Effect of sesamin on serum cholesterol and triglycerides levels in LDL receptor-deficient mice

Abstract Background Sesamin, a major lignan from sesame seeds has been associated with cholesterol reduction in previous reports, but recent studies suggested differences in the response to sesamin intake depending on the model studied as well as the nature of the sesamin preparation used. Aim The effect of pure sesamin epimer on serum lipids was studied in hypercholesterolemic LDL receptor-knockout mice under cholesterol fed condition. Design Animals were randomly assigned to 4 groups, fed an atherogenic diet containing stanol ester, sesamin, combination of stanol ester and sesamin or a control diet with no additions. Results The control group showed an almost 3-fold increase in serum cholesterol levels due to the atherogenic diet but no effect was seen for triglyceride levels. Stanol ester alone or together with sesamin significantly attenuated the elevation of the cholesterol levels. Conclusion Sesamin alone did not affect the elevation of the diet-induced cholesterol level and it did not enhance the effect of stanol ester.

Key words cholesterol – triglycerides – lignans – sesamin – enterolignans – stanol ester
Materials and methods

Animal model

Female ldlr−/− mice (strain number, B6.129S7-Ldlrtm1Her) quality SPF were obtained from the Jackson Laboratory (Bar Harbor, Maine, US), and maintained at the Central Animal Laboratory of Raisio Benecol Ltd. (Raisio, Finland) in accordance with European guidelines (European Treaty Series No. 123, EU No 609/86, Official Journal of the European Communities No. L 358) approved by the Animal Care and Use Committee of the University of Turku (Finland) approval number 1263/02. Animals were 17–18 weeks at the beginning of the study/15–20 g and after 4 weeks of acclimatization, mice were housed (Polycarbonate Macrolon III, Scanbur AS, Denmark) in groups of 1–2/cage and randomly given free access to I–IV diets for 4 weeks. Ambient temperature was 21 ± 3°C, humidity ranged 55 ± 15% and illumination consisted in 12-h dark/light cycle. Free access to community tap water was allowed, except during the experiments. Blood samples were collected for the determination of the concentrations of serum cholesterol and triglycerides at the beginning and at the end of the 4-week dietary intervention. Mice were sedated with CO2/O2-mixture and the blood samples were collected from the orbital sinus (at the beginning) or through a cardiac puncture (at the end). At the beginning of the study the blood was collected into 500 μl gel tubes and allowed to clot at least for 1 h. Samples were then centrifuged for 15 min at 4000 rpm. Serum was separated and stored at −20°C until analyses. At the end of the intervention, blood samples were collected in heparin tubes and centrifuged within 1 h. The plasma samples were frozen at −20°C and stored until analyses. About 50 μl was used for cholesterol and triglyceride analyses and the rest was stored at −20°C for further analyses. During the intervention, the animals were weighed twice a week and observed daily (morning and afternoon) for general well being.

Test compounds and diets

Plant-derived stanol ester was produced by Raisio Benecol Ltd. (Raisio, Finland). Sesamin epimer (CAS No. 607–80-7, >95% purity, Fig. 1A) was synthesized by Industrial Research Ltd. (Wellington, New Zealand). During acclimatization/isolation period the diet was RM1 (E) SQC, (Special Diet Service, Witham Essex, UK). Experimental atherogenic diets containing 0.25% of cholesterol were prepared at Raisio Benecol Ltd. based on Clinton-Cybulsky diet premix (D12106pxc, Research Diets Inc., New Brunswick, NJ) in which the test compounds were administered, as follows: control (I) diet (n = 8), stanol ester (II) diet (n = 8, 7 mg/kg), sesamin (III) diet (n = 8, 1 mg/kg) and combined (IV) diet (n = 7, 7 mg/kg stanol ester and 1 mg/kg sesamin). Diets were stored at +2 to +8°C and allowed to warm to room temperature before delivery to the animals (twice a week) in 300-ml glass cups attached on the cage floor.

Rationale for dose selection

Fat and cholesterol levels in the atherogenic diet were based on the literature [6]. The use of stanol ester as control test model has been proven several times to induce cholesterol reduction (Raisio Benecol Ltd., unpublished results) and the concentration in this study was selected to exert sufficient but not the maximal response, enabling the possible combination effect with sesamin. Sesamin concentration, that was adopted from previous reports [7–9] allowing comparison of the results was also considered as a rationale level to be used in clinical trials.

