

EXPRESSION OF LIPOPROTEIN LIPASE IN THE RENAL
ARTERY AND VEIN OF THE DOMESTIC PIG – AN
ENZYME-HISTOCHEMICAL STUDY

A. VODENICHAROV¹, P. ATANASSOVA², P. YONKOVA¹,
G. KOSTADINOV¹ & H. HRISTOV¹

¹Department of Veterinary Anatomy, Histology and Embryology, Faculty of
Veterinary Medicine, Trakia University, Stara Zagora; ²Department of
Anatomy, Histology and Embryology, Faculty of Medicine,
Medical University, Plovdiv; Bulgaria

Summary

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Lipoproteinlipase (LPL) expression in the wall of renal artery and vein of 8 (4 male and 4 female), 10–12 months old slaughtered for consumption Landrace pigs, was studied. In the renal artery, the LPL expression was observed as a irregular thick layer mostly on the luminal surface of endothelial layer and close to both intimal and media sides of the internal elastic membrane. The latter showed a various degree of fragmentation. In two of the animals lipoproteinlipase expression was also established in the *Tunica media*, between smooth muscle cells. In the renal vein, LPL expression was observed as a slim line on the luminal surface of the endothelium. No positive reaction was detected next to the internal elastic membrane. A large amount of LPL-positive cells was observed in the connective tissue of the venous middle shell of one of investigated animals. On the basis of obtained results, a presumption was made that LPL expression in pig renal artery and vein had a species-related and individual features.

Key words: lipoprotein lipase, pig, renal artery, renal vein

INTRODUCTION

Lipoprotein lipase (LPL) is an important enzyme, involved in the intravascular metabolism of triglyceride-rich lipoproteins, ingested with food (Goldberg, 1996). It hydrolyzes the triglycerols of chylomicrons, circulating in the bloodstream, to fatty acids and glycerol (Fawcett & Jensh, 2002). LPL is synthesized and secreted by various types of cells, among which the endothelial cells of capillaries and arteries. In fat tissues and muscles, the enzyme is synthesized and secreted in a catalyti-

cally active form, by the adipocytes and myocytes, respectively and is then transported to the surface of the capillary endothelium (Pentikäinen *et al.*, 2002). The immunohistochemical expression of LPL is proved in all layers of the arterial wall – the intima, the smooth-muscle cells of the media, and the adventitia (Jonasson *et al.*, 1987; Sako *et al.*, 2003; Kojama *et al.*, 2004). LPL belongs to the mammalian triacylglycerol lipase gene family, which also includes pancreatic, liver, and endo-

thelial lipases (Pentikäinen *et al.*, 2002). The endothelial lipase, synthesized and secreted mostly by the endothelial cells of the blood vessels, is determined as a new member of the lipase gene family (Kojama *et al.*, 2004).

The immunological studies of Ruge *et al.* (2004) showed that LPL in the kidneys of mice and minks is produced by the epithelial cells of the tubules. It was also established that this enzyme can deposit in the glomerules from the blood that passes through them.

According to Camps *et al.* (1990), expression of LPL in the large blood vessels is observed in the smooth-muscle cells of the media, while in the kidneys, a strong immunofluorescence of the vascular endothelium was established, especially in the glomerules.

Recently, in our studies on renal blood vessels of a control group of dogs, and a group fed a high-calorie diet, a positive expression of LPL only in the blood vessels of the latter was established, while no such reaction was observed in the control group (Yonkova *et al.*, 2007).

In available literature, we could not find any data from similar studies on renal blood vessels of pigs, that is why we aimed to study the expression of LPL in the wall of the renal artery and vein on material, obtained from pigs, slaughtered for consumption.

MATERIALS AND METHODS

We studied the renal artery and vein of 8 pigs (4 male and 4 female) from the Belgian Landrace breed, aged 8–12 months, weighing 90–120 kg, with a well-developed layer of subcutaneous fat. The material for the study was obtained immediately after the animals were slaughtered in a slaughterhouse, and transported in a

refrigerated case to the laboratory. After careful preparation of the structures in the area of the chylus with the aid of a magnifying glass, little pieces (1 cm³) with included renal artery and vein, as well as surrounding tissues, were formed and frozen in a cryostat at –20⁰ C. From them, 5–7 µm cryostat cuts were prepared, upon which the enzyme-histochemical reaction for detection of lipoproteinlipase was performed by the Tween method of Gomori (Pearse, 1960). Positive reaction was registered by appearance of clusters of dark brown granules. The detection of the reaction was performed by a semi-quantitative analysis using the score system of Atanassova (2000) to present enzyme activity as followed: (0) – lack of enzyme activity; (+) – weak, (++) – medium and (+++) – strong enzyme activity.

