on food intake and body weight gain[1]

Treatment	Food intake (g/day)	Body weight gain (g/8 week)
Control[2]	22.88^{a}	100.24^{a}
EPO[3]	22.72^{a}	76.63^{b}

[1] Rats were fed for 16 weeks. Means within column by the same letter are not significantly different ($p<0.05$).
[2] Milk with no EPO addition no cholesterol removal.
[3] 10% EPO-added cholesterol-reduced milk. (2 ml/day).

Table 6. Effects of experimental diets on the change of blood triacylglycerol, total cholesterol and high-density lipoprotein in rats fed for 16 weeks[1] (mg/dl)

Treatment	Total CH		TG		HDL	
	Initial	Final	Initial	Final	Initial	Final
Control[2]	120.8^{a}	166.0^{b}	50.8^{b}	51.0^{b}	41.8^{b}	53.3^{b}
EPO[3]	122.4^{a}	197.0^{a}	59.3^{a}	66.2^{a}	36.2^{a}	63.8^{a}

[1] Means within column by the same letter are not significantly different ($p<0.05$).
[2] Milk with no EPO addition no cholesterol removal.
[3] 10% EPO-added cholesterol-reduced milk (2 ml/day).

choleterol diets to essential fatty acid-deficient rats and found that plasma cholesterol levels rose sharply when the dietary fat was safflower oil, but not at all with EPO. Our results did not agree with these results (Horrobin and Manku, 1983; Huang et al., 1984; Sugano et al., 1986); we did not observe a hypocholesterolemic effect of polyunsaturated fatty acids in rats given high-cholesterol diets containing EPO at the 10% level. However, the increase of HDL cholesterol may compensate for the increase of total cholesterol level in this study.

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