Analysis

Serum cholesterol and triglycerides were determined by Ecoline CHOD-PAP and Ecoline 25 GPO-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany), respectively. Control serum was Qualitrol HS N (Merck KGaA, Darmstadt, Germany). Serum metabolic profile of lignans was carried out by HPLC with coulometric electrode array detection [10] and the absence of plant lignans other than sesamin epimer in the diets was confirmed by isotope-dilution GC-MS [11].

Statistics

Graph Pad Prism software version 3.02 (GraphPad Software Inc., CA) was used for statistical data treat-
The differences in changes from the basal concentrations during the intervention between the groups were analyzed by ANOVA. Pair-wise comparisons between the groups were performed by Tukey’s Multiple Comparison Test. Two-way ANOVA was used to analyze differences in the body weights between the groups. A P-value less than 0.05 was considered as statistically significant. All analyses were performed as two-sided tests.

Results

Body weight gain

The body weights of the animals increased in all groups during the 4-week intervention period (Fig. 2). The increment tendency in group I was less than those in the other groups but analysis did not reveal a significant group × week interaction (P = 1.00). However, ANOVA showed a significant group effect (P = 0.0002), which indicates that the body weights differed between the groups during the intervention.

Effects of the atherogenic diet and test compounds on serum cholesterol and serum triglyceride concentrations

The atherogenic diet (group I) induced an almost 3-fold increase in the s-cholesterol levels but no effect was seen in the triglyceride levels. Stanol ester alone (group II) or together with sesamin (group IV) significantly attenuated the elevation of the cholesterol levels. Sesamin alone (group III) either normalize the elevation of cholesterol levels nor did it enhance the effect of stanol ester (group II versus group IV). Diet-induced changes in the triglyceride levels did not differ between groups (P = 0.89). Results are presented in Fig. 3.

Effects of the atherogenic diet and test compounds on plasma lignan profile after intervention

Presence of sesamin epimer in the diets resulted in the appearance of enterolignans in plasma, although concentrations varied greatly between animals (Table 1). Significantly increased levels of enterodiol (P = 0.00), but not enterolactone were found.

Discussion

As one of the major components of sesame seeds, the lignan sesamin has received a great deal of interest regarding its potential as a hypocholesterolemic agent, especially after the positive results reported by Hirata et al. [7] in humans. Thereafter, different approaches have been used to confirm this observation in which different sources of dietary sesamin have been used. Studies using sesame seed-based diets provided results that are difficult to discuss since the...
cholesterol. Sesamin has been reported to inhibit
its inhibitors are very effective in lowering plasma
enzyme in the cholesterol biosynthetic pathway and
6]. Hepatic HMG-CoA reductase is the rate-limiting
modulation of the serum cholesterol concentration [5,
and in addition, they are prone to diet-induced
synchronously elevated serum cholesterol concentration,
receptors, the animals used in this trial have a spon-
dietary absorption. Due to the lack of functional LDL-
endogenous cholesterol synthesis and reduction of
hypocholesterolemic effect of sesamin i.e. reduction of
mechanism it has been proposed to reduce plasma
cholesterol. However, in this study no reduction of
serum cholesterol or triglyceride levels was observed
in the group supplemented with sesamin alone (group
III) and therefore it is concluded that sesamin does
not affect HMG-CoA reductase in this animal model.
Similarly, cholesterol supplementation (0.25%, w/w)
for 4 weeks increased plasma total cholesterol levels
approx. 3-fold in the absence of a functional LDL
receptor. However, the introduction of stanol ester in
the atherogenic diet significantly reduced the total
plasma cholesterol level, independently of the pres-
ence of sesamin. It is known that plant stanols inhibit
the absorption of dietary cholesterol and the reab-
sorption (enterohepatic circulation) of endogenous
cholesterol from the gastrointestinal tract [29]. It
seems though that sesamin does not affect dietary
cholesterol absorption in this animal model.

The metabolism of sesamin seems to play an
essential role in its further biological action. It has
been recently suggested that in humans, sesamin is
absorbed through the portal vein reaching the liver
where extensive metabolism takes place generating
demethylenated (catechol) derivatives secreted
as glucuronidates via the bile [26]. The same metab-
olites were found after in vitro fermentation of pure
sesamin standard with human fecal inoculum and
furthermore extensive in vivo conversion to enterol-
lactone was reported after sesame seed supplemen-
tation in humans [11]. We hypothesized then that the
absorption of sesamin could certainly take place via
the portal vein and that transformation to catechol
derivatives could be possible, as well as further
absorption to the general circulation where they may
exert the biological actions proposed for sesamin.
Nevertheless, in this study as in our previous trial
[11], extensive conversion of sesamin to enteroligna-
s was observed suggesting that non-absorbed sesamin
and possibly biliary catecholic sesamin derivatives
undergo metabolism by gut microflora leading to the
production and further absorption of enteroligna-
s. Postprandial levels of sesamin in plasma differed
considerably between rats [12] and humans [11],
suggesting a different metabolic pathway depending
on species.

