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ORIGINAL CONTRIBUTION

Lupin protein compared to casein lowers the LDL cholesterol:HDL cholesterol-ratio of hypercholesterolemic adults

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Abstract
Background Lupin protein had hypocholesterolemic effects in laboratory animals. However, the effect in humans has not been elucidated till now.
Aim of the study To investigate the effect of lupin protein on circulating cholesterol in plasma and lipoproteins of hypercholesterolemic subjects.
Subjects and methods A randomised, double-blind, placebo-controlled, parallel trial (23 females and 20 males completed the trial) was conducted to compare the effects of lupin protein versus casein as control protein on plasma lipids and amino acids. Thirty-five grams of the test protein were consumed daily for 6 weeks.
Results Both lupin protein and casein resulted in a reduction of circulating plasma cholesterol (-0.50 ± 0.64 and -0.47 ± 0.79 mM; $P < 0.05$) from baseline to week 6. The reduction of plasma cholesterol was mainly caused by a reduction of LDL cholesterol in the lupin protein group (-0.31 ± 0.46 mM; $P < 0.05$), while in the casein group HDL cholesterol significantly declined (-0.17 ± 0.15 mM; $P < 0.05$). Comparing the lupin protein group with the casein group yielded a difference in the net changes from baseline to week 6 in the LDL:HDL cholesterol-ratio of -0.24 (95% CI: -0.007, -0.479; $P < 0.05$). No significant differences in net changes were observed for plasma concentrations of triglycerides, glucose, homocysteine, taurine and most of the amino acids.
Conclusions Lupin protein compared to casein slightly lowered the concentration of LDL cholesterol in hypercholesterolemic subjects, without altering HDL cholesterol. No or minor effects of lupin protein were observed on circulating glucose, homocysteine and plasma amino acids.

Keywords Lupin protein · Plasma lipids · Hypercholesterolemic subjects

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Introduction

Dyslipidemia and its implication for atherogenesis and cardiovascular events is a major problem in human health. In view of the fact that elevated levels of blood lipids, especially LDL cholesterol, play a central role in the genesis of atherosclerosis, therapeutic and dietary approaches to their treatment and prevention are highly relevant. Substitution of soy for animal proteins in the diet of hypercholesterolemic laboratory animals or subjects appears to be an option to reduce serum cholesterol [e.g. 1, 7, 15, 19]. Evaluation of the cholesterol-lowering properties of soybean protein in humans was provided by a widely quoted meta-analysis which found a mean change in LDL cholesterol by −12.9% [1]. In 1999, the US Food and Drug Administration authorised a health claim for the cholesterol-lowering potential of at least 25 g per day of soy protein primarily for individuals with elevated blood cholesterol [4].

Another protein-rich legume besides soy is lupin. Soy and lupin belong to the same plant family and are characterised by a similar protein content and composition. In contrast to soy, lupin seed contains negligible amounts of isoflavones [2, 6, 14]. The sweet varieties of lupin have been cultivated for centuries for domestic animal feed, but

also for human nutrition, mainly in several parts of Australia, Europe and South America. Their use in the food industry is being developed, and lupin protein is beginning to replace soybean protein in several food products [11]. Recent studies have shown that protein from lupin is also capable of lowering circulating LDL cholesterol in hypercholesterolemic rats [2, 14] and pigs [9]. However, it is currently not known whether lupin protein may also exert hypolipemic effects in humans. The present study aimed to investigate the effects of lupin protein compared to casein on plasma lipoproteins in humans. Hypercholesterolemic participants were selected for this trial since lupin protein has been shown to lower circulating plasma lipids much stronger in rats fed a hypercholesterolemic diet than in those fed a normolipemic diet [2] and also soy protein exerts a definite hypocholesterolemic effect only in hypercholesterolemic subjects, whereas minimal or no changes occur in normocholesterolemic subjects [1].

