Journal of Food and Nutrition Research, 2019, Vol. 7, No. 8, 554-559
Available online at http://pubs.sciepub.com/jfnr/7/8/1
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-8-1

Effects of a Non Dairy Cheese Cream Containing Fermented Soybean Extract on Lipid Profile and Lipoproteins in Dyslipidemic Patients

Giuseppe Derosa[1,2,3,*], Davide Romano[1], Angela D'Angelo[1,2], Pamela Maffioli[1]

[1]Centre of Diabetes and Metabolic Diseases, Department of Internal Medicine and Therapeutics, University of Pavia and Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy
[2]Laboratory of Molecular Medicine, University of Pavia, PAVIA, Italy
[3]Center for Prevention, Surveillance, Diagnosis and Treatment of Rare Diseases, Fondazione IRCCS Policlinico San Matteo, PAVIA, Italy
*Corresponding author: giuseppe.derosa@unipv.it

Received May 17, 2019; Revised July 03, 2019; Accepted August 09, 2019

Abstract **Aim:** to evaluate the effects of a non dairy cheese cream containing fermented soybean extract compared to a dairy cheese cream on lipid profile and lipoproteins in dyslipidemic patients. **Methods:** patients were randomized to take, twice a day on a slice of bread, a non dairy cheese cream, containing fermented soybean extract 75% (Valsoia Lo spalmabile®), or a placebo dairy cheese cream for 3 months, in a double-blind, placebo-controlled study design. We evaluated, at baseline and after 3 months: anthropometric parameters, fasting plasma glucose (FPG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), lipoprotein (a) [Lp(a)], apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B), high-sensitivity C-reactive protein (Hs-CRP). **Results:** we did not record any variation of FPG. Total cholesterol, LDL-C and Tg decreased after 3 months with the active treatment, both compared to baseline and placebo ($p < 0.05$ for both). No variations were recorded with placebo. High density cholesterol did not change in neither group. We recorded a decrease of Apo-B and Hs-CRP with the active treatment, but not with placebo, compared to baseline ($p < 0.05$ for both), even if, in group to group comparison, only Apo-B resulted lower compared to placebo ($p < 0.05$). Lp(a) and Apo-A1 did not differ between treatments or compared to baseline. **Conclusions:** a non dairy cheese cream, containing fermented soybean extract 75%, better improved lipid profile compared to placebo dairy cheese cream in dyslipidemic patients.

Keywords: *cheese cream, lipid profile, non dairy cheese cream, soybean extract*

Cite This Article: Giuseppe Derosa, Davide Romano, Angela D'Angelo, and Pamela Maffioli, "Effects of a Non Dairy Cheese Cream Containing Fermented Soybean Extract on Lipid Profile and Lipoproteins in Dyslipidemic Patients." *Journal of Food and Nutrition Research*, vol. 7, no. 8 (2019): 554-559. doi: 10.12691/jfnr-7-8-1.

1. Introduction

In the latest years the interest about the effects of soy and its derivatives on human health has increased. In literature, soy consumption has been associated with various beneficial effects including a lower risk of cardiovascular events and osteoporosis. Soy consumption seems to also play a small part in the prevention of some types of cancer such as, for example, breast cancer. The soy and its isoflavones appear to also alleviate symptoms linked to menopause [1,2]. These positive effects seem to be attributable to the fiber content of soya, its degree of saturated fat and its role in the modulation of lipid metabolism. In epidemiological studies, the consumption of soy, soy isoflavones, or both, were inversely correlated with circulating levels of total cholesterol (TC) [3,4], low density lipoprotein-cholesterol (LDL-C) [4], and triglycerides (Tg) [5], and positively correlated with the levels of high density lipoprotein-cholesterol (HDL-C) [6]. Human studies also supported the hypothesis that an average consumption of 47 g of soy each day results in a reduction of 9.3% in TC, of 12.9% in LDL-C and 10.5% of Tg [7,8,9]. On this basis, the Food and Drug Association (FDA) approved soy as part of a proper diet [10]. Soybeans are a rich source of isoflavones, heterocyclic phenols with structural similarity to estradiol-17 beta and selective estrogen receptor modulators. Soy lowers lipid profile throughout isoflavones absorbed in the body after being activated by lactobacillus. A previous study published in literature [11] evaluated the efficacy of a food supplement combination based on isoflavones and berberine (ISB) in the treatment of menopausal symptoms and dyslipidemia. The isoflavones and berberine combination treatment significantly lowered plasma TC (-13.5% vs -0.2%), LDL-C (-12.4% vs + 0.8 %) and Tg (-18.9% vs -1.3%) and improved menopausal symptoms compared with calcium and vitamin D (3) treatment.

