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LIPOPROTEIN LIPASE mRNA EXPRESSION IN DIFFERENT TISSUES OF FARM ANIMALS

Thesis Presented By

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B.V.Sc. Tanta University ,2003

For the Degree of
Master of Veterinary Sciences
(Biochemistry)

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2007

Kafr-Elsheikh University
Faculty of Veterinary Medicine
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ACKNOWLEDGMENT

First of all prayerful thanks to our merciful ***GOD****, for all gifts who gave me allover my life.*

I can not find enough words to express my deep feeling towards ***Prof. Dr. Ibrahim F. Hassan,*** *Professor of Biochemistry, Faculty of Veterinary Medicine, Kafr El-Sheikh University for his continuous encouragement, helpful advice and unfailing interest that contributed so much effort he did in preparation and completion of this work.*

I wish to express my deep respect, extreme thankfulness appreciation and profound gratitude to ***Prof. Dr. Khaled A. Kahilo,*** *Professor of Biochemistry and Head of Physiology and Biochemistry Department, Faculty of Veterinary Medicine, Kafr El-Sheikh University for his continuous help, advices, providing facilities and for every thing he did for completion of this work.*

I am highly indebted to ***Dr. Azza M. El-Kattawy,*** *Assistant Professor of Biochemistry, Faculty of Veterinary Medicine, Kafr El-Sheikh University, for his continuous help, support, critical comments and advice during the course of this investigation.*

Sincere thanks to ***Dr. Mohamed Soliman and Dr. Samir El-Shazly*** *for their help in experimental part of this work*

Thanks are due to all staff members of Physiology and Biochemistry Department, Faculty of Veterinary Medicine Kafr El-Sheikh University.

Dedication to

My Father &

Mother

My Sister and

My Brothers

My Fiancee

LIST OF ABBREVIATION

APOC	Apo protein C
APOE	Apo protein E
Arg	Arginine
ASP	Aspargine
cAMP	Cyclic adenosin monophosphate
CMS	Chylomicrons
DEPC	Diethyl pyrocarbonate
dNTPs	deoxy nucleotide tri phosphate
EDTA	Ethylene diamine tetra acetic acid
EL	Endothelial lipase
FA	Fatty acid
Fas	Fatty acids
FFA	Free fatty acid
HDL	High density lipoprotein
His	Histidine
HL	Hepatic lipase
HSPG	Heparin sulfate proteoglycans
LPL	Lipoprotein lipase

LRP	LDL receptor related protein
Lys	Lysine
PCR	Polymerase chain reaction
PL	Pancreatic lipase
PPAR	Peroxisome proliferation activator receptor
PPAR-α	Peroxisome proliferation activator receptor alpha
PPGF	Platelet-derived growth factor.
RT-PCR	Reverse transcriptase polymerase chain reaction
Ser	Serine
TAE	Tris -Acetic acid- EDTA buffer
TAG	Triacyl glycerol
TNF	Tumor necrosis factor
VLDL	Very low density lipoprotein
μg	Micro gram
μl	Micro liter

CONTENTS

INTRODUCTION

INTRODUCTION

Lipoprotein lipase (LPL) is the key enzyme responsible for hydrolysis of triacyl glycerol (TAG) **(Camps *et al.*, 1991)**. Thus triacyl glycerols that are unloaded from chylomicrons (CMS), and very low density lipoproteins (VLDL) are hydrolyzed by LPL that are functional at the surface of endothelial cell. The liberated fatty acids are available for tissues as energy sources especially in muscle or for storage in the form of TAG in adipose tissue. It is generally assumed that, the rate-limiting factor for energy delivery from lipoproteins. The amount of active LPL available at the endothelium is correlated to LPL activity in tissue cells **(Borensztajn, 1987, Eckel, 1987 and Olivecrona, 1991)**.

LPL activity may be true in the most physiological conditions **(Olivecrona *et al.*, 1990).** The tissue LPL and its regulation became the subject of active investigation in ruminants **(Chilliard, 1993)**. Thus the study of LPL is of particular interest in tissues of meat producing ruminants, since LPL controls TAG partitioning between adipose tissues and muscles, there by increasing fattening or providing energy in the form of fatty acids for muscle growth **(Hocquette *et al.*,1998)**.