Studies with rats demonstrated that lupin protein acts hypolipemic by modulating the transcription levels of genes involved in lipid metabolism [2, 16] and previous data show that lupin protein up-regulates LDL receptor in human hepatoma cells [14]. Since, we focused our study on the effects of lupin protein on circulating cholesterol, the mRNA concentration of sterol regulatory element-binding protein (SREBP)-2, a transcription factor responsible for the transcription activation of the LDL receptor and 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase [5], and mRNA concentration of LDL receptor and HMG-CoA reductase were measured in the mononuclear blood cells of the subjects. Plasma concentrations of amino acids were measured because lupin protein compared to casein is characterised by low amounts of methionine and lysine, and high amounts of cysteine and arginine which in turn could alter the levels of sulphur and basic amino acids and its derivates.

Subjects and methods

Subject screening and selection

Subjects between 21 and 70 years of age were recruited in the Halle area. Inclusion criterion was moderate hypercholesterolemia (5.7–7.9 mM). The exclusion criteria were use of lipid-lowering medication, chronic diseases and food allergies. Subjects who planned to lose weight during the time scheduled for the study were also excluded. Finally, 56 participants (25 men and 31 women) were assigned to one of two treatment groups by computer randomization. The study procedures were in accordance with the Declaration of Helsinki of 1975 as revised in 1983 and were approved by the Ethics Committee of the University of Halle-Wittenberg. Written informed consent was obtained from all subjects.

Study design and dietary treatment

The trial was performed as a double-blind parallel intervention study. The study protocol included a 10-day run-in period and a 6-week treatment period. The participants received 35 g per day of either lupin protein from *L. angustifolius* or casein as control protein during the treatment period. Casein was obtained from Meggle (Wasserburg, Germany) and was not further processed. Essentially isoflavone-free lupin protein which was extracted from de-oiled lupin seeds was obtained from the Fraunhofer Institute and contained 16 mg alkaloids per kg protein isolate, in which lupanine was the most abundant quinolizidine alkaloid, followed by 13α-hydroxylupanine and angustifoline (IVV, Freising, Germany). Amino acid compositions of the diet proteins were analysed [2] and shown in Table 1. The test proteins were included into snack bars (produced by Fraunhofer Institute), which had to be consumed in two rations per day. The protein bars (ration per day) consist of 15 g wheat flour, 40 g honey, 1 g baking powder, 6 g hazelnut flavour and 35 g of the test protein. Honey and wheat flour were included to get a consumable texture; hazelnut flavour was added to make the bars comparable in taste. Since the protein bars consumed per day provided 1,525 kJ, the participants were

Table 1 Analysed amino acid composition of the experimental proteins (mol/kg)

	Casein	Lupin protein
Alanine	0.31	0.30
Arginine	0.18	0.67
Aspartic acid + asparagine	0.50	0.75
Cysteine	0.03	0.12
Glutamic acid + glutamine	1.45	1.78
Glycine	0.10	0.21
Histidine	0.21	0.16
Isoleucine	0.34	0.31
Leucine	0.67	0.54
Lysine	0.50	0.24
Methionine	0.18	0.02
Phenylalanine	0.29	0.22
Proline	0.88	0.39
Serine	0.51	0.43
Threonine	0.33	0.22
Tryptophan	0.05	0.05
Tyrosin	0.24	0.16
Valine	0.52	0.26

advised to consume the bars as replacement of common isocaloric snacks.

We collected 10 ml of fasting blood at the screening, and again at baseline and at 6 weeks of intervention. The subjects kept diaries to report the amount of foods consumed which were analysed by PRODI nutritional software (4.5/03 expert, Wissenschaftliche Verlagsgesellschaft Stuttgart). Subjects were asked to maintain their usual lifestyles and dietary habits.

Blood lipid analyses

Blood was collected in heparinized tubes and plasma was separated by centrifugation at 1,500×*g* for 10 min at 4 °C. Plasma lipoproteins VLDL, LDL and HDL were separated by step-wise ultracentrifugation (900,000×*g* at 4 °C for 1.5 h; Mikro-Ultrazentrifuge, Sorvall Products, Bad Homburg, Germany) by appropriate density cuts (VLDL, $0.95 < \rho < 1.006$ kg/l; LDL, 1.006 kg/l $< \rho <$ 1.063 kg/l; HDL, $\rho > 1.063$ kg/l). Concentrations of cholesterol and triglycerides in plasma and lipoproteins were examined using enzymatic reagent kits (DiaSys Diagnostic Systems, Holzheim, Germany, no. 1.1300 99 90 314 and 1.5760 99 90 314).