However, in the above study also berberine was administered, so the effects of lipid profile could be also due to berberine, that proved to have hypocholesterolemic and hypoglycemic effects [12-16]. Soy protein products are widely available in supermarkets, and lower-fat soy products are easily obtainable; however, the amount of soy protein in a single serving of various soy products is heterogeneous. No randomized, placebo-controlled, clinical studies have been published about the effects of soy on lipid profile in human.

For this reason, the aim of this study was to evaluate the effects of a non dairy cheese cream (Valsoia Lo spalmabile®), containing fermented soybean extract 75%, compared to a diary cheese cream, on lipid profile and some lipoproteins in dyslipidemic patients.

2. Materials and Methods

2.1. Study Design

This 3-months, double-blind, randomized, placebo-controlled, clinical trial was conducted at the Department of Internal Medicine and Therapeutics, University of Pavia and Fondazione IRCCS Policlinico S. Matteo, Pavia (Italy). The study protocol was approved by the institutional review board and was conducted in accordance with the 1994 Declaration of Helsinki [17] and its amendments and the Code of Good Clinical Practice. All patients provided written informed consent to participate in this study after a full explanation of the study had been given.

2.2. Patients

Caucasian patients, aged ≥18 of either sex, were eligible for inclusion if they had a condition of euglycemia [fasting plasma glucose (FPG) <100 mg/dL], hypercholesterolemia according to National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) criteria [18] (TC between 200-260 mg/dL), and with Tg < 400 mg/dL. They were overweight [19], and also normotensive according to the World Health Organization criteria (Systolic Blood Pressure [SBP] < 140 mmHg and Diastolic Blood Pressure [DBP] < 90 mmHg) [20]. Furthermore, they had normal thyroid function. Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

We enrolled patients whose LDL-C levels were not adequately controlled, and that were found intolerant to statins at high doses. The list of statins taken by patients at the enrollment is listed in Table 1a and Table 1b. Subjects were considered intolerant if, on actual statin dosage, they have experienced: an increase of CPK greater than 3 until 10 times the upper limits of the laboratory (ULN), and/or a rise in the value of transaminases greater than 3 until 5 times the ULN, and/or the onset of asthenia, myalgia or rhabdomyolysis.

Patients were excluded if they had secondary dyslipidemia, impaired hepatic function (defined as plasma aminotransferase level higher than three times the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex); endocrine (included diabetes mellitus), or gastrointestinal disorders, current evidence of ischemic heart disease, heart failure, or stroke, weight change of > 3 Kg during the preceding 3 months, malignancy, and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Excluded medications (within the previous 3 months) included: anorectic agents, laxatives, β-agonists (other than inhalers), cyproheptadine, anti-depressants, anti-serotoninergics, phenothiazines, barbiturates, oral corticosteroids, and anti-psychotics. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded.

2.3. Diet and Physical Activity

At baseline all patients were already following an adequate diet and practicing physical activity. The controlled-energy diet (~600 kcal daily deficit) was based on NCEP-ATP III recommendations [21], that contained 50% of calories from carbohydrates, 30% from fat (< 7% saturated, up to 10% polyunsaturated, and up to 20% monounsaturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d, and 35 g/d of fiber. Standard diet advice was given by a dietitian and/or specialist physician. Dietitians and/or specialists each two weeks provided instruction on dietary intake-recording procedures as part of a behavior-modification program and then from month 1 used the patients' food diaries for counseling. Individuals were also encouraged to increase their physical activity and we standardized the same physical aerobics exercise program by riding a stationary bicycle for 20 to 30 minutes, 3 to 4 times per week.