RESULTS

The light microscope studies showed that, in both blood vessels, there was an expression of lipoproteinlipase, which was manifested at different extents in the layers of vascular walls in the individual animals (Table 1).

In the wall of the renal artery, LPL expression was observed in all test animals on the lumen surface of the intimal endothelial cells, and around the inner elastic membrane. On the lumen surface of the endothelium, the LPL expression was positive in the form of a dark layer with a different width along the vessel's circumference. Around the internal elastic membrane, LPL reactivity was present as a thin layer right next to the two sides (toward the intima and the media) of the membrane (Fig. 1). It should be noted that in all studied arteries, the internal elastic membrane showed different degrees of fragmentation.

Table 1. LPL activity in the walls of renal artery and vein

Blood vessel	Number and gender of dogs							
	1 ♂	2 ♀	3 ♂	4 ♂	5 ♀	6 ♀	7 ♀	8 ♂
<i>Renal artery</i>								
Intimal coat (<i>Tunica intima</i>)	++	+	++	++	++	++	++	++
Medium coat (<i>Tunica media</i>)	0	0	+++	0	0		0	0
<i>Renal vein</i>								
Intimal coat (<i>Tunica intima</i>)	+++	++	++	+++	++	+++	++	+++
Medium coat (<i>Tunica media</i>)	0	0	+++	0	0	0	0	0

(0) – lack of enzyme activity; (+) – weak, (++) – medium and (+++) – strong enzyme activity.

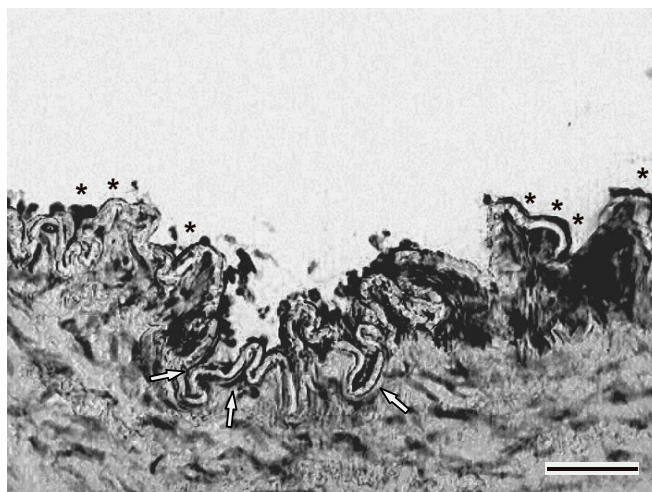


Fig. 1. Lipoproteinlipase expression on the lumen surface (*) of endothelial cells, and on both sides of the internal elastic membrane (arrows). Bar = 50 μ m.

In two of the studied individuals (1 male and 1 female), i.e. 25% of all cases we found a clear LPL expression in the medium coat (*Tunica media*) of the two renal (left and right) arteries. Here, the positive reaction product was observed between the smooth-muscle cells of the media, and, relatively rarely, in the smooth muscle cells themselves (Fig. 2).

In the renal vein's wall, the LPL expression was observed as a thin yet dense layer on the lumen surface of the endothelium. Around the internal elastic membrane, no expression of LPL was established. In one of the male pigs (12.5% of all cases), large amounts of LPL-positive cells in the connective tissue of the media in both studied veins were observed (Fig. 3).

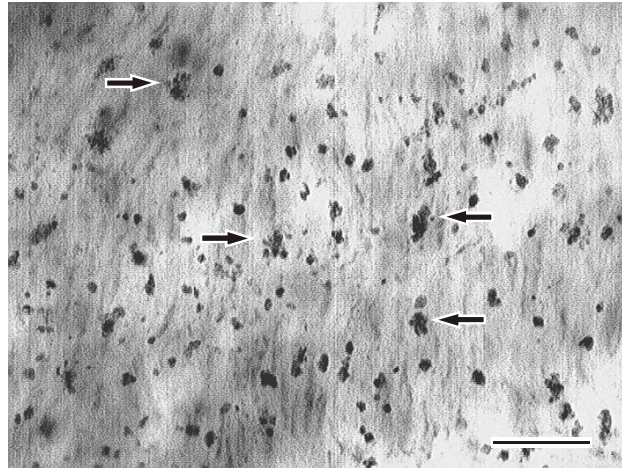


Fig. 2. Part of the media of a renal artery. The arrows point at the positive LPL expression in and around smooth muscle cells. Bar = 40 μ m.

