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ORIGINAL CONTRIBUTION

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Dietary lipid intake influences the level of cholesterol bound to haemoglobin in human erythrocytes

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Abstract *Background* Blood cholesterol levels are affected by diet and in particular by the type of fat intake. We originally showed that a significant but variable amount of cholesterol is firmly bound to haemoglobin (Hb) yielding the Hb-lipid adduct (Hb-Ch) in erythrocytes isolated from normo-lipidemic males. *Aim of the study* To establish whether dietary lipids affect the level of Hb-Ch in human erythrocytes. *Methods* Seventy-four healthy free-living adults were separated according to their serum cholesterol levels into two groups: normo-cholesterolemic (LDL cholesterol <3.4 mmol/l and total cholesterol <5.2 mmol/l) (NC) and hypercholesterolemic (LDL cholesterol ≥3.4 mmol/l) (HC). Habitual dietary information was used to classify subjects in both study groups into sub-groups of low-fat (≤30% total energy as fat) and high-fat consumers (>30% total energy as fat). The NC low-fat consumers were placed on a high-lipid (high-fat and high-cholesterol) diet whereas the HC subjects with high-fat intake were assigned to a low-lipid (low-fat and low-cholesterol) diet. Both types of dietary intervention were allowed to continue for 6 weeks. The main variable under scrutiny was the Hb-Ch concentration. *Results* In both study groups low-fat intake subjects had low levels of Hb-Ch (approx. 0.35 mmol/l RBC) compared with high-fat intake subjects (approx. 0.60 mmol/l RBC), and serum cholesterol was not correlated with Hb-Ch. The two dietary interventions produced substantial changes in the Hb-Ch level that paralleled variation in the serum cholesterol concentration. A high-lipid diet (35% fat, 15% saturated; 580 mg cholesterol) increased Hb-Ch (by approximately 47%, $P < 0.001$) in subjects with low Hb-Ch at onset, whereas a low-lipid diet (28% fat, 9% saturated; 280 mg cholesterol) decreased Hb-Ch (by approximately 40%, $P < 0.001$) in subjects with high Hb-Ch at onset. *Conclusion* High consumption of dietary lipids, including saturated fat and cholesterol, has an important influence on the level of Hb-Ch in human erythrocytes.

Key words dietary lipids – cholesterol – saturated fat – haemoglobin – human erythrocytes

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Introduction

In addition to genetic susceptibility the circulating cholesterol concentration is affected by diet and in particular by the type of fat intake. Consumption of saturated fat has been shown to raise blood cholesterol, whereas consumption of polyunsaturated fat has the opposite effect [6, 11]. Although published data concerning the effects of dietary cholesterol are controversial, carefully controlled metabolic studies have consistently shown that increased dietary cholesterol intake causes an elevated circulating cholesterol concentration [2, 7]. High serum cholesterol is a well-know primary risk factor for coronary heart disease [3, 28]. For this reason the US National Cholesterol Education Programme recommends for the general population that total dietary fat be reduced to 30% or less and saturated fat intake to 10% or less of total calories and that daily dietary cholesterol intake be limited to 300 mg [17, 18]. These guidelines are consistent with the World Health Organisation's dietary recommendations for prevention of chronic diseases [30].

Our previous study involving normo-lipidemic young males indicated that a significant but variable amount of cholesterol, associated with phospholipids, is firmly bound to haemoglobin (Hb) yielding the Hb-lipid adduct termed Hb-Ch [20]. We postulated that Hb-Ch represented a new form of cholesterol in circulation that contributed to the permanent removal of excess non-esterified cholesterol from the circulation [20]. The present study is formulated to determine if dietary lipid intake affects the Hb-Ch content of human erythrocytes.