### Table 1

Enterolignan (enterolactone and enterodiol) values after 4-week
intervention period nmol/l
(Mean ± SEM, n = 8)

| Group          | n  | ENLa | END  | ENL + END       |
|----------------|----|------|------|-----------------|
| I, Control     | 8  | 4.61 ± 0.40 | 6.11 ± 2.10 | 9.18 ± 2.14     |
| II, Stanol ester| 8  | 3.01 ± 0.35 | 10.0 ± 4.69  | 13.0 ± 4.85     |
| III, Sesamin   | 8  | 8.01 ± 0.98 | 569 ± 54.2**| 577 ± 55.0**    |
| IV, Stanol ester + sesamin | 7  | 6.54 ± 1.99 | 388 ± 90.7** | 394 ± 91.8**    |

**P < 0.001 in comparison with Group 1 (Tukey’s Multiple Comparison Test)

ENL, Enterolactone; END, enterodiol
So far, our data suggest that a limited absorption or a too rapid enterohepatic circulation of sesamin could be the explanation for the lack of antihypercholesterolemic effect in mice/hamsters and possibly in humans but this hypothesis needs to be confirmed. Furthermore, the characteristics of our animal model did not allow to study a third mechanism for cholesterol reduction that sesamin could use; the increment of LDL-receptor activity. This hypothetical mechanism has been suggested for other dietary components [30], and it is a likely explanation of the negative results reported in this paper.

In conclusion, possible confounding factors have been minimized with the use of a specific animal model and the selection a pure sesamin epimer. It can be therefore stated that sesamin does not seem to affect cholesterol biosynthesis or absorption in mice. The so far contradictory results hinder the extrapolation to humans, and only a clinical trial with separate epimeric forms and the elucidation of the complete metabolic pathway of sesamin in humans will contribute to clarify the possible utilization of sesamin as a hyocholesterolemic agent.

