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Effect of a phytosterol mixture diet on the plasma level of fatty acids in hypercholesterolaemic rats (PHHC)

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The process of atherogenesis can result in severe atherosclerotic lesions at predisposed sites of the artheries [1]. Such lesions are typically characterized by the presence of inflammatory and fibroproliferatory mechanisms of varying levels [2]. Chemical, mechanical (shear stress) and biological (virus, endotoxin) stimuli as well as contineous abundant plasma levels of cholesterol can develop atherogenetic processes [3, 4].

At present, one of the possible approaches of the therapy is a phytosterol diet. The primary aim of clinical and experimental studies of phytosterol supplementation is the reduction of pathologically increased cholesterolaemia. This beneficial effect in hypercholesterolamic subjects is usually accompanied by the restoration of abnormal lipoprotein metabolism and regression of atherogenesis. The generally accepted mechanism of the phytosterols is the inhibition of cholesterol absorption from the intestine [5].

The changes in plasma levels of free and bound fatty acids associate with hypercholesterolaemia and progresion of atherogenesis [1, 3]. Increased concentrations of free oleic and linolic acids were reported during stage IV of atherogenesis [3]. The saturated fatty acids increase LDL cholesterol and decrease HDL cholesterol and may therefore increase coronary risk [6]. Long-chain fatty acids may influence human susceptibility to pathological processes which involve the interaction of leukocytes with endothelial cells, such as in atherogenesis and in inflammation [7]. In cholesterol-lowering diets, saturated fatty acids can be replaced be different classes of unsaturated fatty acids [6].

It is worth to know how these metabolic effects are modified by chronic supplementation with photysterols. Therefore we decided to evaluate the effects of phytosterol supplementation on changes in plasma concentrations of free and bound oleic, linolic, linoleic and arachidonic acid. To confirm potential effects of phytosterol on this physiological paramethers, we carried out an experimental study of a 60-days lasting diet supplementation with a phytosterol mixture (β-sitosterol 65%, stigmastanol 18%, campesterol 14% and campestanol 3%) in hypercholesterolaemic (PHHC) rats ($n = 12$) and normocholesterolaemic Wistar rats ($n = 12$). The biochemical profile of the PHHC rat, an experimental model of hypercholesterolaemia, is very similar to the human form of the disease [8].

Wistar and PHHC rats were divided into two experimental groups. Each group was maintained on a normal diet and on a diet containing 21 mg/kg of the phytosterol mixture. After 60 days, plasma samples were collected and the profiles of plasma fatty acids were analysed using GC. We focused on the ratio of saturated/unsaturated fatty acids and on changes of the profile of free and bound fatty acids typical for each stage of atherogenesis.

Supplementation of phytosterols to hypercholesterolaemic rats significantly changes plasma levels of fatty acids. The plasma levels of saturated acids (palmitic and stearic) were partially decreased in both rat strains. While in

Table 1: Comparison of free unsaturated fatty acids plasma level in the PHHC and Wistar rats with normal (control) and phytosterol (phyto) diet

	(n)	Free unsaturated fatty acids (%)			
		Oleic	Linolic	Linoleic	Arachidonic
Control Wistar	3	22.29 ± 0.37	12.05 ± 0.28	1.94 ± 0.35	20.81 ± 0.32
Phyto Wistar	3	19.88 ± 0.78	13.02 ± 1.16	3.48 ± 0.68	25.51 ± 2.08
Control PHHC	3	24.03 ± 0.87	15.63 ± 0.43	1.54 ± 0.29	16.65 ± 0.41
Phyto PHHC	3	21.87 ± 1.31	16.74 ± 0.94	3.07 ± 0.80	18.47 ± 1.34

Table 2: Comparison of bound unsaturated fatty acids plasma level in the PHHC and Wistar rats with normal (control) and phytosterol (phyto) diet

	(n)	Bound unsaturated fatty acids (%)			
		Oleic	Linolic	Linoleic	Arachidonic
Control Wistar	3	27.30 ± 2.60	17.37 ± 2.64	1.20 ± 0.33	11.99 ± 1.00
Phyto Wistar	3	37.94 ± 1.61	14.95 ± 1.45	0.57 ± 0.04	10.38 ± 1.37
Control PHHC	3	31.54 ± 0.28	19.69 ± 0.49	0.86 ± 0.04	12.76 ± 0.22
Phyto PHHC	3	30.29 ± 2.48	18.25 ± 2.41	0.68 ± 0.11	9.83 ± 1.85

PHHC rats the saturated/unsaturated fatty acids plasma ratio has changed in favour of unsaturated fatty acids for both free and bound forms, in Wistar rats this ratio was changed only for free forms of fatty acids.
Comparising the plasma level of all unsaturated fatty acids in PHHC rats with a normal diet versus PHHC rats maintained on phytosterols (Table 1, Table 2), we observed a decreased plasma level of bound oleic acid (by 3.96%) and free oleic acid (by 8.99%), a decreased plasma level of bound linolic acid (by 7.31%), bound linoleic acid (by 20.9%), bound arachidonic acid (by 22.96%) and an increased plasma level of free linolic acid (by 7.1%), a significantly increased plasma level of free linoleic acid (by 99.35%) and free arachidonic acid (by 10.93%).
We have previously shown that this phytosterol diet has positive effects on the biochemical lipid profile [9] and on the endothelial physiology of hypercholesterolaemic rats. However, the chronic phytosterol diet increased the plasma level of free unsaturated fatty acids in PHHC as well as in Wistar rats. On the base of this marker, we can speculate the processes of atherogenesis can be shifted from stage II to stage IV [10]. A mitogenic response in vascular smooth muscle cells can be induced as well [11]. However, a causal relationship between phytosterol treatment and development of atherosclerosis remains to be established.

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