Materials and methods

Subjects

Study participants were recruited from three sources: (a) patients from the Department of Nutrition and Metabolism, Belgrade; (b) volunteers working at university research laboratories; and (c) undergraduate and PhD students at the Faculty of Chemistry, University of Belgrade. All participants received a full medical examination and standard laboratory blood tests before enrolment. Subjects that had diabetes mellitus, cardiovascular, liver or renal disease, uncontrolled hypertension, hypo-thyroidism and known family lipid disorders (familiar combined hyperlipidemia or familiar hypercholesterolemia) were excluded. Individuals admitting to smoking more than ten cigarettes per day and those with an alcohol intake of more then 30 g per day were also excluded from the study. Additional criteria for admission included: (1) being between 20 and 60 years of age; (2) having a body mass index (BMI; in kg/m^2) <30 and a percentage of total body fat <35; (3) fasting plasma glucose <6.1 mmol/l, total cholesterol <7.8 mmol/l and triacylglycerol <1.7 mmol/l. In total 74 free-living adults were selected. All of them had stable weight during the month prior to the study, were not on any prescribed medication known to affect lipid metabolism, were not adhering to special diets and were not regular consumers of nutritional supplements (except a daily multi-vitamin/mineral tablet). Participants were either sedentary or moderately active (monitored by a physical activity questionnaire).

According to their baseline serum cholesterol concentration, subjects were categorised into two study groups [18]. A normo-cholesterolemic (NC) group [low-density lipoprotein (LDL) cholesterol <3.4 mmol/l and total cholesterol <5.2 mmol/l] consisted of 38 subjects. The other study group contained 36 hyper-cholesterolemic (HC) subjects (LDL cholesterol ≥3.4 mmol/l). Fulfilment of these criteria was based on data obtained from two fasting serum lipid profiles taken one week apart. According to their habitual total dietary fat intake, subjects from the two different study groups were classified either as low-fat consumers, if their fat intake was 30% or less of energy intake, or high-fat consumers, if their total fat intake contributed more then 30% of total calories.

All study participants provided written informed consent and the whole study was approved by the ethical review boards of the participating institutions in accordance with the principles of the Declaration of Helsinki. The investigation was performed in 2006 during late spring and summer to exclude the seasonal variations in serum lipid [1, 27] and Hb-Ch [20] concentrations.

Anthropometric variables and body composition

Height was measured while the subjects were wearing light clothing and no shoes using a standard tape measure fixed to a wall. The simultaneous measurement of body weight and the percentage of body fat mass was performed while subjects were barefoot using a Tanita body composition analyser (Model TBF-300, Japan). BMI was calculated by dividing the mass in kg by the square of height in metres (kg/m^2).

Dietary assessment method

Information concerning dietary intake was obtained at the beginning of the study (the habitual diet) and at

the end of each dietary intervention period (see below) using a semi-quantified food frequency questionnaire (FFQ). The FFQ contained 142 food items and beverages commonly consumed in Serbia. Food items were listed in food groups (milk and dairy products, meat and fish, meat products, oils and fats, eggs, cereals and grain products, fruits and vegetables, nuts, "fast food", canned products and beverages). For each food item the following information was recorded: (a) frequency of consumption as number of times per week (1–7), 1–3 times per month or never and (b) number of daily portions eaten (slices, units or standard servings) with estimated quantities. A nutritionist, for accuracy and completeness, checked all the completed questionnaires. The consumption frequency was transformed into average daily values for the calculation in grams of each food product or food group. Nutrient calculations were performed using US Department of Agriculture food-composition tables [29] or Serbian food-composition tables [9] for certain local foodstuffs.

Dietary interventions

Twenty-four HC subjects with a reported high-fat intake were placed on a low-lipid (low-fat and low-cholesterol) diet for 6 weeks. The main goal of the dietary intervention was to restrict both dietary total fat and daily cholesterol intake. The recommended energy intake was the participant's calculated energy intake for weight maintenance. Food that was encouraged during the low-lipid diet included lean meat, fat-free (skimmed) milk and low-fat dairy products, whole grain/high-fibre food, fruit and vegetables. Fish was recommended as a main meal once or twice a week. Restricted food included high-fat meat and dairy products, eggs, fried food, creamed sauces, high-fat pastries and sweets. Over the course of the low-lipid diet three participants left the study, for reasons not related to the study.

Sixteen NC subjects assigned as low-fat consumers were placed on a high-lipid (high-fat and high-cholesterol) diet for 6 weeks. There were no restrictions on the type of fat or the cholesterol level in the dietary intake. Food types encouraged during the high-lipid diet period included meat (poultry, beef and pork) and high-fat meat products, whole dairy products, eggs, butter, various nuts and moderate amounts of fruits and vegetables. All subjects from this group completed the diet.

