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Role of Tissue Lipoprotein Lipase Activity Localization in the Pelvic Urethra in Male Cats

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Abstract: The pelvic urethra has been investigated in seven sexually mature, clinically healthy European short-hair male cats, aged 12-18 months, weighing 2.8-4 kg, obtained from a licensed animal breeder. All experiments were carried out under strict observance of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and law on Animal Protection in the Republic of Bulgaria. Cryostat cross sections of 5-7 μm were used for detection of lipoprotein lipase (LPL) by Tween method of Gomori. The reaction was positive when clusters of dark-brown lead sulfide precipitates were present. The localization of tissue LPL expression was determined by light microscopy. The intensity of the reaction was assessed by a semi-quantitative analysis using the score system. The enzyme histochemical investigation of feline pelvic urethra showed that the highest intenstiy of LPL expression, occurred in the muscular layer that was mainly of skeletal muscle. A medium intensity was observed in epithelial cells of disseminated part of the prostate and in the lumen of its glandular tubules. The urethral propria exhibited weak LPL expression, whereas no LPL activity was detected in the urethral lumen epithelium.

Key Words: Lipoprotein lipase, pelvic urethra, tomcats.

Doku Lipoprotein Lipaz Aktivitesinin Erkek Kedilerde Urethra'nın Pars Pelvina'sındaki Lokalizasyonunun Rolü

Özet: Lisanslı bir hayvan yetiştiricisinden elde edilmiş, 12-18 aylık yaşta, 2,8-4 kg ağırlığında, klinik olarak sağlıklı ve seksüel olgunluğa ulaşmış, Avrupa Short-hair ırkı yedi adet erkek kedide urethra'nın pars pelvina'sı incelenmiştir. Tüm deneyler, Avrupa Deneysel ve Diğer Bilimsel Amaçlar için Kullanılan Omurgalı Hayvanları Koruma Konseyi'ne ve Bulgaristan Cumhuriyeti'ndeki Hayvanları Koruma Kanunu'na çok sıkı riayet edilerek uygulanmıştır. Lipoprotein lipaz (LPL) tespiti için Gomori'nin Tween metodu vasıtasıyla 5-7 mikrometrelik cryostat kesitler kullanıldı. Koyu kahverengi kurşun sülfit presipitat kümeleri gözlendiğinde sonuç pozitifti. Dokudaki LPL ekspresyonunun lokalizasyonu ışık mikroskobu ile saptandı. Reaksiyonun yoğunluğu skorlama sistemi kullanan yarı kantitatif bir analiz ile tayin edildi. Kedi urethra'sının pars pelvina'sının enzim histokimyasal incelemesi gösterdi ki, LPL enziminin en yoğun ekspresyonu kas katmanında, özellikle iskelet kaslarında meydana gelmekte idi. Orta yoğunlukta ekspresyon ise prostatın pars disseminata'sında gözlendi. Urethra'nın propriya katmanı zayıf LPL ekspresyonu gösterirken, urethra'nın lumen epitelyumunda hiçbir LPL aktivitesi gözlenmedi.

Anahtar Sözcükler: Lipoprotein lipaz, uretra'nın pars pelvina'sı, erkek kediler.

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Introduction

In mammals, lipoprotein lipase (LPL) belongs to the group of triglyceride lipases, together with pancreatic, hepatic and endothelial lipases[13].

LPL is an enzyme with limited expression, that is involved in triglyceride and lipoprotein catabolism and that provides free fatty acids to adipose tissue and in their accumulation in muscles as a source of energy[3].

The expression of LPL activity in men has been investigated and compared in subcutaneous fat deposits in the abdomen and the subcutis, in the region of the gluteal muscle in both genders. In both studied regions, LPL activity was higher in women, whereas in men the enzyme was more active in the abdominal subcutaneous area as compared to the gluteal subcutaneous region. In both genders, LPL activity was found to be higher in abdominal subcutaneous region than in gluteal regions[1].

