

OCCURRENCE OF LIPIDS IN THE LIVER OF THE HYPERTRIGLYCERIDEMIC RATS

Zdenka Zemanova^{a*}, Miluse Strnadova^a, Zuzana Jirsova^b, Petra Klusonova^a

^a Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

^b Department of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, Czech Republic
e-mail: zemanova@biomed.cas.cz

Received: October 10, 2008; Accepted: December 20, 2008

Key words: Lipid accumulation/Prague Hereditary Hypertriglyceridemic rats/Liver histochemistry/OilRed/Sudan Black/Local glucocorticoid metabolism

Aims: The purpose of this study was to demonstrate the accumulation and distribution of lipids in the liver of the adult Prague hereditary hypertriglyceridemic (HHTg) rats. They reveal an increased expression of 11 β -hydroxysteroid dehydrogenase 1 (11HSD1), which locally increases concentration of corticosterone in the liver. We studied the effect of the 11HSD1 inhibition on the lipid content.

Methods: Samples of liver of three groups of adult female rats – HHTg, HHTg treated for 14 days with 50 mg/kg/day carbenoxolone (HHTg+CBX) and control Wistar rats, were examined histochemically. Cryosections of the samples were stained with Oil red O or Sudan black B to demonstrate different kinds of lipids. Extent and intensity of staining was evaluated semiquantitatively.

Results: The orientational analysis showed a higher extent and intensity of the staining of the liver of HHTg and HHTg+CBX rats (equal in both hypertriglyceridemic groups) than that of the control Wistar rats. Oil red O stained unsaturated fatty acids and neutral fats, mainly triglycerides. The difference was on average 30 per cent. Staining of phospholipids with Sudan black B showed similarly the higher positivity in the hypertriglyceridemic groups than in controls.

Conclusions: Staining for triglycerides and phospholipids demonstrated a higher amount of lipids in the liver of HHTg and HHTg+CBX female rats than in controls. The inhibition of 11HSD1 activity had no effect on the lipid content in the liver of the HHTg rats.

INTRODUCTION

The increasing number of people in advanced countries suffers from a cluster of associated metabolic disorders that are collectively termed metabolic syndrome and that include hypertension, glucose intolerance, hyperinsulinemia, hypertriglyceridemia, hepatic steatosis and obesity. Similar symptoms can be found in patients with Cushing's syndrome which is characterised by elevated plasma level of glucocorticoids and thus the disturbances of metabolic syndrome seem to be not only insulin – but also steroid-sensitive.

11 β -hydroxysteroid dehydrogenase 1 (11HSD1) is an oxidoreductase highly expressed in liver and adipose tissue catalysing an interconversion of corticosterone into 11-dehydrocorticosterone and *vice versa*. Both activities, reductase and oxidase of 11HSD1, were found *in vitro*, however conversion of 11-dehydrocorticosterone to corticosterone predominates in intact tissues¹. Study on transgenic mice with selective overexpression of 11HSD1 in liver, a non-obese model of metabolic syndrome, showed enormous hepatic steatosis in these animals².

Prague hereditary hypertriglyceridemic rats (HHTg) were previously described as a genetic model of metabolic syndrome. They suffer from hypertriglyceridemia, insulin resistance and hypertension³. Our pilot experiments revealed an increased activity and expression of 11HSD1

in the liver of female HHTg rats. We therefore decided to study the role of 11HSD1 in accumulation and distribution of lipids in the liver of these animals using chronic administration of carbenoxolone (CBX), a nonselective inhibitor of steroid dehydrogenases. We examined the lipid content in the rat liver using lipid histochemistry.

MATERIALS AND METHODS

Experimental groups

Lipid content was investigated in the liver of three experimental groups with 10 animals: adult Prague hereditary hypertriglyceridemic rats (HHTg), HHTg rats treated with carbenoxolone (14 days, 50 mg/kg/day dissolved in drinking water, HHTg +CBX), and Wistar rats as normotriglyceridemic controls.