Study participants from both diet groups attended initial individual one-hour teaching sessions led by experts in nutritional counselling. The counselling varied with each participant's unique needs but often included help with the fat and cholesterol content of food items, types of fat and food preparation. Recommendations for food choices were given during these sessions. Subjects were instructed to select only commercially available food products and were encouraged to maintain their regular physical activity and lifestyle during the diets. Counselling attempted to resolve any problems encountered in meeting the dietary goals was reinforced at regular intervals throughout the intervention period. All participants found that modifying their fat intake was easy and did not report any symptomatic adverse effects. The dietary interventions took place between July and September 2006 to minimise seasonal effects and academic stress.

Blood sampling and analytical determinations

Blood samples were obtained in the morning after an overnight (>12 h) fast. Venous blood was collected into tubes containing EDTA (1 g/l) or no anticoagulant. Serum and plasma were separated by centrifugation at $1500 \times g$ for 15 min within 1 h. All measurements described below were carried out in duplicate using fresh serum or erythrocyte lysates.

The serum concentrations of glucose, triacylglycerol and total cholesterol were measured using enzymatic kits (EliTech Diagnostic, Sées, France). Serum high-density lipoprotein (HDL) cholesterol was measured in the supernatant following the precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium chloride [16]. The intra- and inter-assay coefficients of variation of the methods were less than 4% and less than 8%, respectively. LDL cholesterol was calculated using the Friedewald equation [5]. After removing the plasma and buffy coat, erythrocytes were washed three times with isotonic saline. The content of total red blood cell (RBC) cholesterol and Hb-Ch was determined as previously described [20].

Statistical analysis

Results are presented as mean ± standard deviation (SD). Data sets indicated normal distributions. Paired and un-paired comparisons were made using the Student's t test. Linear regression analysis was performed to evaluate the strength of association between two variables. Differences were considered significant at a P value < 0.05.

Results

The baseline characteristics of the participants are presented in Table 1.

Table 1 Baseline characteristics of the normo-cholesterolemic (NC) and hyper-cholesterolemic (HC) study subjects

Characteristic	NC group	HC group
Number of individuals	38	36
Male	21	20
Female	17	16
Age (years)*	31 ± 7	45 ± 9[3]
Body mass index (kg/m^2)*	24.2 ± 2.2	27.0 ± 2.7[3]
Body fat (%)*	22.6 ± 4.1	26.3 ± 4.3[3]
Serum (mmol/l)*		
Fasting glucose	4.67 ± 0.34	4.78 ± 0.43
Triacylglycerol	0.96 ± 0.25	1.16 ± 0.30[1]
Total cholesterol	4.78 ± 0.28	6.43 ± 0.62[3]
LDL cholesterol	3.08 ± 0.24	4.64 ± 0.58[3]
HDL cholesterol	1.26 ± 0.07	1.26 ± 0.10
RBC lipids (mmol/l RBC)*		
Total cholesterol	3.00 ± 0.30	3.08 ± 0.28
Hb-cholesterol	0.52 ± 0.23	0.55 ± 0.20

Values are given as mean ± SD
*Significantly different from the NC group: [1]$P < 0.05$, [2]$P < 0.01$ and [3]$P < 0.001$ (Student's *t*-test)

The NC and HC study groups contained an unbiased gender distribution. NC subjects were much younger and both their BMI and body fat percentage were significantly lower ($P < 0.001$, for all comparisons). There were an equivalent number of smokers in each group and only a minority of subjects reported moderate physical exercise (data not shown). Fasting serum glucose concentration was normal in all subjects. Differences in the total cholesterol ($P < 0.001$) and LDL cholesterol ($P < 0.001$) between the two study groups were evident. However, identical HDL cholesterol concentrations were found. The difference in the triacylglycerol concentration between the groups was also statistically significant ($P < 0.05$).

There was no significant difference in both the mean total RBC cholesterol and Hb-Ch concentration between the two study groups.

Habitual dietary information was used to classify participants from both study groups into sub-groups of low-fat and high-fat consumers (Table 2).

Total dietary fat intake greater than 30% of total calories (high-fat consumers) was observed among most participants (58% in the NC group and 67% in the HC group). The mean contribution of saturated fat to total energy supply was greater than 10% for all sub-groups. Subjects classified as high-fat consumers, from both study groups, consumed significantly less carbohydrate ($P < 0.001$) but significantly more total fat ($P < 0.001$), saturated fat ($P < 0.01$), monounsaturated fat ($P < 0.01$), polyunsaturated fat ($P < 0.05$) and more dietary cholesterol ($P < 0.001$) than low-fat consumers. In contrast, the reported average daily caloric intake as well as intake of protein and dietary fibre was similar (Table 2). Even in subjects with a customary total dietary fat intake of 30% or less of total calories (low-fat consumers) the average daily consumption of dietary cholesterol exceeded 300 mg.

