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LIPOPROTEINS AND OTHER CLINICAL-CHEMISTRY PARAMETERS UNDER THE CONDITIONS OF LACTO-OVO-VEGETARIAN NUTRITION

Volker Richter, Kathrin Purschwitz, Anne Bohusch, Hermann Seim, Christoph Weisbrich, Wolfgang Reuter[+], Dietlind Sorger[++], Fausi Rassoul
Department of Clinical Chemistry and Pathobiochemistry, University Leipzig/
Working group health promotion and prevention of atherosclerosis (AGA e. V.) Leipzig
[+]Clinic of Internal Medicine IV, University Leipzig
[++]Department of Nuclear Medicine, University Leipzig, Germany

ABSTRACT

The aim of this study was to examine concentration and composition of lipoproteins and levels of apolipoproteins in serum of 46 lacto-ovo-vegetarians in comparison with 49 non-vegetarians. Other clinical-chemistry parameters were also included in this study. Both male and female lacto-ovo-vegetarians showed a favourable lipoprotein profile characterized by low concentrations of atherogenic LDL-cholesterol, low ratios of cholesterol/HDL-cholesterol, LDL-cholesterol/ HDL-cholesterol and a high ratio HDL_2-cholesterol/HDL_3-chol-esterol. The favourable lipoprotein profile of vegetarians manifested as a low ratio of apolipoprotein B/apolipoprotein A-I and a low concentration of lipoprotein particles containing apolipoprotein B together with apo C-III. These parameters were significantly different between the vegetarians and the non-vegetarians.

The α-tocopherol/LDL-cholesterol molar ratio was significantly higher in vegetarians in comparison with non-vegetarians which may be important with respect to protection against atherosclerosis. Total and free carnitine concentrations were reduced in serum of lacto-ovo-vegetarians. However this small reduction does not reflect a carnitine deficiency. The favourable serum lipoprotein profile among vegetarians observed in this study may result not only from nutritional conditions but also from special life-style factors within this group.

KEY WORDS: Lacto-ovo-vegetarians, Lipoproteins, Apolipoproteins, α-tocopherol

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Address reprint request to : Prof. Dr. V. Richter, Department of Clinical Chemistry and Pathobiochemistry, University Leipzig, Paul-List-Straße 13/15, 04103 Leipzig, Germany.
PH: ++49 341 97 22 271 FAX: ++49 341 97 22 279
Supported by Eden-Stiftung Bad Soden /TS, Germany

INTRODUCTION

It is well recognised that a great number of risk factors are associated with an increased risk for cardiovascular disease. Amoung these, the most important are atherogenic lipoprotein profile, hypertension and cigarette smoking (1). Although no single dietary factor is universally associated with cardiovascular disease, saturated fatty acids and cholesterol in the diet are generally positively correlated with coronary artery disease (2). A diet consisting largely of low-caloric vegetables and fruit markedly reduces risk factors for atherosclerosis (3). Epidemiological studies have shown a decreased risk for coronary heart disease among vegetarians. In contrast the incidence of cardiovascular diseases is high in the general population of countries consuming diet rich in animal fat. There are several reports discussing differences in morbidity and mortality between vegetarians and non-vegetarians in different countries (4, 5, 6). Vegetarians and general population differ both with respect to nutrition and other life-style factors (7, 8). These factors may have implications for lipoprotein metabolism and risk for atherosclerosis. In Germany the most important vegetarian studies were the Heidelberg, Gießen and Berlin studies (6, 7, 9). Investigations on population basis among residents of Leipzig/Germany showed significant differences in ratios of cholesterol/ high density lipoprotein (HDL) cholesterol, mean body mass index and various life-style factors between general population and vegetarians (10). These differences may be related to the primary role of dietary and life-style factors in determining the lipid-mediated risk for atherosclerosis. Several reports showed beneficial metabolic effects that result from low total and saturated fat, low cholesterol, high fiber diet (11, 12). The favourable lipid profile of vegetarians compared with non-vegetarians could result from lower intake of saturated fat and higher ratios of polyunsaturated to saturated fatty acids in the diet (13). Shifting from conventional diet to vegetarian diet alters serum lipid and lipoprotein profile (14, 15).