Table 1a. Dosage of statins taken at the study enrollment in the group treated with active treatment.

DOSAGE	LOVASTATIN	PRAVASTATIN	SIMVASTATIN	ATORVASTATIN	ROSUVASTATIN
10 mg (n)	5	2	10	1	4
20 mg (n)	4	2	9	0	1
40 mg (n)	8	8	9	1	0

n: number of patients.

Table 1b. Dosage of statins taken at the study enrollment in the group treated with placebo

DOSAGE	LOVASTATIN	PRAVASTATIN	SIMVASTATIN	ATORVASTATIN	ROSUVASTATIN
10 mg (n)	3	4	8	2	2
20 mg (n)	3	2	7	2	1
40 mg (n)	6	9	11	3	0

n: number of patients.

2.4. Treatment

Patients were randomized to take, 20 g twice a day on a slice of bread, a non dairy cheese cream, containing fermented soybean extract 75% (Valsoia Lo spalmabile®), or a placebo dairy cheese cream for 3 months, in a double-blind, placebo-controlled study design. For the composition of administered treatments, see Table 2 and Table 3.

Both active and placebo treatment were supplied as identical, white cheese cream in coded boxes to ensure the blind status of the study. Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. Medication compliance was assessed by counting the number of boxes returned at the time of specified clinic visits. Throughout the study, we instructed patients to take their first dose of treatment on the day after they were given the study product. At the same time, all unused boxes were retrieved for inventory. All treatments were provided free of charge.

2.5. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of waist circumference (WC), abdominal circumference (AC), hip circumference (HC), height and body weight, calculation of body mass index (BMI), assessment of FPG, TC, LDL-C, HDL-C, Tg, lipoprotein (a) [Lp(a)], apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B), high-sensitivity C-reactive protein (Hs-CRP). All variables were assessed at baseline, and after 3 months from randomization.

All plasmatic variables were determined after a 12-hour overnight fast. Venous blood samples were drawn by a research nurse for all patients between 8:00 AM and 9:00 AM. We used plasma obtained by addition of Na_2-EDTA, 1 mg/ml, and centrifuged at 3000 g for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at -80°C for ≤3 months. All measurements were performed in a central laboratory.

Body mass index was calculated by the investigators as weight in kilograms divided by the square of height in meters. Waist circumference was measured midway between the lateral lower rib margin and the iliac crest and its reduction was determined with a Gulick anthropometric spring-loaded tape measure (Model 5829, Bell Medical Services, Neptune, NJ, USA).

Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV) < 2 % [22]. Total cholesterol and Tg levels were determined using fully enzymatic techniques [23,24] on a clinical chemistry analyzer (Hitachi 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0 % and 2.1 % for TC measurement, and 0.9 % and 2.4% for Tg measurement, respectively. HDL-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [25]; intra- and interassay CsV were 1.0 % and 1.9 %, respectively. LDL-C level was calculated using the Friedewald formula [26].

Apo A-1 and Apo B were measured by immuno-turbidimetric assays (Boehringer-Mannheim, Mannheim, Germany); the intra- and interassay CsV were 5 % and 3 %, respectively [27,28]. Lipoprotein(a) [Lp(a)] was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) method, that is insensitive to the presence of plasminogen, using the commercial kit Macra-Lp(a) (SDI, Newark, Delaware, USA) [29,30]; the intra- and interassay CsV of this method were 5 % and 9 %, respectively.

High-sensitivity C reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Delaware, USA). The intra- and interassay CsV were 5.7 % and 1.3 %, respectively [31].

