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# EFFECT OF INULIN INTAKE ON THE CONTENT AND SUSCEPTIBILITY TO OXIDATIVE DAMAGE OF CHOLESTEROL IN RAT LIVER PLASMA MEMBRANES

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**Abstract**

The influence of the prebiotic inulin on the level and sensitivity to oxidative attack has been investigated in rat liver plasma membranes. The obtained results showed that the oral administration of inulin in experimental animals induced a slight decrease of the content of cholesterol in liver plasma membranes. In addition, the susceptibility of cholesterol to oxidation, which was tested with cholesterol oxidase assay, was lower in membranes isolated from inulin-fed rats. Studies were also performed on the changes in the asymmetric distribution of cholesterol between the two leaflets of the membrane bilayer in order to elucidate the reasons for the observed differences in cholesterol sensitivity to oxidation. Basically, the alterations in the oxidation of cholesterol molecules could be due either to differences in the localization of cholesterol in the external membrane monolayer and/or to changes in the structural organization of the membranes. Our studies revealed a slight reduction of the cholesterol molecules in the outer membrane monolayer, which could be one possible reason for the observed reduction of cholesterol susceptibility to oxidative damage.

**Key words:** cholesterol, oxidation, inulin, prebiotic, plasma membrane lipids

**Introduction.** Prebiotics are short-chain non-digestable carbohydrates which affect the serum lipids and the gastro-intestinal microflora [1]. Studies with rodents have shown that inulin and oligofructose could decrease the plasma

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levels of cholesterol and triacylglycerols. In addition, effect on liver lipogenesis has been reported as a result of inulin uptake. However, studies in man have produced some controversial data concerning the influence of prebiotic intake on the liver lipids and their susceptibility to oxidative damage. Since the effect of prebiotics on liver lipids is one major issue concerning prebiotic influence on molecular level, we carried out studies on the alterations in the content and susceptibility to oxidative attack of cholesterol in liver plasma membranes in rats as a result of inulin administration. This is a topic of great interest because lipid peroxidation is an important factor in the pathogenesis of serious diseases, involving atherosclerosis, Alzheimer's disease, diabetes, etc. [2]. In addition, since the liver plays a central role in metabolizing therapeutic drugs and a vast range of environmental contaminants, the composition of the liver cell membranes and the sensitivity of the membrane lipid components to oxidative damage and the possibility to affect these parameters by prebiotic intake are very important in medico-biological aspect.

**Materials and methods.** The experimental rats were on diet enriched with 10 g inulin/kg body weight dissolved in water. Rat liver plasma membranes from rats on inulin-enriched diet and control rats were obtained as described elsewhere [3]. Lipid extraction was performed according to BLIGH and DYER [4]. The cholesterol content in the membrane fractions was assayed by gas chromatography [5]. Cholesterol oxidase assay was performed exactly as described by MOORE et al. [6]. The fluorescence of NBD-cholesterol was measured at 502 (excitation beam) and 520 (emission beam). Calculation of the amount of NBD-cholesterol accessible to dithionite quenching was performed as described previously [7].

**Results.** The content of cholesterol in plasma membranes isolated from livers of rats on inulin-enriched diet is shown in Fig. 1. As evident from the figure,

Table 1

Quenching of NAD-cholesterol fluorescence in the outer membrane leaflet of liver plasma membranes isolated form control rats and rats on inulin-enriched diet

| Membranes | Fo | Fr | Fap | % quenchable probe |
|---|---|---|---|---|
| Control | $265 \pm 7.8$ | $165 \pm 5.7$ | $112 \pm 8.9$ | $65.4 \pm 2.5$ |
| Inulin | $261 \pm 9.5$ | $169 \pm 3.6$ | $110 \pm 6.7$ | $61.0 \pm 1.7^*$ |

Fo – fluorescence intensity before addition of sodium dithionite;

Fr – fluorescence intensity after treatment with sodium dithionite;

Fap – apparent fluorescence with no NBD cholesterol;

Each value represents the mean of three separate experiments.