The long term effect of vegetarian nutrition with respect to lipoprotein constellation is of special interest. Earlier studies showed low plasma levels of total and low density lipoprotein (LDL) cholesterol in vegetarians (16, 17, 18). HDL-cholesterol/apo A-I ratios appeared higher in vegetarians compared to a group of non-vegetarians (17). According to the lipoprotein family concept plasma lipoproteins are viewed as a mixture of discrete lipoprotein families defined by their apolipoprotein composition (19). The effects of vegetarian nutrition on families of lipoprotein particles have not yet been systematically studied. The present study was directed to characterization of apolipoprotein-specific lipoprotein particles (Lp A-I, Lp A-I:A-II, Lp B:C-III) and to evaluation of the distribution of other lipoprotein subfractions. Additional several clinical-chemistry parameters were also included in the study.

MATERIAL AND METHODS

Participants

Ninety five subjects (49 non-vegetarians and 46 lacto-ovo-vegetarians) were included in the study. Informed consent was obtained from all participants. The Ethical Committee of the University of Leipzig approved the project plan.
Among lacto-ovo-vegetarians 18 were male and 28 were female subjects. Recruitment of vegetarians was carried out during meetings of the German Society of Vegetarians (Deutscher Vegetarierbund e. V.). The age – matched controls consisted of 18 men and 30 women. The mean age of male vegetarians and control subjects was 42 years, that of female participants was 33 years. Recruitment of control subjects was carried out among the staff of the University Leipzig, several factories and social institutions as in earlier investigations (10).

Thus the control group may be representative with respect to general population. The objectives of this study were to compare lipoprotein profile and other clinical-chemistry parameters between this group and lacto-ovo-vegetarians. Evaluation of dietary and life-style characteristics both within the control and the vegetarian group was conducted after subjects were recruited. Patients with diabetes mellitus, gout, hypo-and hyperthyreosis and diseases of liver and kidney were excluded.

Evaluation of dietary and life-style factors and sample collection

Most of the vegetarians included in the present study were members of the German Society of Vegetarians. Evaluation of dietary and life-style factors (smoking behaviour and alcohol consumption), anthropometric data and measurement of blood pressure and sample collection were done during regular meetings of vegetarians. Firstly, in a health-habits questionaire, these practising vegetarians were asked to categorize themselves as vegan, lacto-vegetarian and lacto-ovo-vegetarian. Additionally they also completed a food-frequency questionaire. Diet history was obtained by a team of well-trained interviewers. They were members of the Working group health promotion and prevention of atherosclerosis (AGA e.V.) Leipzig. Diet history and life-style factors of control subjects were evaluated by the same team of interviewers. The amount of intake per meal and the frequency of intake in 7 days were ascertained through interviews. The food consumption data were analyzed using the EBIS program (J. Erhardt, Robert-Bosch Krankenhaus Stuttgart/ Universität Hohenheim, Germany). Subjects were classified as lacto-ovo-vegetarians if they had not eaten meat, meat products, fish and fish products. However, they did not avoid milk, eggs and theyr products. The duration of vegetarian diet was at least two years, in the middle and high age groups of vegetarians the duration was ten years and more.
Fasting venous blood was collected and the serum was separated by low grade centrifugation. The serum was analysed immediately or aliquotes were stored frozen at –80 °C until analysed.