2.6. Statistical Analysis

An intention-to-treat (ITT) analysis was conducted in patients who had received ≥1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received ≥1 dose of trial medication after randomization and had undergone a subsequent tolerability observation. The null hypothesis that the expected lipid profile change from baseline until the end of the study did not differ significantly between active treatment and placebo was tested using analysis of variance and analysis of covariance (ANCOVA) models [32]. Similar analyses were applied to the other variables. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. A 1-sample *t* test was used to compare values obtained before and after treatment administration; 2-sample *t* tests were used for between-group comparisons. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean (SD). For all statistical analyses, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Study Sample

A total of 129 patients were enrolled in the trial. Of these, 65 (50.4%) were randomized to the active treatment and 64 (49.6%) to placebo. One hundred and twenty-three subjects completed the study; there were 6 patients (3 males and 3 females) who did not complete the study and the reasons for premature withdrawal was non-compliance to treatment (1 male and 1 female in the placebo group, 1 male and 1 female in the active treatment group) and withdrawn of the consent (1 female in placebo group and 1 male in the active treatment group).

3.2. Anthropometric Parameters

No variations of body weight or BMI or circumferences were recorded (Table 4).

Table 2. Composition of non dairy cheese cream (Valsoia Lo spalmabile®) and placebo dairy cream

ACTIVE TREATMENT (VALSOIA LO SPALMABILE®)	PLACEBO DAIRY CREAM
Fermented soya extract 75% [water, soya beans (8.4%), live cultures]	Pasteurized milk
Coconut oil	Cream
Dietary fibre	Salt
Thickening agents: carrageenan-sodium alginate	Thickening agents (sodium alginate, flour of bean seeds, carrageenan)
Stabiliser: pectin	
Calcium phosphate, sea salt, vitamin D2	

Table 3. Nutritional composition of non dairy cheese cream (Valsoia Lo spalmabile®) and placebo dairy cream for 100 g of product

NUTRIENTS	ACTIVE TREATMENT (VALSOIA LO SPALMABILE®)	PLACEBO DAIRY CREAM
Energy	209 kcal, 861 kj	280 kcal, 1145 kj
Fats	20 g (17 g saturated)	27.7 g (17.5 g saturated)
Carbohydrates	1.8 g (1.3 sugars)	2.7 g (2.6 sugars)
Fibers	3.3 g	0.1 g
Protein	2.9 g	4.5 g
Salt	0.5 g	0.3 g
Calcium	120 mg	/

Table 4. Patients data during the study in placebo and active treatment group

glimepiride + metformin group	PLACEBO		ACTIVE TREATMENT	
	BASELINE	3 MONTHS	BASELINE	3 MONTHS
N	64	61	65	62
sex (M/F)	30/34	29/32	33/32	31/31
Age (years)	51.2 ± 10.4	-	53.8 ± 11.7	-
Smoking status (M/F)	15/12	15/11	13/17	13/15
Height (m)	1.67 ± 0.04	-	1.68 ± 0.05	-
Weight (Kg)	74.2 ± 9.4	73.8 ± 9.1	76.1 ± 10.8	75.8 ± 10.3
BMI (Kg/m^2)	26.7 ± 2.0	26.6 ± 1.9	26.9 ± 2.2	26.8 ± 2.1
WC (cm)	91.3 ± 7.2	90.1 ± 7.0	90.4 ± 7.0	89.5 ± 6.8
HC (cm)	106.4 ± 9.7	105.8 ± 9.2	106.8 ± 9.9	106.1 ± 9.3
AC (cm)	93.6 ± 6.4	93.4 ± 6.1	94.2 ± 6.9	93.7 ± 6.2
FPG (mg/dL)	89.5 ± 8.2	87.7 ± 7.4	90.3 ± 8.5	85.4 ± 6.7
TC (mg/dL)	226.4 ± 22.7	231.8 ± 25.3	230.2 ± 24.9	202.5 ± 13.6*^
LDL-C (mg/dL)	157.5 ± 14.6	161.3 ± 15.1	161.8 ± 15.4	136.7 ± 7.2*^
HDL-C (mg/dL)	46.5 ± 5.9	45.2 ± 5.1	46.3 ± 5.8	48.7 ± 6.7
Tg (mg/dL)	112.1 ± 35.2	126.4 ± 41.8	110.3 ± 37.4	85.6 ± 23.5*^
Lp(a) (mg/dL)	22.8 ± 16.5	24.9 ± 17.2	24.6 ± 17.1	21.3 ± 15.7
Apo A-I (mg/dL)	132.7 ± 24.9	129.6 ± 22.4	131.2 ± 23.6	135.7 ± 26.5
Apo B (mg/dL)	120.1 ± 19.4	125.2 ± 22.8	122.5 ± 20.9	112.2 ± 14.6*^
Hs-CRP (mg/L)	1.3 ± 0.7	1.1 ± 0.6	1.3 ± 0.7	0.9 ± 0.5*