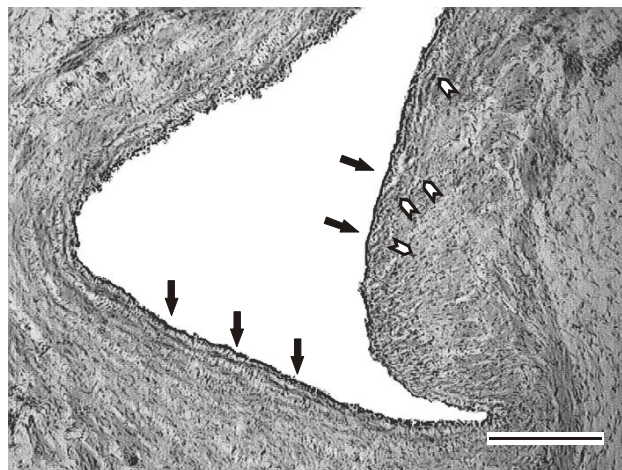


Fig. 3. Wall of a renal vein. LPL expression on the lumen surface of the endothelium (arrows) and in the media (arrow heads). Bar = 120 μ m.

In the adventitia of the studied arteries and veins, the expression of LPL enzyme activity was weak.

DISCUSSION

Data from the performed light-microscope tests showed convincingly the presence of

LPL activity in the renal artery and vein of domestic pigs. Even though the animals, from which the material was obtained, had a well-developed layer of subcutaneous fat, only in a relatively small portion of them – 25% for arteries, and 12.5% for veins, did we discover reaction for LPL in the middle coat. The reaction product was

observed mainly around the smooth muscles, and rarely in them. In this regard, our results about the renal artery differ, to an extent, from the data of Camps *et al.* (1990) about the presence of LPL in smooth-muscle cells in the media of large arteries, one of which is the renal artery. We consider that the established positive LPL expression, mainly outside the smooth-muscle cells in the media, probably originates from the circulating LPL, and is related to components of the extracellular matrix, as suggested by Pentikäinen *et al.* (2000).

The observed deposition of LPL around the internal elastic membrane, fragmented at varying extent, is described for the first time, yet this localization is probably related to the initial processes of atherogenesis in the arterial wall. Because we did not observe any LPL-positive cells (mostly macrophages) in the intima, we consider that this is most probably a case of lipids penetrating from the bloodstream.

An item of interest is the marked expression of LPL on the lumen surface of endothelial cells of the renal vein, since we could not find information on such a finding in any reference. The strongly expressed enzyme activity gives us reason to believe that the vein endothelium takes an active role in the destruction of chylomicrons in the blood that exits the kidney. It could be, however, hard to determine whether LPL is synthesized in the endothelium of the renal vein.

Unlike the dogs who became obese after a high-calorie diet, for which there was a LPL expression in the media of the renal artery and vein in 100% of the cases (Yonkova *et al.*, 2007), in our research, this percentage was significantly lower. According to us, this could be due to species-related, as well as individual peculi-

arities, associated above all to an acquired resistance towards the infiltration of lipids in the vascular wall. The grounds for this suggestion is the statement of Anestiady & Nagornev (1982), who believed that the individual reactivity of an organism is highly significant in the process of formation and regression of lipid deposits in arteries.

The results from our research give us a reason to suggest that the observed expression of LPL in the media of the renal artery and vein in a comparatively low percentage of studied animals is most probably caused by individual characteristics. It should not be overruled that there is a possibility along with age advancement and the increasing extent of fatness LPL expression to be found in more, if not all, animals. It is obvious that further research into the matter of age-related peculiarities is necessary.

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Correspondence:

Assoc. Prof. A. Vodenicharov
Department of Veterinary Anatomy,
Histology and Embryology,
Faculty of Veterinary Medicine,
Trakia University, Student's Campus,
6000 Stara Zagora, Bulgaria