Determination of parameters of lipoprotein metabolism

Cholesterol, HDL-cholesterol and triglyceride concentrations were determined enzymatically using test kits of Boehringer Mannheim GmbH, Germany. HDL subfractionation was performed with polyethylene glycol 20.000 (Quantolip HDL_2/HDL_3) of Immuno GmbH Heidelberg, Germany. LDL-cholesterol was determinated following precipitation of LDL with polyvinylsulfate (Boehringer Mannheim GmbH). According to this method the LDL-cholesterol concentration was calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation (20). Apolipoproteins A-I and B were analysed immunonephelometrically using antisera obtained from Behring AG Marburg, Germany. Lipoprotein particles defined by their apolipoprotein composition (LpA-I, LpA-I:A-II, LpB:C-III) and apo C-III-Lp non B were measured by electroimmunoassay (21,22). Anti apo A-II antibodies incorporated in excess in the gel permit to block the family of Lp A-I:A-II particles, when the Lp A-I particles go on to migrate and react with anti apo A-I, to give formation to an immunoprecipitation rocket with a height proportional to the concentration of Lp A-I particles. In order to characterize particles Lp B:C-III and HDL-apo C-III, firstly total apo C-III was measured and secondly anti apo B antibodies were added to serum. After precipitation of all classes of apo B containing lipoproteins apo C-III-Lp non B was measured. The difference between the rockets obtained on neat serum and on traited serum, gives the concentration of Lp B:C-III particles. Electroimmunoassay kits were supplied by Sebia Fulda, Germany.

Determination of other clinical-chemistry parameters

Parameters of lipoprotein metabolism were measured together with other clinical-chemistry parameters: glucose, uric acid, protein and albumin in serum, α-tocopherol, ß-carotene, ascorbic acid, vitamin B_{12}, folic acid, calcium, magnesium, zinc, blood iron, ferritine, transferrine. Determinations were carried out under routine conditions in the central laboratory and in clinical laboratories of the university. Carnitine concentrations were measured by a radioenzymatic assay based on carnitine acetyltransferase reaction and incorporation of acetyl moiety of [1-^{14}C]-acetyl-CoA into acetylcarnitine.

RESULTS

89 percent of vegetarians and 80 percent of control subjects included in the present study were nonsmokers. 70 percent of vegetarians generally avoided alcohol consumption. In 30 percent of vegetarians moderate drinking (1 drink per week or less) was evaluated. In contrast, moderate drinking was evaluated in almost all of the control subjects (95 percent of male and 90 percent of female subjects, 3 or 2 drinks per week which corresponds to the drinking behaviour of general population).

The percentage of vegetarians and non-vegetarians within classes of different concentrations of serum cholesterol is shown in Table 1. The prevalence of hypercholesterolemia > 6.5 mmol/l is low among vegetarians in comparison with non-vegetarians.

Table 1: Percentage of vegetarians and non-vegetarians within classes of different concentrations of serum cholesterol

Cholesterol (mmol/l)	Non-vegetarians (n=49)	Vegetarians (n=46)
≤ 4.0	6%	21%
> 4.0 – 5.2	30%	40%
> 5.2 – 6.5	42%	37%
> 6.5	22%	2%

Both male and female lacto-ovo-vegetarians included in the study show a favourable lipoprotein profile characterized by low concentrations of atherogenic LDL-cholesterol and low ratios of cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol. Although there is no statistically significant difference with respect to HDL-cholesterol, the ratio of HDL_2-cholesterol/HDL_3-cholesterol is higher in vegetarians in comparison with non-vegetarians. This is due to elevated HDL_2-cholesterol concentrations. Vegetarians had an increased α-tocopherol to LDL-cholesterol molar ratio (Table 2).

TABLE 2: Lipids, lipoproteins and lipoprotein composition in male and female lacto-ovo-vegetarians and non-vegetarian control subjects