Histochemistry of lipids in the liver

Animals were killed by decapitation, liver were removed, immediately frozen in liquid nitrogen and stored until use at – 84 °C. They were processed for cryosections 8 μ m thick, fixed in Baker solution for 1 hour, rinsed in water (tap water followed by distilled water), rinsed in 50 per cent ethanol and stained – either with Oil red O or Sudan black B⁴. Staining with Oil red O (Sigma) saturated in 70 per cent ethanol lasted 1 hour. The sec-

Table 1. Intensity and area of the lipid staining in sections of the liver samples (percentage of the total).

Experimental group (n)	Oil red O			Sudan black B		
	+	±	(±)-0	+	±	(±)-0
HHTg (9)	55 %	11 %	33 %	44 %	56 %	0
HHTg + CBX (6)	63 %	37 %	0	43 %	57 %	0
Wistar (7)	25 %	50 %	25 %	25 %	75 %	0

Explanations: HHTg – Prague hereditary hypertriglyceridemic rats; HHTg + CBX – HHTg rats treated with carbenoxolone; extent and intensity of staining: strong „+“, mild „±“, weak, or none „(±)-0“; n – number of animals processed for histological examination.

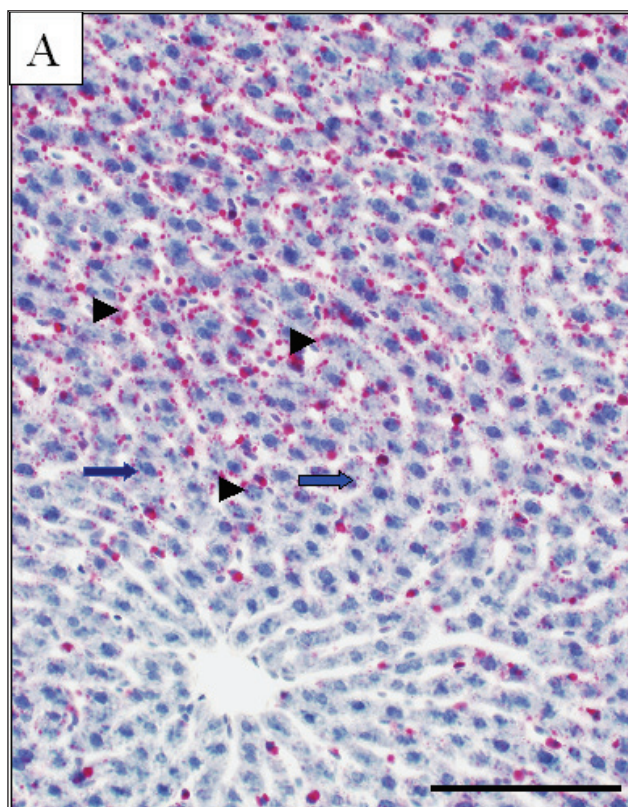


Fig. 1 A. Oil red O staining of lipid droplets in hepatocytes (full arrows) and Ito cells (arrowheads) of the liver of HHTg rat; bar = 100 μm.

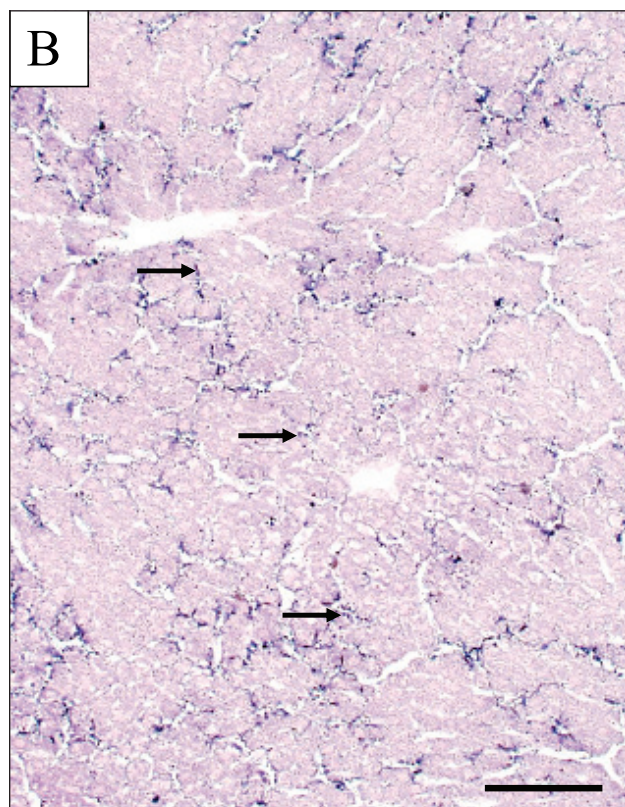


Fig. 1 B. Sudan black B dark blue staining of phospholipids in perisinusoidal localization in the periportal zone of the liver lobule in HHTg rat (arrows); bar represents 100 μm.