Analysis of plasma glucose and amino acids

Plasma glucose was measured immediately after blood collection with a glucose reagent kit (Ecoline S +Diasys Diagnostic Systems GmbH, no: 12531 99 90 335). For calculation a standard solution (Ecoline S +Diasys Diagnostic Systems GmbH, no: 12500 99 90 030) was used. The concentrations of free amino acids in the plasma were measured as isoindole derivatives by HPLC [13] after pre-column derivatization. The plasma concentrations of total homocysteine (free homocysteine, homocysteine disulfides and mixed disulfides), cysteine and taurine were determined by HPLC [21].

Isolation of mononuclear cells and gene expression analysis

The mononuclear cells (lymphocytes and monocytes) were isolated from blood with Histopaque®-1077 (Sigma, Taufkirchen, Germany) according to the manufacturers' protocol. For determination of mRNA expression levels of the LDL receptor (gene bank no: NM 000527.2; for: 5′ CCCCGCAGATCAACCCCCACTC 3′, rev: 5′ AGACC CCCAGGCAAAGGAAGACGA 3′), SREBP-2 (gene bank no: NM 004599.2; for: 5′ CGCCACCTGCCCCTCTCCTT CC 3′, rev: 5′ TGCCCTGCCACCTATCCTCTCACG 3′) and HMG-CoA reductase (gene bank no: M11058.1; for: 5′ TACCATGTCAGGGGTACGTC 3′, rev: 5′ CAAGCCTA GAGACATAATCATC 3′) total RNA were isolated using TRIZOL™ (Invitrogen, Karlsruhe, Germany) according to the manufacturers' instructions. Calculation of the relative mRNA concentrations was made using the amplification efficiencies and the C_t values [12]. The relative mRNA quantities of SREBP-2, LDL receptor and HMG-CoA reductase related to the reference gene *β*-actin (NM 001101.2; for: 5′ GAGCGGGAAATCGTGCGTGAC 3′, rev: 5′ GCCTAGAAGCATTTGCGGTGGAC 3′) were determined by realtime RT-PCR [2].

Statistical analyses

The trial was designed to have 80% power to detect a 13% change in LDL cholesterol concentrations (the suggested difference in LDL cholesterol between the two groups were estimated considering meta-analysis data of human studies with soybean protein [1]) between the two groups (assuming the SD to be 15%) at the 5% level of significance in a two-tailed test. The required number of subjects per group was 22; to allow for 25% dropouts, 56 subjects were recruited and randomly assigned to one of the two groups. Statistical analyses were performed with the use of MINITAB statistical software (version 13, Minitab, State Collage, PA, USA). The data are presented as mean values and standard deviations (SD). Non serial differences between the groups were compared by Student's *t*-test or Kruskal–Wallis analysis, depending on whether data were normally distributed or not. Baseline and treatment values were compared by the paired *t*-test. Change scores (final–initial) for casein and lupin protein were compared by the Student's *t*-test. We used an estimate of the difference in the change from baseline to week 6 (with a 95% CI) between the groups as a measure of the treatment effect. At a *P*-level of 0.05 or smaller, results were considered significantly different.

Results

Consumption of the protein bars was well accepted by most of the subjects. However, of the 56 subjects initially enrolled, 13 subjects (seven from the casein group and six from the lupin protein group) dropped out (10 were lost to follow-up, two withdrew consent or were unable to comply with the study protocol, one complained of gastrointestinal problems). Means of the two groups showed no differences in the baseline characteristics (Table 2, 3), except plasma threonine which was lower in the lupin protein group than in the casein group ($P < 0.05$, Table 4). Also the nutrient intakes, gathered at the end of the run-in phase, were not different between the two groups (data not shown); the

Table 2 Baseline characteristics of the two study groups and the entire study population[a]