No significant differences in serum lipids were observed between NC subjects with different habitual fat intakes (Table 2). HC high-fat consumers, however, had significantly higher total and LDL cholesterol ($P < 0.01$, for both comparisons) and triacylglycerol ($P < 0.05$) concentrations than HC low-fat consumers. Inter-study group comparisons revealed that the mean Hb-Ch concentration was much higher

Table 2 Characteristics of habitual dietary intake, baseline serum and RBC lipid values of the study subjects

Characteristic	NC group		HC group	
	Low-fat consumers	High-fat consumers	Low-fat consumers	High-fat consumers
Number of individuals	16	22	12	24
Nutrition intake*				
Energy (kcal/day)	2280 ± 295	2350 ± 325	2410 ± 195	2480 ± 310
Protein (% of energy)	18.3 ± 1.7	18.4 ± 1.9	18.4 ± 1.5	18.1 ± 2.0
Carbohydrate (% of energy)	54.7 ± 2.6	47.8 ± 3.4[3]	53.2 ± 2.3	47.9 ± 2.5[3]
Total fat (% of energy)	27.0 ± 1.9	33.8 ± 2.2[3]	28.4 ± 1.2	34.0 ± 1.9[3]
Saturated fat	10.4 ± 1.2	13.4 ± 1.5[2]	10.8 ± 0.9	12.9 ± 1.3[2]
Monounsaturated fat	11.2 ± 1.1	14.5 ± 1.5[2]	12.3 ± 1.1	15.2 ± 1.3[2]
Polyunsaturated fat	5.4 ± 0.5	5.9 ± 0.6[1]	5.3 ± 0.4	5.9 ± 0.7[1]
Cholesterol (mg/day)	345 ± 100	497 ± 125[3]	365 ± 90	531 ± 108[3]
Dietary fibre (g/day)	19.2 ± 3.8	18.6 ± 2.9	18.8 ± 2.5	18.0 ± 3.4
Serum lipids (mmol/l)*				
Triacylglycerol	0.96 ± 0.26	0.97 ± 0.24	1.02 ± 0.25	1.24 ± 0.32[1]
Total cholesterol	4.71 ± 0.31	4.86 ± 0.24	6.02 ± 0.26	6.65 ± 0.65[2]
LDL cholesterol	3.03 ± 0.31	3.14 ± 0.17	4.32 ± 0.33	4.82 ± 0.62[2]
HDL cholesterol	1.24 ± 0.08	1.28 ± 0.06	1.24 ± 0.06	1.27 ± 0.11
RBC lipids (mmol/l RBC)*				
Total cholesterol	2.86 ± 0.26	3.08 ± 0.33[1]	2.90 ± 0.29	3.16 ± 0.35[1]
Hb-Cholesterol	0.35 ± 0.07	0.62 ± 0.11[3]	0.37 ± 0.09	0.64 ± 0.12[3]

Values are given as mean ± SD
*Significantly different from low-fat consumers: [1]$P < 0.05$, [2]$P < 0.01$ and [3]$P < 0.001$ (Student's *t*-test)

($P < 0.001$) in high-fat dietary consumers when compared with low-fat consumers (0.62 mmol/l RBC vs. 0.35 mmol/l RBC in the NC group and 0.64 mmol/l RBC vs. 0.37 mmol/l RBC in the HC group, respectively). Significantly higher total RBC cholesterol levels in sub-groups of high-fat consumers corresponded entirely to the increase in Hb-Ch content (Table 2). The highest concentrations of Hb-Ch (approximately 0.8 mmol/l RBC) were found in individuals with the highest reported intake of both saturated fat (approximately 17% of total calories) and daily cholesterol (approximately 700 mg).

Given the wide spectrum of values within study groups, we explored inter-relationships among the various lipid parameters by linear regression analysis.