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References

1. Sugano M, Akimoto K (1993) Sesamin: a multifunctional gift from nature. J Chinese Nutr Soc 18:1–11
2. Namiki M (1995) The chemistry and physiological functions of sesame. Food Rev Int 11:282–329
3. Kushiro M, Takahashi K, Ide T (2004) Species differences in the physiological activity of dietary lignan (sesamin and episesamin) in affecting hepatic fatty acid metabolism. Br J Nutr 91:377–386
4. Kushiro M, Masaoka T, Hageshita S, Takahashi Y, Ide T, Sugano M (2002) Comparative effect of sesamin and episesamin on the activity and gene expression of enzymes in fatty acid oxidation and synthesis in rat liver. J Nutr Biochem 13:289–295
5. Ishibashi S, Brown MS, Goldstein JL, Gerard JD, Hammer RE, Herz J (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenosine-mediated gene delivery. J Clin Invest 92:883–893
6. Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, Cybulsky MI (1999) Hyperlipidemia and atherosclerotic lesion development in LDL receptor-deficient mice fed defined semipurified diets with and without cholate. Arterioscler Thromb Vasc Biol 19:1938–1944
7. Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Nakamura H (1996) Hypocholesterolemic effect of sesame lignan in humans. Atherosclerosis 122:135–136
8. Satcithanandam S, Chanderbhan R, Kharroubi AT, Calvert R, Klurfeld D, Tepper SA, Krichevsky D (1996) Effect of sesame oil on serum and liver profiles in the rat. Int J Vit Nutr Res 66:386–392
9. Sugano M, Inoue T, Koba K, Yoshida K, Hirose N, Shinmen Y, Akimoto K, Amachi T (1990) Influence of sesame lignans on various lipid parameters in rats. Agric Biol Chem 54:2669–2673
10. Peñalvo JL, Nurmi T, Haajanen K, Al-Maharik N, Botting NP, Adlercreutz H (2004) Determination of lignans in human plasma by liquid chromatography with coulometric electrode array detection. Anal Biochem 332:384–393
11. Peñalvo JL, Heinonen S-M, Aura A-M, Adlercreutz H (2005) Dietary sesamin is converted to enterolactone in humans. J Nutr 153:1056–1062
12. Umeda-Sawada R, Ogawa M, Igarashi O (1999) The metabolism and distribution of sesame lignans (sesamin and episesamin) in rats. Lipids 34:633–637
13. Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu S, Yamada H (1991) Inhibition of cholesterol absorption and synthesis in rats by sesamin. J Lipid Res 32:629–638
14. Nakabayashi A, Kitagawa Y, Suwa Y, Akimoto K, Asami S, Shimizu S, Hirose N, Sugano M, Yamada Y (1995) Alfa-tocopherol enhances the hypocholesterolemic action of sesamin in rats. Int J Vit Nutr Res 65:162–168
15. Kamal-Eldin A, Frank J, Razdam A, Tengblad S, Basu S, Vessby B (2000) Effects of dietary phenolic compounds on tocopherol, cholesterol, and fatty acids in rats. Lipids 35:427–435
16. Umeda-Sawada R, Fujisawa Y, Igarashi O (1994) Effect of sesamin on cholesterol synthesis and on the distribution of incorporated linoleic acid in lipid subfractions in cultured rat cells. Biosci Biotechnol Biochem 58:2114–2115
17. Gu JY, Wakizono Y, Dohi A, Nonaka M, Sugano M, Yamada K (1998) Effect of dietary fats and sesamin on the lipid metabolism and immune function of Sprague-Dawley rats. Biosci Biotechnol Biochem 62:1917–1924
18. Mizukuchi A, Umeda-Sawada R, Igarashi O (2003) Effects of dietary fat level and sesamin on the polyunsaturated fatty acid metabolism in rats. J Nutr Sci Vitaminol 49:320–326
19. Yamashita K, Nohara Y, Katayama Y, Namiki M (1992) Sesame seed lignans and gamma-Tocopherol act synergistically to produce Vitamin-E activity in rats. J Nutr 122:2440–2446
20. Ikeda S, Tohyama T, Yamashita K (2002) Dietary sesame seed and its lignans inhibit 2,7,8-trimethyl-2(2′-carboxyethyl)-6-hydroxychroman excretion into urine of rats fed γ-tocopherol. J Nutr 132:961–966
21. Utsunomiya T, Chavali SR, Zhong WW, Forse RA (2000) Effects of sesame-supplemented dietary fat emulsions on the ex vivo production of lipopolysaccharide-induced prostaglandins and tumor necrosis factor [alpha] in rats. Am J Clin Nutr 72:804–808
22. Nakano D, Itoh C, Ishii F, Kawanishi H, Takaoka M, Kiso Y, Tsuruoka N, Takanaka T (2003) Effects of sesamin on aortic oxidative stress and endothelial dysfunction in deoxycorticosterone acetate-salt hypertensive rats. Biol Pharm Bull 26:1701–1705
23. Hirose N, Doy F, Ueki T, Akazawa K, Chijiwa K, Sugano M, Akimoto K, Shimizu S, Yamada H (1992) Suppressive effect of sesamin against 7,12-dimethylbenz[a]anthracene induced rat mammary carcinogenesis. Anticancer Res 12:1259–1265
24. Kamal-Eldin A, Pettersson D, Appelqvist LA (1995) Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum. Lipids 30:499–505
25. Kiso Y (2004) Antioxidative roles of sesamin, a functional lignan in sesame seed, and its effect on lipid- and alcohol-metabolism in the liver: a DNA microarray study. Biofactors 21:191–196
26. Tsuruoka N, Kidokoro A, Matsumoto I, Abe K, Kiso Y (2005) Modulating effect of sesamin, a functional lignan in sesame seeds, on the transcription levels of lipid- and alcohol-metabolizing enzymes in rat liver: a DNA microarray study. Biosci Biotechnol Biochem 69:179–188
27. Akimoto K, Kitagawa Y, Akamatsu T, Hirose M, Sugano M, Shimizu S, Yamada H (1993) Protective effects of sesamin against liver damage caused by alcohol or carbon tetrachloride in rodents. Ann Nutr Metab 37:218–224
28. Ogawa T, Makino T, Hirose N, Sugano M (1994) Lack of influence of low blood cholesterol levels on pancreatic carcinogenesis after initiation with N-nitroso bis(2-oxopropyl)amine in Syrian golden hamsters. Carcinogenesis 15:1663–1666
29. Miettinen TA (2001) Cholesterol absorption inhibition: a strategy for cholesterol-lowering therapy. Int J Clin Pract 55:710–716
30. Kirk EA, Sutherland P, Wang SA, Chait A, Leboeuf RC (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not ldl receptor-deficient mice. J Nutr 128:954–959