	Casein group	Lupin protein group	All subjects
Age (y)	43.3 (11.8)	44.4 (12.2)	43.9 (11.8)
Height (cm)	172 (7)	172 (6)	172 (7)
Weight (kg)	76.3 (13.2)	77.4 (17.2)	76.9 (15.2)
BMI (kg/m^2)	25.7 (4.0)	26.2 (5.0)	25.9 (4.5)

[a] Values are means (standard deviation); casein group $n = 21$ (male: 9, female: 12), lupin protein group $n = 22$ (male: 11, female: 11)

overall energy intake was 10,509 kJ (SD 3,453) in the casein group and 10,751 kJ (5,133) in the lupin protein group. Of the overall energy consumed, protein-derived energy in the casein group was 14.5% (2.7) and 14.1% (3.1) in the lupin protein group, which corresponds to 91.2 g (40.5) and 86.5 g (39.4) protein, respectively.

After 6 weeks of intervention the change in body weight was −0.04 kg (SD 0.39) in the group who consumed casein and +0.14 kg (0.58) in the group who consumed lupin protein ($P = 0.265$).

After 6 weeks of intervention the individuals of the casein and the lupin protein group had lower plasma cholesterol concentrations than at baseline ($P < 0.05$). The changes in plasma cholesterol were not different between the two groups (Table 3). From baseline to week 6, the concentration of LDL cholesterol remained unchanged in the casein group, while it decreased in the subjects who received lupin protein ($P < 0.05$, Table 3). In subjects who received casein the concentration of HDL cholesterol decreased from baseline to week 6 of intervention ($P < 0.05$), while there was no change in the group who received lupin protein (Table 3). At week 6 the concentration of HDL cholesterol was not different between the two groups. However, subjects who received lupin protein developed a more favourable change of LDL cholesterol:HDL cholesterol-ratio than subjects from the casein group ($P < 0.05$); the net change was −0.24 mM (95% CI: −0.007; −0.479).

After 6 weeks of supplementation, the concentration of plasma triglycerides decreased in the group who received casein ($P < 0.05$), whereas no change in plasma triglycerides was observed in the group who received the lupin protein (Table 3). The concentration of VLDL triglycerides was not influenced by the dietary protein and the changes in plasma and VLDL triglyceride concentrations did not differ between the two treatment groups. The concentration of glucose was not influenced by the dietary protein (Table 3).

Most of the plasma amino acid concentrations remained unchanged by consumption of the dietary test proteins (Table 4). The plasma concentrations of alanine and glycine decreased from baseline to week 6 in the casein group and the plasma concentration of methionine decreased from baseline to week 6 in the lupin protein group. Differences in the net changes between the two groups were observed only with methionine which declined in the lupin protein group (Table 4). At the end of the experimental period, individuals who received lupin protein had lower plasma concentrations of lysine, threonine and valine than individuals who received casein, but no differences in the net changes were observed. The plasma concentrations of arginine, asparagine, cysteine, glutamine, glutamic acid, histidine, homocysteine, isoleucine, leucine, phenylalanine, serine, taurine, tryptophan and tyrosin were not influenced by the dietary treatment.

Table 3 Concentrations of cholesterol and triglycerides in plasma and lipoproteins and plasma concentrations of glucose of the two study groups at the beginning of the study and after 6 weeks of intervention with casein or lupin protein[a]