Endothelial lipase is a relatively new member of the group of lipoprotein enzymes and it occupies a central place in the metabolism of high-density lipoproteins. In men, it is expressed in endothelial cells, macrophages and smooth muscle cells[10].

LPL expression was negatively correlated to the accumulation of visceral fat deposits[9].

There is a positive correlation of the distribution of muscle fibres and serum high-density lipoproteins and this fact was due to the high capacity for energy metabolism of these muscle fibres and the high density of surrounding capillaries. Therefore, these fibres metabolize the fatty acids released by LPL and, serum high-density lipoprotein cholesterol levels subsequently increase[14].

LPL activity was investigated in guinea pigs, where the enzyme was shown to become catalytically active in endothelial vascular cells, and its synthesis – to occur in smooth muscle cells, the glandular epithelium of tubuloalveolar glands, renal glomeruli and parenchymal cells. LPL also participates in the distribution of fatty acids, derived from triglycerides, between the adipose and muscle tissue in guinea pigs[5,6].

In mice, it has shown, that LPL was produced and secreted in catalytically active form by adipocytes and myocytes, and then, transported to the capillary endothelium. This hydrolase metabolized triglycerides in chylomicrons, releasing fatty acids[11].

LPL activity is studied in the white adipose tissue in rats, located around the epididymes, the uterus, kidneys as well in subcutaneous and intramuscular adipose tissue. The high levels of this enzyme reduced serum concentrations of triglycerides and cholesterol[7].

LPL activity in skeletal muscles remained unchanged following continuous physical exercise in dogs, probably due to transportation of enzyme substrate from muscle cells to the lumen of blood vessels in muscles[4].

LPL expressioin the cardiac tissue of the heart and skeletal muscles in rats, and gave evidence for its expression in these tissues[8].

The scarce literature data about the localization of LPL expression in the pelvic urethra in male cats motivated the performance of the present enzyme histochemical investigation. It aimed to establish those parts of the normal pelvic urethra of male cats, where LPL activity was present.

Material and Methods

The pelvic urethra of 7 sexually mature, clinically healthy male European shorthair cats at the age of 12–18 months and weighing 2.8 to 4 kg was investigated. The animals were obtained from a licensed animal breeder.

The animals were anesthetized with 15 mg/kg Zoletil® 50 (tiletamine hydrochloride 125 mg and zolazepam hydrochloride 125 mg in 5 ml of the solution) Virbac, France

Cats were euthanized with intravenous injection of 50 mg/kg Thiopental 1g (thiopental sodium, Sandoz, Kundl-Austria) into the cephalic vein. The material for the study was obtained immediately after opening of abdominal and pelvic cavities and removal of prostate gland. The experiment was carried out under strict observance of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the **European Convention** for the **Protection** of Pet Animals, and Law on Animal Protection in the Republic of Bulgaria – part IV (Experiments with animals, art 26, 27, 28), part VII (Euthanasia of Animals, art. 45, 46, 47, 48) and part VIII (Protection and welfare of animals, art 52, 53).

After careful removal with the aid of a magnifying glass, pieces of the pelvic urethra (1 cm^3) were frozen in a cryostat at –20°C and used for enzyme histochemical detection of LPL by the Tween method[12]:

- obtaining cryostat cross sections of 5-7 µm and fixation in 10% neutral formalin for 10 min;
- washing with distilled water, placing in incubation medium and putting the cross sections in a thermostat for 24 hours at 37 °C;
- washing with distilled water and treatment with 1% lead nitrate in a thermostat at 56 ˚C for 10 min;
- washing with distilled water and treatment with 1% yellow ammonium sulfide till the appearance of lead sulfide precipitate (clusters of dark brown granules).

The reaction is considered positive if clusters of dark brown lead sulfide precipitates were present.

The localization of tissue LPL expression was determined by light microscopy (Primo Star (Zeiss, Germany), and results were recorded with a digital camera Prog Res CT3 (Germany).