Data are means ± SD
*p< 0.05 vs baseline; ^p< 0.05 vs 3 months of placebo
BMI: body mass index; WC: waist circumference; HC: hip circumference; AC: abdominal circumference; FPG: fasting plasma glucose; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; Lp(a): lipoprotein (a); Apo A-I: apolipoprotein A-I; Apo B: apolipoprotein B; Hs-CRP: high-sensitivity C reactive protein.

3.3. Metabolic Parameters

We did not record any variation of FPG. Total cholesterol, LDL-C and Tg decreased after 3 months with the active treatment, both compared to baseline and placebo ($p < 0.05$ for both). No variations were recorded with placebo. High density cholesterol did not change in neither group.

We recorded a decrease of Apo-B and Hs-CRP with the active treatment, but not with placebo, compared to baseline ($p < 0.05$ for both), even if, in group to group comparison, only Apo-B resulted lower compared to

placebo ($p < 0.05$). Lp(a) and Apo-A1 did not differ between treatments or compared to baseline (Table 4).

4. Discussion

The cholesterol-lowering effects of soy protein as compared with animal protein have been recognized in animals for more than 80 years [33]. Carroll reviewed the evidence that soy protein produced less hypercholesterolemia and less atherosclerosis in laboratory animals than animal protein [34]. Our study showed that a non dairy cheese cream, containing fermented soybean extract 75%, better improved lipid profile compared to placebo dairy cheese cream in dyslipidemic patients, in particular we recorded a reduction of -12.1 % of TC, a reduction of -15.5 % of LDL-C and a reduction of -22.4 % with Tg. The reduction we recorded in this study was similar to the one reported by Cianci et al. [11] that administered isoflavones and berberine together. Different hypotheses have been made about the mechanisms responsible for the effects of soy protein on serum lipoproteins. Some studies suggested that alterations in bile acid or cholesterol absorption may contribute to altered cholesterol homeostasis [35]; however, Fumagalli et al. [36] found no differences in the fecal excretion of bile acids or sterols by human subjects. Other studies suggested that alterations in the ratio of serum glucagon to serum insulin may affect hepatic cholesterol synthesis; others suggested that serum free thyroxine concentrations may be higher when the diet contains soy protein [37]. Huff et al. [38] affirmed that turnover of VLDL is increased in humans when soy protein is substituted for meat and dairy protein. Other colleagues [39] observed that the LDL-receptor activity of monocytes is eight times greater in human subjects receiving soy protein than in those eating control diets; moreover, Setchell [40] suggested that soy estrogens may contribute to the cholesterol-lowering effects of soy protein.

Of course our study has some limitations, for example the short duration of the study; moreover, we did not assess if the positive effects of soy on lipid profile were maintained after the interruption of therapy.

5. Conclusions

A non dairy cheese cream, containing fermented soybean extract 75%, better improved lipid profile compared to placebo dairy cheese cream in dyslipidemic patients.

References

[1] D'Adamo, C.R., Sahin, A., "Soy foods and supplementation: a review of commonly perceived health benefits and risks," *Altern Ther Health Med*, 20(1). 39-51. Winter.2014.

[2] Nagata, C., Mizoue, T., Tanaka, K., Tsuji, I., Tamakoshi, A., Matsuo, K., Wakai, K., Inoue, M., Tsugane, S., Sasazuki, S.; Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan, "Soy intake and breast cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population,". *Jpn J Clin Oncol*, 44(3). 282-295. Mar.2014.

[3] Clarkson, T.B., "Soy, soy phytoestrogens and cardiovascular disease,". *J Nutr*, 132. 566S-569S. Mar.2002.