	Casein group			Lupin protein group			*P*-value[b]	
	Baseline	6 Weeks	Changes	Baseline	6 Weeks	Changes	6 Weeks	Changes
Cholesterol								
Plasma (mM)	5.78 (0.82)	5.32[c] (0.77)	−0.47 (0.79)	5.66 (0.64)	5.17[c] (0.59)	−0.50 (0.64)	0.509	0.900
LDL (mM)	3.65 (0.64)	3.50 (0.73)	−0.15 (0.62)	3.61 (0.65)	3.30[c] (0.64)	−0.31 (0.46)	0.380	0.384
HDL (mM)	1.70 (0.38)	1.54[c] (0.35)	−0.17 (0.15)	1.75 (0.48)	1.67 (0.42)	−0.08 (0.22)	0.294	0.150
LDL:HDL-ratio	2.29 (0.79)	2.43 (0.83)	+0.13 (0.37)	2.24 (0.74)	2.13 (0.70)	−0.11 (0.35)	0.239	0.043
Triglycerides								
Plasma (mM)	1.59 (1.00)	1.26[c] (0.70)	−0.33 (0.59)	1.24 (0.53)	1.32 (0.72)	+0.08 (0.56)	0.715	0.068
VLDL (mM)	0.95 (0.72)	0.88 (0.76)	−0.07 (0.37)	0.72 (0.49)	0.80 (0.63)	+0.09 (0.52)	0.822	0.482
Glucose								
Plasma (mM)	4.90 (0.88)	5.14 (0.78)	+0.21 (0.90)	4.89 (0.92)	5.10 (0.75)	+0.21 (1.02)	0.861	0.992

[a] Values are means (SD); casein group $n = 21$ (male: 9, female: 12), lupin protein group $n = 22$ (male: 11, female: 11)

[b] Comparison between the casein and the lupin protein group (Student's *t*-test)

[c] Significantly different from baseline within a group, $P < 0.05$ (paired *t*-test)

Table 4 Plasma concentrations of amino acids, homocysteine and taurine of the two study groups at the beginning of the study and after 6 weeks of intervention with casein or lupin protein[a]

	Casein group			Lupin protein group			*P*-value[b]	
	Baseline	6 Weeks	Changes	Baseline	6 Weeks	Changes	6 Weeks	Changes
Alanine (μM)	438 (103)	402[c] (71)	−35.5 (72.5)	393 (85)	384 (80)	−9.5 (86.5)	0.442	0.312
Arginine (μM)	55.6 (24.8)	55.8 (17.8)	+0.2 (22.2)	54.1 (19.0)	58.3 (18.4)	+4.2 (22.6)	0.669	0.575
Asparagine (μM)	57.1 (12.9)	56.3 (8.9)	−0.8 (10.9)	52.6 (8.6)	53.2 (10.6)	+0.6 (6.8)	0.325	0.639
Cysteine (μM)	186 (35)	190 (42)	+4.5 (22.1)	208 (61)	210 (48)	+2.0 (30.8)	0.199	0.789
Glutamine (μM)	570 (89)	563 (86)	−6.9 (59.1)	571 (81)	577 (111)	+5.9 (67.4)	0.664	0.529
Glutamic acid (μM)	46.8 (22.4)	42.6 (19.0)	−4.1 (16.5)	42.9 (14.6)	45.0 (16.5)	+2.1 (9.4)	0.667	0.141
Glycine (μM)	212 (60)	186[c] (46)	−26.7 (28.8)	221 (91)	211 (67)	−10.3 (38.6)	0.178	0.139
Histidine (μM)	99.3 (14.3)	95.4 (20.3)	−3.9 (13.8)	91.4 (12.0)	86.0 (11.0)	−5.2 (11.0)	0.080	0.760
Total homocysteine (μM)	12.8 (2.7)	13.1 (3.0)	+0.3 (1.9)	15.8 (5.6)	15.0 (4.8)	−0.8 (2.2)	0.185	0.115
Isoleucine (μM)	71.5 (19.5)	76.5 (16.5)	+5.0 (13.2)	72.4 (19.2)	73.1 (19.3)	+0.6 (14.0)	0.550	0.318
Leucine (μM)	142 (36)	148 (29)	+6.0 (19.2)	139 (33)	139 (32)	−0.2 (14.6)	0.344	0.257
Lysine (μM)	207 (25)	213 (32)	+5.5 (29.7)	189 (35)	173 (35)	−15.2 (44.6)	0.001	0.095
Methionine (μM)	34.9 (5.1)	35.5 (5.4)	+0.6 (4.0)	35.6 (6.5)	33.0[c] (5.3)	−2.6 (5.2)	0.168	0.046
Phenylalanine (μM)	61.8 (11.5)	64.5 (7.8)	+2.6 (10.5)	59.5 (8.7)	60.0 (8.7)	+0.5 (8.9)	0.097	0.501
Serine (μM)	110 (23)	107 (23)	−2.7 (23.0)	97.5 (22.6)	98.1 (23.1)	+0.6 (18.1)	0.238	0.608
Taurine (μM)	55.7 (12.6)	49.7 (15.8)	−6.0 (12.9)	58.3 (24.3)	58.0 (14.3)	−0.3 (19.2)	0.104	0.311
Threonine (μM)	167 (48)	164 (42)	−2.3 (33.7)	137 (26)	129 (29)	−8.4 (34.0)	0.003	0.568
Tryptophan (μM)	57.0 (14.1)	58.2 (9.7)	+1.3 (10.7)	53.2 (9.0)	54.0 (10.4)	+1.0 (11.2)	0.129	0.810
Tyrosin (μM)	70.6 (14.3)	74.0 (15.2)	+3.5 (11.9)	67.8 (16.7)	68.3 (15.6)	+0.5 (16.3)	0.247	0.524
Valine (μM)	234 (46)	252 (40)	+18.7 (40.3)	223 (46)	221 (42)	−2.4 (29.6)	0.020	0.065