Parameter	Non-vegetarians (Men, n=19)	Vegetarians (Men, n=18)	Non-vegetarians (Women,n=30)	Vegetarians (Women,n=28)
Cholesterol (mmol/l)	5.70 ± 0.89	*4.88 ± 1.18	5.44 ± 1.26	*4.76 ± 0.84
Triglycerides (mmol/l)	1.76 ± 0.71	#1.17 ± 0.75	1.17 ± 0.59	1.01 ± 0.41
LDL-cholesterol (mmol/l)	3.71 ± 0.82	*2.99 ± 1.05	3.51 ± 1.17	*2.76 ± 0.76
HDL-cholesterol (mmol/l)	1.18 ± 0.27	1.22 ± 0.29	1.39 ± 0.31	1.47 ± 0.26
HDL_3-cholesterol (mmol/l)	0.98 ± 0.20	0.94 ± 0.21	1.17 ± 0.32	1.05 ± 0.16
HDL_2-cholesterol (mmol/l)	0.20 ± 0.11	*0.28 ± 0.10	0.22 ± 0.11	#0.42 ± 0.14
Cholesterol / HDL-cholesterol	5.0 ± 1.3	*4.0 ± 1.0	4.2 ± 1.7	*3.3 ± 1.0
LDL-cholesterol / HDL-cholesterol	3.3 ± 1.0	*2.5 ± 0.9	2.8 ± 1.3	#1.9 ± 0.8
HDL_2-cholesterol / HDL_3-cholesterol	0.21 ± 0.09	#0.30 ± 0.06	0.18 ± 0.09	#0.40 ± 0.12
HDL-triglycerides / HDL-cholesterol	0.16 ± 0.09	0.14 ± 0.07	0.15 ± 0.09	0.13 ± 0.05
α-tocopherol (mmol/l)	0.022 ± 0.006	*0.029 ± 0.01	0.028 ± 0.009	*0.036 ± 0.009
α-tocopherol / LDL-cholesterol	0.006 ± 0.003	*0.010 ± 0.003	0.008 ± 0.002	*0.013 ± 0.004

Values are means ± SD, *P < 0.05; #P < 0.01 relative to non-vegetarians, MANN-WHITNEY test was used for statistic analysis

Levels of apolipoproteins and lipoprotein particles defined by their apolipoprotein composition are shown in Table 3. According to the well known sex-difference regarding HDL and ther constituents female subjects are characterized by higher apo A-I concentrations in comparison to male subjects. The favourable lipoprotein profile of vegetarians is reflected in a low ratio of apo B/apo A-I and a low concentration of LpB:C-III particles. Vegetarian women have higher apo A-I concentrations in comparison with non-vegetarian women.

TABLE 3: Apolipoproteins and lipoprotein particles defined by their apolipoprotein composition in male and female lacto-ovo-vegetarians and non-vegetarian control subjects

Parameter	Non-vegetarians (Men, n=19)	Vegetarians (Men, n=18)	Non-vegetarians (Women, n=30)	Vegetarians (Women, n=28)
Apo B (g/l)	1.19 ± 0.21	1.07 ± 0.22	1.13 ± 0.38	1.00 ± 0.29
Apo A-I (g/l)	1.66 ± 0.40	1.71 ± 0.26	1.82 ± 0.24	*1.99 ± 0.26
Apo B / Apo A-I	0.76 ± 0.22	*0.62 ± 0.14	0.65 ± 0.31	*0.51 ± 0.16
Lp A-I (g/l)	0.52 ± 0.20	0.49 ± 0.16	0.59 ± 0.17	0.53 ± 0.15
Lp A-I:A-II (g/l)	1.14 ± 0.34	1.22 ± 0.22	1.23 ± 0.19	1.46 ± 0.23
Apo C-III (mg/l)	39.1 ± 15.3	*25.5 ± 10.3	34.3 ± 9.5	27.1 ± 7.9
Lp B:C-III (mg/l)	14.3 ± 6.9	10.6 ± 7.3	11.1 ± 3.6	*9.1 ± 4.9

Values are means ± SD, *P < 0.05 relative to non-vegetarians, MANN-WHITNEY test was used for statistic analysis

Serum glucose, uric acid, protein and albumin concentrations were found to be in the normal range both in vegetarians and non-vegetarians. As shown in Table 4 lacto-ovo-vegetarians had decreased total and free serum L(-)-carnitine concentrations in comparison with non-vegetarians.