tions were then briefly rinsed in 50 per cent ethanol followed by tap water and by staining of nuclei with Harris hematoxylin for 1 min. The sections were then rinsed in tap water by customary procedure and mounted in glycerine jelly. Staining with Sudan black B (Merck) lasted 20 min and the counterstain of nuclei was performed with Kernechtrot for 5–7 min. The other steps were similar.

The dyes stained different kinds of lipids: Oil red stained unsaturated fatty acids and neutral lipids (mostly triglycerides). Sudan black coloured predominantly phospholipids in fixed sections. Evaluation of the colouring

extent and intensity was performed semiquantitatively, discriminating strong „+“, mild „±“, and weak, hardly visible or none colouring as „(±)“ or „0“ (Table 1). The strong colouring exceeded more than half of the section area, the mild colouring occupied medium extent and intensity of the section and the weak colouring was that, which was rare and hardly visible. The staining procedure was repeated in several sections of any sample to get representative results. Besides extent and intensity of the section staining we still evaluated its distribution in the liver sections and localization of lipid droplets in various cell types.

RESULTS AND DISCUSSION

Most of lipid droplets stained with Oil red O was distributed in the liver of control Wistar rats equally in both the peripheral periportal zone and the central venous zone of liver lobules. Lipid droplets appeared evenly in both hepatocytes and perisinusoidal cells (so-called Ito cells, fat-storing cells or lipocytes)^{5,6}. In the liver of HHTg rats there appeared a slight preponderance of stained lipids in parenchymal cells, and in HHTg +CBX rats just opposite, in the Ito cells.

Sudan black B stained liver lobules more intensely at their periphery, in periportal zone (in 70–80 % sections). The phospholipids were demonstrated mostly outside of hepatocytes, in the perisinusoidal cells in all experimental groups, but more frequently in control rats (in 90 %) and less frequently in HHTg +CBX rats (in 60 %).

The extent and intensity of the lipid staining of the samples of both triglyceridemic groups was higher than that of controls (Table 1) and was nearly identical.

CONCLUSIONS

We demonstrated a higher lipid content in the liver sections of HTG rats. Treatment of HTG rats with CBX did not change markedly the lipid deposition in the liver. The study will continue by the detailed analysis of the distribution of different kinds of lipids in the liver cells.

ACKNOWLEDGMENTS

Supported by the Grant Agency of the Academy of Sciences (grant KJB 500110703).

REFERENCES

1. Walker BR, Andrew R. Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. Review. *Ann N Y Acad Sci.* 2006; 1083:165-84.
2. Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, Seckl JR, Mullins JJ. Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A.* 2004; 101:7088-93.
3. Štolba P, Dobesová Z, Husek P, Opltová H, Zicha J, Vrána A, Kunes J. The hypertriglyceridemic rat as a genetic model of hypertension and diabetes. *Life Sci* 1992; 51:733-40.
4. Bayliss High OB, Lake BD, Lipids. In: Bancroft J.D. SA, Turner D.R. (Ed.), *Theory and Practice of Histological techniques*, Churchill Livingstone, New York, Edinburgh, London, Madrid, Melbourne, 1996, pp. 213-42.
5. Ito T, Itoshima T, Ukida M, Tobe K, Kiyotoshi S, Kawaguchi K, Ogawa H, Yamamoto H, Hattori S, Kitadai M, et al. Scanning electron microscopy of Ito's fat-storing cells in the rat liver. *Acta Med Okayama* 1984; 38:1-9.
6. Enzan H, Himeno H, Hiroi M, Kiyoku H, Saibara T, Onishi S. Development of hepatic sinusoidal structure with special reference to the Ito cells. *Microsc Res Tech* 1997; 39:336-49.