[a] Values are means (SD); casein group $n = 21$ (male: 9, female: 12), lupin protein group $n = 22$ (male: 11, female: 11)

[b] Comparison between the casein and the lupin protein group (Student's *t*-test)

[c] Significantly different from baseline within a group, $P < 0.05$ (paired *t*-test)

An increase of the mRNA abundance of SREBP-2 from baseline to week 6 was seen in the group who received lupin protein but not in the group who received casein (Fig. 1). The mRNA abundance of the LDL receptor in mononuclear blood cells increased from baseline to week 6 in both groups ($P < 0.05$, Fig. 1), whereas the mRNA concentration of HMG-CoA reductase declined. The mRNA concentrations of SREBP-2, LDL receptor and HMG-CoA reductase were not different between the groups.

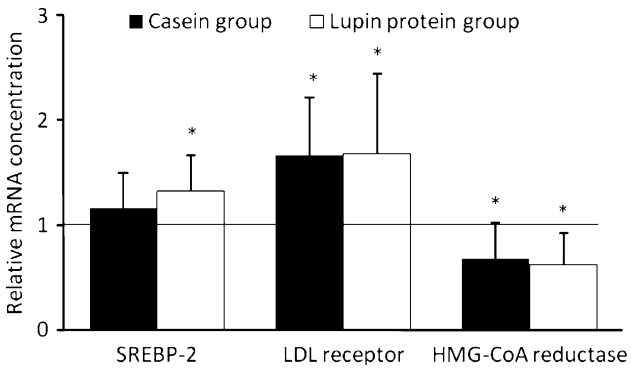

Fig. 1 Relative mRNA concentrations of sterol regulatory element-binding protein (SREBP)-2, LDL receptor and 3-hydroxy-3-methyl-glutaryl (HMG-CoA) reductase in mononuclear cells of the subjects after 6 weeks of intervention with casein or lupin protein relative to baseline (= 1.00). Bars are means with standard deviations. For normalization β-actin mRNA concentration was used. *Significantly different from baseline within a group, $P < 0.05$ (paired *t*-test). Casein group $n = 21$ (male: 9, female: 12), lupin protein group $n = 22$ (male: 11, female: 11)

Discussion

The present study investigated the effects of lupin protein on plasma cholesterol concentration in hypercholesterolemic subjects who would most benefit from a cholesterol reduction. This study showed that the intake of the proteins over a period of 6 weeks lowered the plasma cholesterol concentration of the subjects, irrespective of the type of protein. However, in the casein group the reduction of circulating plasma cholesterol was primarily caused by a reduction of HDL cholesterol (−9.4%), whereas in the lupin protein group the observed reduction of plasma cholesterol was mainly caused by a decrease of LDL cholesterol, with insignificant effects on HDL cholesterol. The mean reduction of the LDL cholesterol was