TABLE 4: Total and free serum L(-)-carnitine in male and female lacto-ovo-vegetarians and non-vegetarian control subjects

Parameter	Non-vegetarians (Men, n=19)	Vegetarians (Men, n=18)	Non-vegetarians (Women, n=30)	Vegetarians (Women, n=28)
Total carnitine (μmol/l)	46.1 ± 9.2	*37.3 ± 8.6	39.4 ± 9.1	*31.5 ± 10.0
Free carnitine (μmol/l)	41.3 ± 8.4	*33.6 ± 9.3	33.2 ± 8.2	*27.1 ± 6.3

Values are means ± SD, *P < 0.05 relative to non-vegetarians, MANN-WHITNEY test was used for statistic analysis

Mean serum concentrations of vitamin B_{12} were low in male lacto-ovo-vegetarians (160 ± 110 pmol/l) and in female vegetarians (240 ± 150 pmol/l). In contrast mean levels of

folic acid (37 ± 8 nmol/l), ascorbic acid (8.5 ± 2.0 µg/ml), ß-carotin (6.5 ± 1.3 µg/ml) and α-tocopherol (33 ± 11 µmol/l) were within the reference range. Also serum concentrations of calcium (1.13 ± 0.10 mmol/l), magnesium (0.91 ± 0.7 mmol/l), zinc (15.9 ± 2.1 µmol/l) and copper (18.8 ± 3.0 µmol/l) were within the normal range. Both male and female vegetarians had a lower mean body-mass index, waist-hip ratio and lower mean blood pressure in comparison with control subjects. There was a positive correlation between body-mass index and systolic blood pressure in men (correlation coefficient $r = + 0.48$; $P < 0.001$) and women ($r = + 0.41$; $P < 0.001$). Waist-hip ratio and systolic blood pressure were also positively associated in men ($r = + 0.47$; $P < 0.001$) and women ($r = + 0.29$; $P < 0.001$).

DISCUSSION

There is a wealth of evidence that several life-style factors associated with the „western" culture - a high caloric diet rich in animal fat, physical inactivity and smoking - are important determinants of risk for atherosclerosis. Risk factors seldom occur in isolation and, when they cluster, they augment the risk for cardiovascular events. Therefore the most striking aspect of recent guidlines on coronary heart disease prevention has been the emphasis on the total burden of risk for atherosclerosis to wich an individual is exposed. This approach acknowledges that coronary heart disease has a multifactoral etiology, and that risk factors have a multiplicative effect.

Prospective epidemiological studies were able to show lower mortality of vegetarians compared with the general population, which was mainly due to a low cardiovascular and cancer death rate (4, 5, 6). Vegetarians and general population differ not only with respect to nutrition but also regarding several life-style factors which are determinants of lipoprotein profile. Recently within Leipzig lipid screening projects including over 10.000 inhabitants of Leipzig and 177 vegetarians the determination of capillary blood cholesterol and high-density lipoprotein (HDL) cholesterol by means of the Reflotron dry-chemistry system (Boehringer Mannheim GmbH, Germany) and the evaluation of risk for atherosclerosis and life-style factors was carried out (10, 23). Both vegetarian men and women showed a lower mean body mass index than the general population. The prevalence of smoking was very low. Furthermore the prevalence of elevated blood pressure was low in comparison with the general population. Vegetarians were characterized by decreased plasma cholesterol concentrations and ratios of cholesterol/HDL-cholesterol. In general population the prevalence of hypercholesterolaemia > 6.5 mmol/l was 28 % in male and 35 % in female subjects. In contrast, the corresponding values of vegetarians were 5 % and 9 %, respectively. These results reveal the primary role of nutritional and general life-style factors in determination of the lipid profile on population basis.

Because hyper- and dyslipoproteinemias constitute a major risk for development of atherosclerosis, investigation of lipoprotein constellation of vegetarians in relation to other clinical-chemistry parameters is of special interest. According to the results of the present study vegetarians are characterized by low serum levels of total cholesterol, LDL-cholesterol and triglycerides. Both male and female vegetarians have lower ratios of total cholesterol to HDL-cholesterol, LDL-cholesterol to HDL-cholesterol and apo B to apo A-I in comparison with non-vegetarians. There is no significant difference in the levels of HDL-cholesterol in vegetarians and non-vegetarians. However changes of HDL composition were found. Both male and female vegetarians are characterized by an elevated ratio of HDL_2-cholesterol to HDL_3-cholesterol. It is known that physical activity affects the concentration of the HDL_2 subfraction. In the present study levels of activity between vegetarians and non-vegetarians were not measured. Further investigations should include the evaluation of activity level, because in general a high degree of physical activity is related to high HDL_2-cholesterol concentrations.