Within either the NC or the HC group and both low-fat consumer sub-groups the Hb-Ch level failed to correlate significantly with habitual fat and cholesterol intake (data not shown). A relationship was found between Hb-Ch concentration and the habitual dietary lipid intake values in sub-groups of high-fat consumers. There were significant ($P < 0.05$, for all comparisons) correlations between Hb-Ch and the total fat ($r = 0.42$), saturated fat ($r = 0.46$) and dietary cholesterol ($r = 0.49$) intake of NC high-fat consumers ($n = 22$). In the HC high-fat consumers ($n = 24$) Hb-Ch was only correlated with the habitual intake of saturated fat ($r = 0.48$, $P < 0.05$) and dietary cholesterol ($r = 0.53$, $P < 0.01$).

To further investigate if dietary lipid intake was associated with the level of Hb-Ch in human erythrocytes, we examined the effect of two six-week dietary interventions with different fat and cholesterol content.

HC subjects with a reported high-fat dietary intake were placed on a low-lipid (low-fat and low-cholesterol) diet. The average calorie intake in the low-lipid diet group was approximately distributed as 54% carbohydrate, 18% protein and 28% fat (9% saturated) with a daily cholesterol intake of 280 mg. NC subjects assigned as low-fat consumers were placed on a high-lipid (high-fat and high-cholesterol) diet. The daily total calories of the high-lipid diet group were distributed approximately as 46% carbohydrate, 19% protein and 35% fat (15% saturated). The mean cholesterol content was 580 mg per day. Accordingly, the intake of several nutrients was significantly altered by the diets (Table 3).

During the low-lipid dietary intervention the HC high-fat consumers mainly reduced their saturated fat and daily cholesterol intake by 32 and 44%, respectively ($P < 0.001$). The largest dietary change for NC low-fat consumers during the high-lipid diet was a substantial increase ($P < 0.001$) in their saturated fat (by 40%) and cholesterol (by 68%) intake. The modifications in the total dietary fat intake resulted in concomitant changes in total energy intake. However, mean body weight and the percentage of body fat did not significantly alter after both intervention diets (data not shown).

Table 3 Calculated composition of the study intervention diets*

Variable	NC group ($n = 16$)		HC group ($n = 21$)	
	High-lipid diet	Percentage change	Low-lipid diet	Percentage change
Energy (kcal/day)	2480 ± 265	+8.8[1]	2325 ± 225	−6.4
Protein (% of energy)	19.0 ± 1.5	+3.7	17.7 ± 1.2	−2.2
Carbohydrate (% of energy)	46.1 ± 3.3	−15.7[2]	54.3 ± 3.6	+13.4[2]
Total fat (% of energy)	34.9 ± 3.0	+29.2[3]	28.0 ± 2.7	−17.6[3]
Saturated fat	14.6 ± 1.3	+40.4[3]	8.8 ± 0.7	−31.8[3]
Monounsaturated fat	14.5 ± 1.5	+29.5[3]	12.4 ± 1.4	−19.7[3]
Polyunsaturated fat	6.8 ± 0.7	+7.4[1]	5.8 ± 0.7	+15.2[2]
Cholesterol (mg/day)	580 ± 145	+68.1[3]	280 ± 60	−43.7[3]
Dietary fibre (g/day)	18.8 ± 3.0	−2.1	20.4 ± 2.8	+13.3[2]

Values are given as mean ± SD
*From respective habitual diet: [1]$P < 0.05$, [2]$P < 0.01$ and [3]$P < 0.001$ (Paired student's t-test)

The mean changes in serum lipid and RBC cholesterol concentrations after both diets are summarised in Table 4.

Compared with the corresponding baseline concentrations, overall total cholesterol was 7% lower ($P < 0.01$) after consumption of the low-lipid diet but 4% higher ($P < 0.05$) after the high-lipid diet. The calculated LDL cholesterol concentration followed the same pattern [−8% and +5%, respectively ($P < 0.01$)]. Although not significant, there was a trend for the triacyglycerol concentration to be higher in subjects after consuming both diets. The HDL cholesterol concentration was not significantly affected by the diets. In sharp contrast, the Hb-Ch level dramatically changed in response to alterations in dietary lipid intake. The low-lipid diet reduced the Hb-Ch concentration in HC high-fat consumers by approximately 40% [from 0.64 ± 0.12 mmol/l RBC to