−0.31 mM (about −8.6%), which is similar to the LDL cholesterol-lowering effect observed from 38 controlled clinical intervention studies with soy, where a mean intake of 47 g/d soy protein resulted in a reduction of LDL cholesterol by 12.9% compared with an animal protein [1]. We also found that lupin protein compared to casein resulted in a more favourable net change of the LDL:HDL cholesterol-ratio. Along with hypertension and tobacco consumption, increased cholesterol levels account for approximately 50% of all coronary heart diseases [20]. Thus, we assume that lupin protein in exchange of casein could possibly contribute to lower the cardiovascular risk. Although we are aware of the fact that an intake of 35 g lupin protein per day is impracticable, the nutty taste of lupin protein makes it a suitable ingredient for various kinds of foods. However, subsequent studies are necessary to test the efficacy of smaller amounts of lupin protein in reduction of circulating LDL cholesterol.

In contrast to recently described triglyceride-lowering effect of lupin protein in hypercholesterolemic rats [2], this study did not found any favourable effect of lupin protein on circulating triglycerides. By comparison, the soy protein effect on plasma triglycerides varies from a decrease [8, 17] to no effects [18]. The participants of our study were under "uncontrolled" nutrition conditions and plasma triglycerides are known to be influenced by a series of dietary factors such as n-3 polyunsaturated fatty acids, refined carbohydrates, *trans* fatty acids and alcohol [23]. Studies which will focus on the effect of lupin protein on plasma triglycerides should be performed under conditions of controlled nutrient intake.

Lupin protein belongs to the same plant family as soy, and thus it can be assumed that the composition of the proteins shows a great similarity. We assume that the cholesterol-lowering effect of lupin protein compared to casein could be caused by its peptides or amino acids. Conglutin γ is suggested to be one of the cholesterol-lowering peptides in lupin protein [14], and bioactive peptides derived from soy protein hydrolysates have been reported to have cholesterol-lowering property due to their stimulating effects on LDL receptor transcription [3]. In this study, we could show that the mRNA abundance of the LDL receptor in mononuclear blood cells increased from baseline to week 6 of intervention with lupin protein, but also with casein. The up-regulation of LDL receptor and the down-regulation of HMG-CoA reductase could explain the observed decrease of plasma cholesterol from baseline to week 6 of intervention in both treatment groups but did not explain the differences in LDL and HDL changes between both treatment groups. Thus, we assume that the transcript levels of SREBP-2, LDL receptor and HMG-CoA reductase in the mononuclear cells did not reflect the actual changes in circulating LDL cholesterol between the two groups.

Another question was whether such high amounts of lupin protein or casein could alter the concentrations of plasma amino acids or lead to imbalances of circulating amino acids. Lupin protein compared to casein has an about 3-fold higher concentration of arginine, a 3.6-fold higher concentration of cysteine, half of the lysine and only about 10% of the methionine concentration. Despite such strong differences in the amino acid composition of the dietary proteins, the plasma amino acids were not or only moderately changed by the dietary proteins. Among the essential amino acids, the concentration of plasma methionine declined by 7.3% in individuals with lupin protein intake. We suggested that this could have influenced the synthesis of homocysteine and the plasma concentrations of homocysteine. Recently, a high consumption of soy food was inversely associated with plasma homocysteine concentrations in an observational study [10]. Homocysteine is a non-proteinogenic amino acid and is considered to be a strong and independent risk factor for cardiovascular disease [22]. Although we observed a slight decrease of plasma methionine concentration from baseline to week 6 in the group who received lupin protein, the concentration of homocysteine was not influenced. Since levels of other amino acids were also not markedly influenced by lupin protein, we suggest that lupin protein will not cause detrimental effects on amino acid metabolism.

In conclusion, this report examined the effects of lupin protein on circulating plasma lipids compared with casein as a control protein in humans. Lupin protein compared to casein has the potential to improve the LDL:HDL cholesterol-ratio of hypercholesterolemic subjects. Despite strong differences in the amino acid composition of both proteins, amino acid imbalances or detrimental alterations of plasma amino acids were not observed. Further research is warranted to elucidate the active component in lupin protein and the minimum dose required for beneficial health effects.

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