$^*P < 0.01$

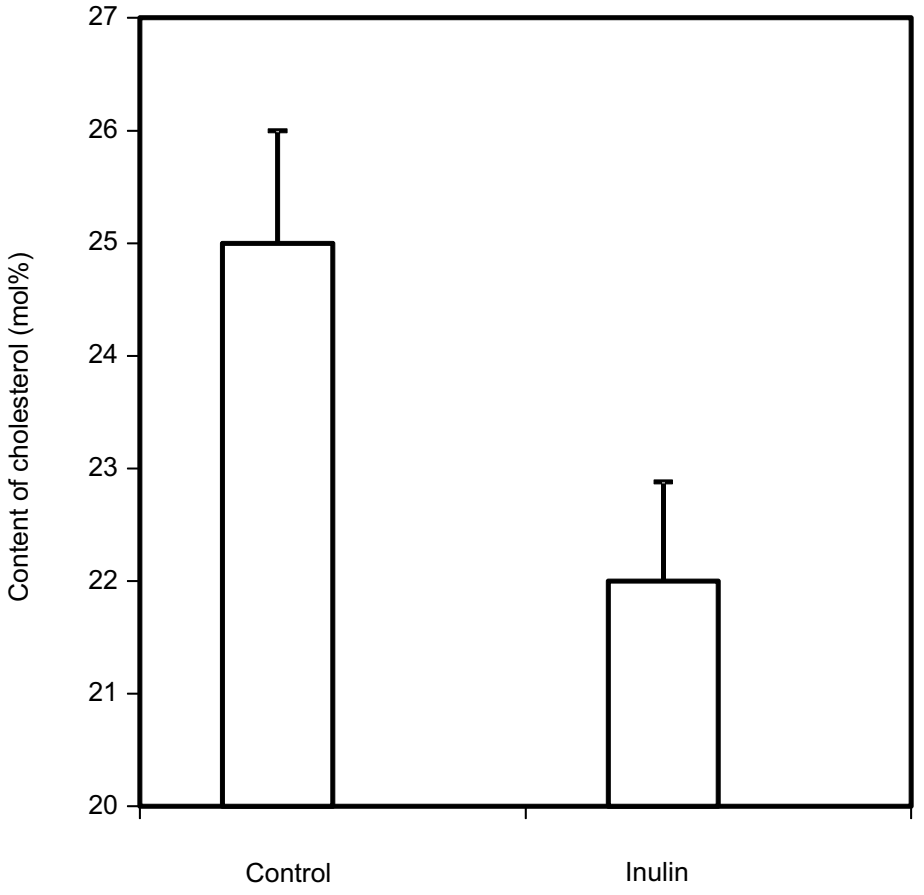


Fig. 1. Content of cholesterol in liver plasma membranes isolated from liver plasma membranes from control rats and rats on inulin-enriched diet, $P < 0.01$

the content of cholesterol, expressed in mol %, was slightly reduced in membranes from inulin-fed rats, compared to the controls. Our further aim was to analyse the effect of inulin intake on the susceptibility of membrane cholesterol to oxidative damage. For this purpose, the membranes isolated from control and inulin-fed rats were exposed to the enzyme cholesterol oxidase, which triggered oxidation to this moiety of cholesterol molecules, which was most readily available to oxidative attack. Figure 2 illustrates the influence of inulin-enriched diet on the susceptibility to oxidation of cholesterol localized in the outer membrane leaflet. Apparently, the oxidative attack was more significant for cholesterol in control membranes, compared to inulin-treated membranes. Based on the observed differences in the sensitivity of cholesterol molecules to oxidation, we studied the differences in the distribution of cholesterol between the two monolayers of the membrane bilayer (Table 1). For this aim was monitored the quenching of the fluorescent probe of NBD-cholesterol, which was localized predominantly in the