Low serum triglyceride concentrations are reflected in low concentrations of HDL-triglycerides because it is known that there is an exchange of triglycerides between triglyceride rich particles and HDL. In the present study there is a tendency for a reduced ratio of HDL-triglycerides to HDL-cholesterol in the serum of lacto-ovo-vegetarians in comparison with non-vegetarians. In another study the concentration of HDL-triglycerides was significantly decreased in the vegetarians (24).

In the present study the concentration of apolipoprotein A-I was significantly higher in vegetarian women than in non-vegetarians. This is in accordance with other observations (25). Fractionation of the apo A-containing lipoproteins of the high density region results in the identification of two major lipoprotein families including particles that contain apo A-I but not apo A-II (termed Lp A-I) and particles that contain both apo A-I and apo A-II (termed Lp A-I:A-II) and several minor lipoprotein particles containing additional apolipoproteins (19, 21). It has been reported that Lp A-I particles are potent agonists for the promotion of cholesterol mass efflux from cholesterol-loaded cells (21). The concentration of these two subpopulations of apolipoprotein A-containing lipoprotein particles was measured in the serum of lacto-ovo-vegetarians and non-vegetarians. However no significant difference regarding the LpA-I concentration was observed.

Data from several studies suggest that the distribution of apo C-III among lipoproteins may play a role in susceptibility of individuals to the development of atherosclerosis (19, 22). Apo C-III inhibits apo C-II-activated lipoprotein lipase and also inhibits the apo E-dependent interaction of lipoproteins with the low density lipoprotein receptor-related protein. Increased amounts of apo C-III in HDL relative to apo C-III in apo B containing lipoproteins are indicative of an effective clearance of triglyceride rich lipoproteins. According to the present study vegetarians are characterized by low Lp B:C-III levels. This may be indicative of a decreased risk for atherosclerosis.

Among several other clinical-chemistry parameters carnitine was also measured. Carnitine, L(-)-ß-hydroxy-γ-trimethylaminobutyrate, plays an important role in fatty acid metabolism. The main biological function of carnitine is the transport of fatty acids trough the inner mitochondrial membrane to sites of ß-oxidation in the mitochondrial matrix. There are several effects of carnitine administration on parameters of lipoprotein metabolism (26). In general, carnitine content is low in foods of plant origin (27). Total and free carnitine concentrations are reduced in serum of lacto-ovo-vegetarians included in this study. However this limited reduction does not reflect a carnitine deficiency. This is in agreement with other investigations in which vegetarians were found to have lower circulating carnitine concentrations than omnivores, but nevertheless their plasma carnitine concentrations remained within normal limits. It is suggested that the kidney adapts to reduced carnitine intake by increasing the efficiency of carnitine reabsorption (28).

As a reflection of a specific eating pattern vitamin B_{12} concentrations of vegetarians were found at the lower end of the reference range. On the other hand, lacto-ovo-vegetarians were characterized by normal or high serum levels of ascorbic acid, α-tocopherol and ß-carotene. The α-tocopherol/LDL-cholesterol molar ratio was significantly higher in both male and female vegetarians in comparison with non-vegetarians. This may be important with respect to protection against atherosclerosis because α-tocopherol is a potent lipid-soluble antioxidant carried in LDL. Antioxidants such as ascorbic acid, carotenoids, and α-tocopherol are hypothesized to help prevent cardiovascular disease by blocking the oxidative modification of LDL which plays an important part in atherosclerosis (29, 30).

In summary, the favourable serum lipoprotein profile of lacto-ovo-vegetarians observed in the present study may be related to the special life-style factors and nutritional conditions within this group in accordance with the known decreased risk for coronary heart disease.

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