Table 4 Serum and RBC lipid values after six weeks of the intervention diets*

Variable	NC group ($n = 16$)		HC group ($n = 21$)	
	High-lipid diet	Percentage change	Low-lipid diet	Percentage change
Serum lipids (mmol/l)				
Triacylglycerol	0.99 ± 0.27	+3.2	1.27 ± 0.30	+2.3
Total cholesterol	4.90 ± 0.33	+4.0[1]	6.20 ± 0.26	−6.8[1]
LDL cholesterol	3.18 ± 0.31	+4.9[2]	4.47 ± 0.59	−7.3[2]
HDL cholesterol	1.26 ± 0.06	+1.4	1.25 ± 0.05	−1.7
RBC lipids (mmol/l RBC)				
Total cholesterol	3.01 ± 0.29	+5.4[1]	2.93 ± 0.30	−7.2[2]
Hb-cholesterol	0.52 ± 0.09	+47.4[3]	0.39 ± 0.10	−39.2[3]

Values are given as mean ± SD
*From respective baseline values: [1]$P < 0.05$, [2]$P < 0.01$ and [3]$P < 0.001$ (Paired student's t-test)

0.39 ± 0.10 mmol/l RBC ($P < 0.001$)]. The Hb-Ch concentration increased by approximately 47% [from 0.35 ± 0.07 mmol/l RBC to 0.52 ± 0.09 mmol/l RBC ($P < 0.001$)] in NC subjects that consumed the high-fat, high cholesterol diet.

Discussion

The objective of the current study was to assess the influence of dietary lipids (total fat, saturated fat and cholesterol) on the level of Hb-Ch in human erythrocytes isolated from normo-cholesterolemic and hyper-cholesterolemic subjects. We found greater Hb-Ch levels in both NC and HC subjects with higher habitual total fat, saturated fat and cholesterol intake compared with subjects with a lower daily consumption of these dietary lipids. The two diets, at opposite ends of the usual clinical spectrum ("pro-atherogenic" high-lipid diet and "anti-atherogenic" low-lipid diet), produced substantial changes in the Hb-Ch level that paralleled variation in the serum cholesterol concentration.

Various methods are commonly used to collect information regarding nutrition. The purpose of any given study defines the level of precision that is needed from dietary intake measurements in order to meet the study objectives [25]. The formation of Hb-Ch and exchange of Hb-Ch lipids with plasma lipoproteins are both very complex and relatively slow processes [20, 21]. Therefore, we used semi-quantitative FFQ, as it is one of the most practical dietary assessment methods covering food intake over a long(er) period of time [25]. The habitual dietary patterns of study participants were compared with respect to their energy intake, macronutrients, dietary fibre and cholesterol. In both study groups we found large disparities between the prevailing and the international recommended dietary intake values. The high intake of saturated fat was largely a consequence of high consumption of animal fats by our subjects. The high daily cholesterol intake, notably in the sub-groups of high-fat consumers, seemed to be related to a high consumption of eggs (a major source of dietary cholesterol). Our findings are in agreement with published data that considered local patterns of food consumption [13, 24]. The consequence of such eating patterns, high in saturated fat and cholesterol and relatively low in polyunsaturated fat and dietary fibre, is a high prevalence of nutrition-related health risks in the adult Serbian population [15, 23].

The quantity of dietary lipids ingested, rather than the serum cholesterol concentration, seems to influence the amount of Hb-Ch found in human erythrocytes. Subjects with different serum cholesterol concentrations (NC vs. HC group) did not differ with respect to their Hb-Ch levels. The marked age difference between the two study groups seemed irrelevant: fasting LDL cholesterol as well as triacylglycerol concentrations increase significantly with age [26]. When we compared the Hb-Ch levels in subjects stratified on the basis of total daily fat consumption (low- vs. high-fat consumers) we found that the Hb-Ch levels were consistently higher in subjects with increased total fat intake. Dietary saturated fat and cholesterol appeared to be important factors that influenced the level of Hb-Ch. The highest individual levels of Hb-Ch were found in individuals with the highest reported intake of both saturated fat and cholesterol. In addition, we found a positive correlation between the Hb-Ch level and the intake of both saturated fat and cholesterol in both high-fat consumer sub-groups. Moreover, we found a negligible amount of Hb-Ch in individuals preferring a vegetarian-style diet that provided less than 5% of energy as saturated fat and less than 100 mg of daily cholesterol intake (our unpublished results).