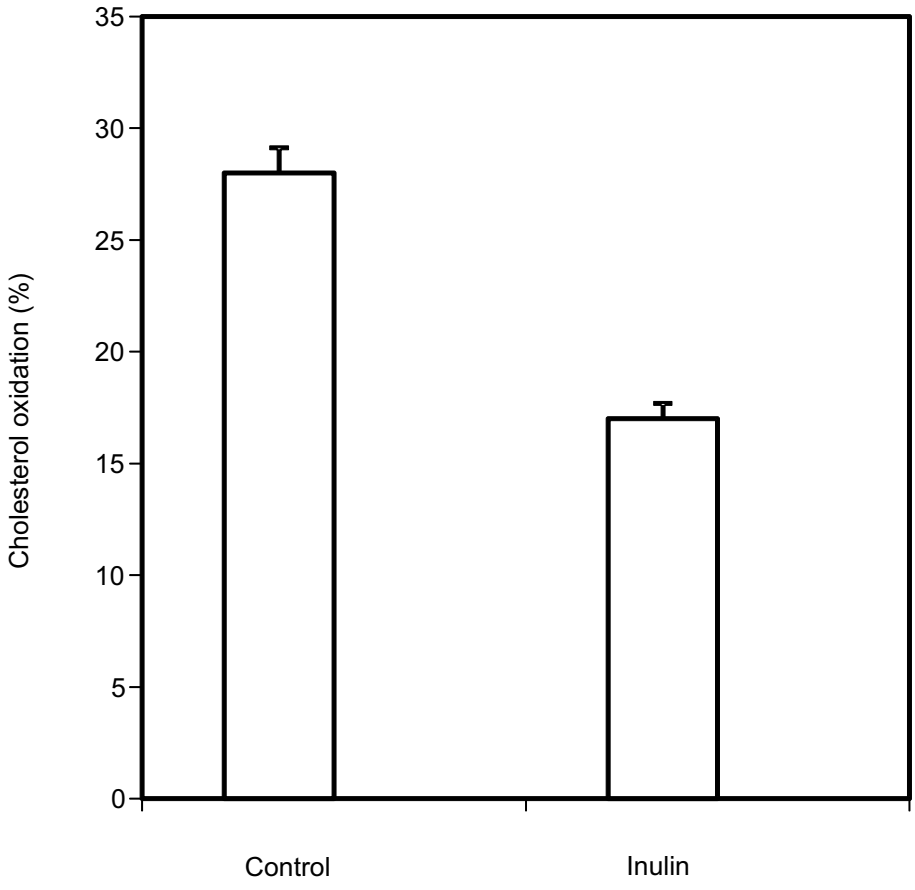


Fig. 2. Susceptibility of cholesterol to oxidation, tested by cholesterol oxidase assay in liver plasma membranes isolated from control rats and rats on inulin-enriched diet, $P < 0.001$

extracellular leaflet of the plasma membrane. The results showed that cholesterol asymmetry was not strongly affected by inulin intake, but a slight reduction in the cholesterol molecules localized in the external membrane leaflet was observed (Table 1).

**Discussion.** Studies on prebiotics are devoted mainly to their influence on the gastro-intestinal tract [8] and to the serum lipids [9]. In addition, there are a few studies related to the effect of prebiotics on liver lipogenesis concerning predominantly triglycerides and fatty acid re-esterification [10]. However, very little is known about the effect of prebiotics on cholesterol in the liver membranes and on its stability against oxidative destruction. In the present work, we analysed the effect of inulin intake on the level and susceptibility to oxidation of cholesterol in rat liver plasma membranes. The obtained results showed that the content of cholesterol, expressed as mol%, was slightly reduced in membranes from inulin-

fed rats. This finding is in accordance with our previous data obtained with rats on fructooligosaccharide (FOS)-enriched diet, which revealed a more marked decrease of cholesterol in liver plasma membranes [11]. In these investigations, we also established a lower degree of cholesterol oxidation, as well as a lower level of lipid peroxides in FOS-fed rats. In the present work were analysed the possible reasons for the observed lower susceptibility to oxidative damage of cholesterol molecules in plasma membranes from inulin-fed rats. For this purpose, we measured the asymmetric distribution of cholesterol between the two leaflets of the membrane bilayer in order to understand whether the observed differences were due to lower availability of cholesterol molecules to the external oxidizing agent, or to some other reason related to the membrane composition and/or structure. The obtained results showed that the asymmetric distribution of cholesterol was not significantly influenced by inulin administration, but a small reduction in the cholesterol molecules localized in the outer membrane monolayer was established. Thus one possible reason for the lower susceptibility of cholesterol to oxidation in prebiotic-fed rats could be the presence of less cholesterol in the external leaflet of these membranes, which means that less cholesterol molecules are available for external oxidative attack. It is also possible that the increase of the content of sphingomyelin in these membranes (from 12 to 17 mol%) could be another factor for the observed higher resistance of cholesterol to oxidative damage in membranes from inulin-fed rats. Our previous studies [11] also supported the antioxidant effect of sphingomyelin in membranes isolated from FOS-fed experimental animals. Of course, other intrinsic membrane-related parameters like alterations in the lateral structural organization, decrease of membrane fluidity or decrease of cholesterol "chemical activity" [12], which could contribute to making the membrane lipids more resistant to oxidative damage, should also be taken into consideration in the interpretation of the reported findings.

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