[4] Forsythe, W.A., Green, M.S., Anderson, J.J., "Dietary protein effects on cholesterol and lipoprotein concentrations: a review,". *J Am Coll Nutr*, 5. 533-549. 1986.

[5] Nagata, C., Takatsuka, N., Kurisu, Y., Shimizu, H., "Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women," *J Nutr*, 128. 209-213. Feb.1998.

[6] Ho, S.C., Woo, J.L., Leung, S.S., Sham, A.L., Lam, T.H., Janus, E.D., "Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population," *J Nutr*, 130. 2590-2593. Oct.2000.

[7] de Kleijn, M.J., van der Schouw, Y.T., Wilson, P.W., Adlercreutz, H., Mazur, W., Grobbee, D.E., Jacques, P.F., "Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study (1-4)," *J Nutr*, 131. 1826-1832. Jun.2001.

[8] Derosa, G., D'Angelo, A., Romano, D., Maffioli, P., "Response to an oral fat load and effects on lipid profile, glycemia and high-sensitivity C-reactive protein after soybean extract consumption." *Arch Med Sci*, 14(4).760-765. Jun.2018.

[9] Anderson, J.W., Johnstone, B.M., Cook-Newell, M.E., "Meta-analysis of the effects of soy protein intake on serum lipids," *N Engl J Med*, 333. 276-282. Aug.1995.

[10] US Food and Drug Administration, "Food labeling: health claims; soy protein and coronary heart disease," *Fed Regist*, 64. 57699-57733. Oct.1999.

[11] Cianci, A., Cicero, A.F., Colacurci, N., Matarazzo, M.G., De Leo, V., "Activity of isoflavones and berberine on vasomotor symptoms and lipid profile in menopausal women", *Gynecol Endocrinol*, 28(9). 699-702. Sep.2012.

[12] Derosa, G., Bonaventura, A., Bianchi, L., Romano, D., D' Angelo, A., Fogari, E., Maffioli, P., "Effects of Berberis aristata/Silybum marianum association on metabolic parameters and adipocytokines in overweight dyslipidemic patients," *J Biol Regul Homeost Agents*, 27(3). 717-728. Jul-Sep.2013.

[13] Derosa, G., Bonaventura, A., Bianchi, L., Romano, D., D'Angelo, A., Fogari, E., Maffioli, P., et al. "Berberis aristata/Silybum marianum fixed combination on lipid profile and insulin secretion in dyslipidemic patients," *Expert Opin Biol Ther*, 13(11). 1495-1506. Nov.2013.

[14] Derosa, G., Romano, D., D'Angelo, A., Maffioli, P., "Berberis aristata combined with Silybum marianum on lipid profile in patients not tolerating statins at high doses," *Atherosclerosis*, 239(1). 87-92. Mar.2015.

[15] Derosa, G., D'Angelo, A., Bonaventura, A., Bianchi, L., Romano, D., Maffioli, P., "Effects of berberine on lipid profile in subjects with low cardiovascular risk," *Expert Opin Biol Ther*, 13(4). 475-482. Apr.2013.

[16] Derosa, G., Romano, D., D'Angelo, A., Maffioli, P., "Berberis aristata/Silybum marianum fixed combination (Berberol(®) effects on lipid profile in dyslipidemic patients intolerant to statins at high dosages: A randomized, placebo-controlled, clinical trial," *Phytomedicine*, 22(2). 231-237. Feb.2015.

[17] Proposed International Guidelines for Biomedical Research Involving Human Subjects. The Council for International Organisation of Medical Sciences. Geneva, 1982.

[18] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, "Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III)," *JAMA*, 285(19). 2486-2497. May.2001.

[19] World Health Organization. Obesity: Preventing and Managing the Global Epidemic, "Report of WHO Consultation on Obesity," Geneva: WHO; June.1997.

[20] 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J Hypertens*, 17. 151-183. 1999.

[21] NCEP, "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report," *Circulation*, 106(25). 3143-4216. Dec.2002.