Dietary fat restriction lowers total and LDL cholesterol concentrations. However, low-fat diets also decrease HDL cholesterol and may increase serum triacylglycerol [10]. Overall triacylglycerol increased and HDL cholesterol decreased from the pre-intervention concentrations in this study population but the differences were not significant. A reduction in total fat and particularly saturated fat intake is mainly the consequence of a lower intake of high-fat meat and dairy products. A substantial reduction in the intake of both animal fat and eggs also decreases the intake of dietary cholesterol. Many high saturated fat content foodstuffs are also sources of dietary cholesterol. Therefore, the marked decrease in the level of Hb-Ch in the sub-group of HC high-fat consumers after the low-lipid diet may be a reflection of its association with lower intakes of both saturated fat and cholesterol. Indeed, a switch from a habitual diet relatively low in dietary lipids to the high fat, high cholesterol diet produced almost a two-fold increase in the Hb-Ch level. Perhaps the biggest concern regarding high-lipid based diets is their association with increased risk of coronary heart disease [14]. The high-fat and high cholesterol dietary intervention during six weeks resulted in only a modest increase in serum cholesterol concentration in the sub-group of NC low-fat consumers.

The serum cholesterol response to dietary fat, and especially to dietary cholesterol, is highly variable among individuals within studied populations [4, 8]. Our results indicate high responsiveness of the Hb-Ch level to changes in dietary lipid intake over a relatively short period of time. Whether or not Hb-Ch represents a useful additional parameter for the assessment of nutrient intake of lipid (saturated fat and cholesterol in particular) remains to be established.

In summary, our study tested the overall influence of dietary lipids on the Hb-Ch level in human erythrocytes of free-living adult subjects. As such it was not designed to decipher the contribution of individual lipids (e.g. saturated fat or cholesterol) on the Hb-Ch level. This could be accomplished by employing more strictly controlled dietary studies in order to delve deeper into our observed phenomena. Epidemiological data consistently show that populations with high-fat diets have increased incidence of coronary heart disease [12]. Our results indicate that typical Western-style diets, rich in saturated fat and cholesterol and relatively low in polyunsaturated fat and dietary fibre, are associated with the accumulation of cholesterol in the interior of erythrocytes. The majority of the cholesterol released from Hb-Ch appears in the plasma HDL fraction in vitro, suggesting that Hb-Ch may play a role in reverse cholesterol transport in vivo [21]. Collectively, the results indicate that human erythrocytes represent a transient storage system for an excess of (potentially detrimental) cholesterol in circulation, most probably of exogenous origin.

The consequence(s) of cholesterol binding to haemoglobin on the function of erythrocytes remain(s) to be established. Our preliminary data indicate that the presence of Hb-Ch does not affect cellular oxygen availability or its transport (unpublished observations). The amount of Hb-Ch does not influence antioxidant enzyme activities nor alter the level of metHb [19], although Hb-Ch could affect membrane lipid peroxidation and superoxide generation in human erythrocytes [22].