The intensity of the reaction was assessed semi-quantitatively using the score system: 0 – lack of enzyme activity; + – weak enzyme activity; ++ – medium enzyme activity and +++ – strong enzyme activity[2].

Results

The enzyme histochemical investigation of pelvic urethra showed that the highest LPL expression intensity was observed in the muscle layer of pelvic urethra that is mainly composed of skeletal muscle. A medium intensity was present in epithelial cells of disseminated prostate and in the lumen of its glandular tubules. The urethral propria demonstrated weak LPL activity whereas no enzyme activity was observed for the urethral lumen epithelium (Table 1).

Table 1. Intensity of the reaction for detection of LPL activity in the pelvic urethra in male cats

Urethra	**0** – lack of activity	**+** weak activity	**++** medium activity	**+++** strong activity
Urethral muscle layer				**+++**
Epithelium of disseminated prostate			**++**	
Urethral propria		**+**		
Urethral epithelium	**0**			

The LPL expression predilection sites were firstly, in the skeleton muscle part of the pelvic urethra (Fig. 1), followed by epithelial cells of disseminated prostate and the lumen of its glandular tubules (Fig. 2).

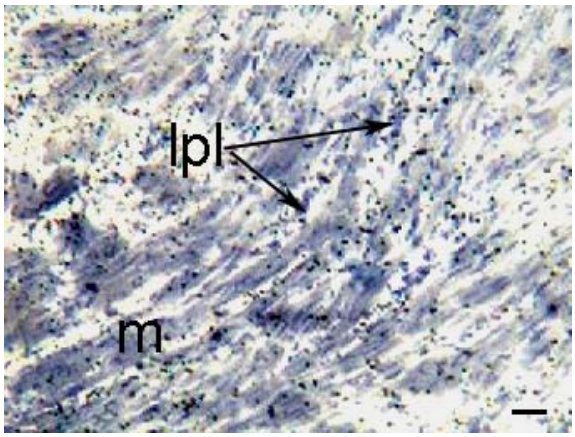

Figure 1.
An area of the pelvic urethra, showing the localization of lipoprotein lipase (lpl) expression in urethral muscle layer (m). Gomori staining. Bar=50 µm.

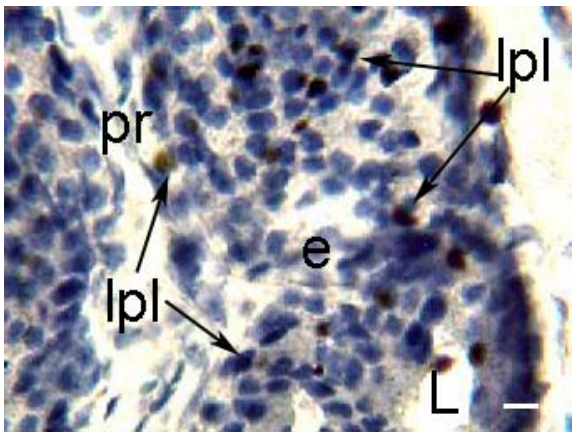

Figure 2.
An area of the pelvic urethra, showing the localization of lipoprotein lipase (lpl) expression in epithelial cells of disseminated prostate (e), lumen of the glandular tubules (L), propria (pr). Gomori staining. Bar=15 µm.

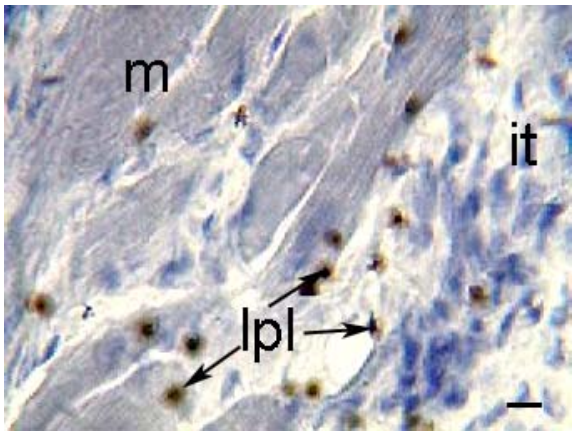

Figure 3.
An area of the pelvic urethra, showing the localization of lipoprotein lipase (lpl) expression in the propria (it) and urethral muscle layer (m). Gomori staining. Bar=40 µm.