[22] European Diabetes Policy Group 1999, "A desktop guide to type 2 diabetes mellitus," *Diabet Med*, 16. 716-730. Sep.1999.

[23] Klose, S., Borner, K, "Enzymatische Bestimmung des Gesamtcholesterins mit dem [Enzymatic dosage of total cholesterolemia by Greiner Selective Analyzer (GSA II)]," *J Clin Chem Clin Biochem*, 15. 121-130. 1978.

[24] Wahlefeld, A.W., "Triglycerides determination after enzymatic hydrolysis," In: Methods of Enzymatic Analysis. Ed. H. U. Bergmeyer, 2nd English ed. Academic Press, New York (USA) 1974; pp. 18-31.

[25] Havel, R.J., Eder, H.A., Bragdon, J.H., "The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum," *J Clin Invest*, 34. 1345-1353. Sep.1955.

[26] Friedewald, W.T., Levy, R.I., Fredrickson, D.S., "Estimation of the concentration of low density lipoprotein in plasma, without use of the preparative ultracentrifuge," *Clin Chem*, 18. 499-502. Jun.1972.

[27] Leblond, L., Marcel, Y.L. "The amphipathic alpha-helical repeats of apolipoprotein A-I are responsible for binding of high density lipoproteins to HepG2 cells," *J Biol Chem*, 266. 6058-6067. Apr.1991.

[28] De Loof, H., Rosseneu, M., Yang, C.Y., Li, W.H., Gotto, A.M. Jr., Chan, L., "Human apolipoprotein B: analysis of internal repeats and homology with other apolipoproteins" *J Lipid Res*, 28. 1455-1465. Dec.1987.

[29] Scanu, A.M., Scandian, L., "Lipoprotein (a): structure, biology and clinical relevance" *Adv Intern Med*, 36. 249-270. 1991.

[30] Uterman, G., Weber, W., "Protein composition of lipoprotein(a)," *J Clin Invest*, 80. 458-465. Apr.1987.

[31] Rifai, N., Tracy, R.P., Ridker, P.M., "Clinical Efficacy of an Automated High-Sensitivity C-Reactive Protein Assay," *Clin Chem*, 45 (12). 2136-2141. Dec.1999.

[32] Winer, B.J., "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill, New York (USA); 1971.

[33] Ignatowsky, M.A., "Influence de la nourriture animale sur l'organisme des lapins," *Arch Med Exp Anat Pathol*, 20. 1-20. 1908.

[34] Carroll, K.K., "Hypercholesterolemia and atherosclerosis: effects of dietary protein," *Fed Proc*, 41. 2792-2796. Sep.1982.

[35] Carroll, K.K., "Review of clinical studies on cholesterol-lowering response to soy protein," *J Am Diet Assoc*, 91. 820-827. Jul.1991.

[36] Fumagalli, R., Soleri, L., Farina, R., Musanti, R., Mantero, O., Noseda, G., Gatti, E., Sirtori, C.R., "Fecal cholesterol excretion studies in type II hypercholesterolemic patients treated with the soybean protein diet," *Atherosclerosis*, 43. 341-353. Jun.1982.

[37] Bakhit, R.M., Klein, B.P., Essex-Sorlie, D., Ham, J.O., Erdman, J.W. Jr., Potter, S.M., "Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations," *J Nutr*, 124. 213-222. Feb.1994.

[38] Huff, M.W., Giovannetti, P.M., Wolfe, B.M., "Turnover of very low-density lipoprotein-apoprotein B is increased by substitution of soybean protein for meat and dairy protein in the diets of hypercholesterolemic men," *Am J Clin Nutr*, 39. 888-897. Jun.1984.

[39] Lovati, M.R., Manzoni, C., Canavesi, A., Sirtori, M., Vaccarino, V., Marchi, M., et al., "Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients," *J Clin Invest*, 80. 1498-1502. Nov.1987.

[40] Setchell, K.D.R., "Naturally occurring non-steroidal estrogens of dietary origin," In McLachlan JA, ed. Estrogens in the environment II: influences on development. New York: Elsevier, 69-85. 1985.