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References

1. Bluher M, Hentschel B, Rassoul F, Richter V (2001) Influence of dietary intake and physical activity on annual rhythm of human blood cholesterol concentrations. Chronobiol Int 18:541–557
2. Clarke R, Frost C, Collins R, Appleby P, Peto R (1997) Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ 314:112–117
3. Consensus conference (1985) Lowering blood cholesterol to prevent heart disease. JAMA 253:2080–2086
4. Denke MA (1995) Review of human studies evaluating individual dietary responsiveness in patients with hypercholesterolemia. Am J Clin Nutr 62:471S–477S
5. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
6. Grundy SM, Denke MA (1990) Dietary influences on serum lipids and lipoproteins. J Lipid Res 31:1149–1172
7. Hopkins PN (1992) Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. Am J Clin Nutr 55:1060–1070
8. Jansen S, Lopez-Miranda J, Salas J, Castro P, Paniagua JA, Lopez-Segura F, Ordovas JM, Jimenez-Pereperez JA, Blanco A, Perez-Jimenez F (1998) Plasma lipid response to hypolipidemic diets in young healthy non-obese men varies with body mass index. J Nutr 128:1144–1149
9. Jokic N, Dimic M, Pavlica M (1999) Tablice hemijskog sastava prehrambenih proizvoda (Chemical composition tables of nutrition products). Kulin Art, Zavod za ekonomiku domacinstva Srbije, Beograd, Srbija (in Serbian)
10. Katan MB, Grundy SM, Willett WC (1997) Should a low-fat, high-carbohydrate diet be recommended for everyone? Beyond low-fat diets. N Engl J Med 337:563–566
11. Katan MB, Zock PL, Mensink RP (1994) Effects of fats and fatty acids on blood lipids in humans: an overview. Am J Clin Nutr 60:1017S–1022S
12. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH, Kromhout D, Nedeljkovic S, Ounsar S, Seccareccia F, Toshima H (1986) The diet and 15-year death rate in the Seven Country Study. Am J Epidemiol 124:903–915
13. Kromhout D, Keys A, Aravanis C, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Toshima H (1989) Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr 49:889–894
14. Kromhout D, Menotti A, Bloemberg B, Aravanis C, Blackburn H, Buzina R, Dontas AS, Fidanza F, Giampaoli S, Jansen A, Karvonen M, Katan M, Nissinen A, Nedeljkovic S, Pekkanen J, Pekkarinen M, Punsar S, Rasanen L, Simic B, Toshima H (1995) Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. Prev Med 24:308–315
15. Kromhout D, Nedeljkovic SI, Grujic MZ, Ostojic MC, Keys A, Menotti A, Katan MB, van Oostrom MA, Bloemberg BP (1994) Changes in major risk factors for cardiovascular diseases over 25 years in the Serbian cohorts of the Seven Countries Study. Int J Epidemiol 23:5–11
16. Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 23:882–884
17. National Cholesterol Education Program (1993) Second report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. NIH Pub. No. 93-3095. National Heart, Lung, and Blood Institute, Bethesda
18. National Cholesterol Education Program (2001) Third report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). NIH Pub. No. 01-3670. National Heart, Lung, and Blood Institute, Bethesda
19. Nikolic M, Nikolic-Kokic A, Stanic D, Blagojevic DP, Vranic D, Jones DR, Niketic V, Spasic MB (2007) Does cholesterol bound to haemoglobin affect the anti-oxidant enzyme defence system in human erythrocytes? J Serb Chem Soc 72:339–346

20. Nikolic M, Stanic D, Antonijevic N, Niketic V (2004) Cholesterol bound to hemoglobin in normal human erythrocytes: a new form of cholesterol in circulation? Clin Biochem 37:22–26
21. Nikolic M, Stanic D, Baricevic I, Jones DR, Nedic O, Niketic V (2007) Efflux of cholesterol and phospholipids derived from the haemoglobin-lipid adduct in human red blood cells into plasma. Clin Biochem 40:305–309
22. Nikolic M, Vranic D, Spiric A, Batas V, Nikolic-Kokic A, Radetic P, Turubatovic L, Blagojevic DP, Jones DR, Niketic V, Spasic MB (2006) Could cholesterol bound to haemoglobin be a missing link for the occasional inverse relationship between superoxide dismutase and glutathione peroxidase activities? Biochem Biophys Res Commun 348:265–270
23. Pavlovic M, Grujic V (2001) Nutritive risk factors within population. In: Skoric D (ed) Proceedings 1st International Symposium "Food in the 21st Century" 14–17 November, Subotica, Yugoslavia. Institute for Agriculture and Vegetables, Novi Sad, pp 474–481
24. Pavlovic M, Grujic V, Oshaug A (2005) Nutrition and physical activity of the population in Serbia. World Rev Nutr Diet 94:51–59
25. Rutishauser IHE (2005) Dietary intake measurements. Public Health Nutr 8:1100–1107
26. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, McNamara JR, Ordovas JM (1995) Lipoproteins, nutrition, aging, and atherosclerosis. Am J Clin Nutr 61:726S–740S
27. Shahar DR, Froom P, Harari G, Yerushalmi N, Lubin F, Kristal-Boneh E (1999) Changes in dietary intake account for seasonal changes in cardiovascular disease risk factors. Eur J Clin Nutr 53:395–400
28. Stamler J, Wentworth D, Neaton JD (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screeners of the multiple risk factor intervention trial (MRFIT). JAMA 256:2823–2828
29. US Department of Agriculture, Agricultural Research Service (2005) USDA national nutrient database for standard reference, release 18. Nutrient data laboratory home page, http://www.nal.usda.gov/fnic/foodcomp
30. WHO technical report series 916 (2003) Diet, nutrition and the prevention of chronic disease. Report of the joint WHO, FAO expert consultation, Geneva, Switzerland