In the urethral propria, the enzyme was observed less frequently (Fig. 3), whereas in the urethral epithelium, no LPL activity was observed (Fig. 4).

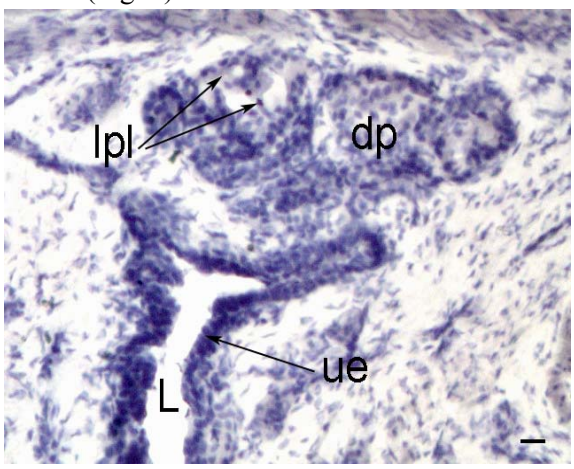

Figure 4.
An area of the pelvic urethra, showing the localization of lipoprotein lipase (lpl) expression in epithelial cells of disseminated prostate (de), urethral lumen (L) and urethral epithelium (ue). Gomori staining. Bar=30 μm.

Discussion

The present investigation describes for the first time a considerable presence of LPL expression in the pelvic urethra of male cats, with predominance of enzyme activity in the urethral muscular layer.

The data established by us, showing the highest LPL activity in the muscular layer of male feline pelvic urethra, correspond to findings in humans, referring to the importance of LPL expression for the catabolism of triglycerides and lipoproteins as sources of free fatty acids, accumulated in muscles as a source of energy. Therefore, the LPL activity in feline urethral muscle could probably have a similar significance[3,13].

Comparing our data with the results about the metabolism of LPL-release fatty acids by skeletal muscle fibres in men, it could be assumed that the predominance of LPL activity in the urethral musculature in male cats could be also related to the energy metabolism at this site[1,9,14].

Our results showing LPL activity in the muscle part of pelvic urethra correlate with the findings about the expression of this enzyme in human smooth muscle cells[10].

The dominating LPL expression in urethral musculature in disseminated prostate supports the studies in guinea pigs and mice, which have shown that the synthesis of the enzyme occurred in smooth muscle cells and the glandular epithelium of tubuloalveolar glands and that LPL participated in the distribution of fatty acids released from triglycerides between the adipose and the muscle tissue[5,6,11]. Therefore, the presence of LPL in these areas is related to the involvement of the enzyme in secretory processes that take place in disseminated part of the prostate and the accumulation of fatty acids in the muscular part of pelvic urethra.

The observed high and medium LPL intensity in the musculature and the epithelium of disseminated prostate corresponded to the view, that the high activities of this enzyme reduced serum triglyceride and cholesterol concentrations. It could be therefore presumed that in the pelvic urethra, LPL would exhibit the same properties[7].

The observed high LPL activity in the muscular part of the urethra could be also interpreted taking into consideration the opinion that LPL was expressed in the adipose tissue of the heart and the skeletal muscles of rats, as well as the view that LPL activity remained constant after continuous physical exertion in dogs [4,8]. Therefore the strong LPL expression in the musculature of urethra is a reliable and constant marker of the contractile capacity of pelvic urethra in male cats.

The results of the present investigation, on the background of the lack of literature data about the localization of LPL activity in pelvic urethra of male cats, could be useful for explaining some enzyme histochemical features of this part of the urethra in this carnivore species. On the other hand, the present data could be utilized for both evaluation of the reproductive performance of male cats and for diagnostics of pathological adipose lesions in this anatomical region.

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