Some quantitative studies at whole animal level have suggested an important function of LPL activity in muscle, tissues with regard to the total TAG removal capacity of the body in both single stomached **(Borensztajn, 1987)** and in ruminant species **(Pethick and Dunshea, 1993).**

In adult sheep fed on maintenance ration about 55-60% of total amount of free fatty acids originate from hydrolysis of circulating TAG by LPL, and the heart and the skeletal muscle mass together could utilize approximately 40% of non esterified fatty acids entry rate **(Pethick and Dunshea, 1993)**.

The level of LPL transcriptase is positively related to LPL activity in bovine tissues **(Hocquette *et al.*, 1998).** So it seems to us very important to study LPL expression not only in adipose tissues but also in other tissues.

Aim of the work:

The present study was performed to throw light on the polymerase chain reaction (PCR) for quantitation of mRNA coding required for synthesis of LPL in various bovine tissues.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1. Lipoprotein Lipase:

Lipoprotein lipase (LPL) regulates the plasma levels of triglycerides and HDL **(Merkel *et al.*, 2002)**.

The LPL gene in human is located on chromosome 8P22 spans ~30 kb and is divided into 10 exons, and has substantial sequence homology among most of the species that have been examined by **Wang and Schotz (2002)**. The cDNA codes for a 475 amino acid protein including a 27-amino acid signal peptide. The catalytic center as noted by **Brunzell (1995)** is formed from three amino acids listed: Ser ^{132}, Asp^{156} and His^{241}

Ong *et al.*, (1994) reported that, lipoprotein lipase (LPL) is a key enzyme in lipid metabolism and is found predominantly in adipose tissue and muscle.

Peter *et al.*, (1993) reported that, there is a significant correlation between the ability of tissue to incorporate the fatty acids of triacylglycerol (TAG) of lipoproteins and the activity of the LPL. LPL is located in the wall of blood capillaries and anchored by proteoglycan chains of heparin sulphate. LPL has been found in the extract of heart, adipose tissue, spleen, lung,

renal medulla, aorta, diaphragm and lactating mammary gland. Normal blood does not contain appreciable quantities of LPL.

After injection of heparin, lipoprotein lipase is released from heparin sulphate binding and released into circulation. That is accompanied by clearing of lipemia. In liver, the lipase enzyme is released by large quantities of heparin and called heparin releasable hepatic lipase which has a properties different from that of lipoprotein lipase. In adipose tissue, insulin enhances both synthesis of LPL in adipocytes and LPL translocation to luminal surface of the capillary endothelium.

Robert *et al.*, (1993) stated that, heparin is combined to LPL present in the capillary wall and causes releasing of LPL to the circulation.

Havel *et al.*, (1960) showed that, plasma heparin was unable to clear chylomicrons (CMS) from the blood stream following injection in patients with sever forms of genetic hyperlipidemia.

Lipoprotein lipase (LPL) is synthesized within the fat cell and transported to it's site of action on the capillary endothelium of adipose tissue. The activity of LPL of adipose tissue is regulated at multiple levels among which transcription, translocation and glycosylation **(Eckel, 1989)**

2. Role of Lipoprotein Lipase:

Lipoprotein lipase (LPL) hydrolyses triacylglycerol progressively to diacyl glycerol and then to monoacyl glycerol that finally hydrolyzed to free fatty acids (FFA) and glycerol. Some of the released free fatty acids return to circulation and attached to albumin and finally transported into tissues. These FFA are resterified for subsequent lipid storage. LPL is present in many tissues especially in adipose tissue **(Eckel, 1987 and Peter *et al.*, 1993)**

Peter (1993) recorded that, LPL causes loss more than 90% of triacylglycerol of chylomicron and loss of Apoprotein C (APOC) to become HDL but retained Apoprotein E (APO E) and resulting chylomicron remnant. Chylomicron remnant is about half of the diameter of parent chlomicron and mainly composed of cholesterol and cholesterol ester because of the loss of triacyl glycerol.

Borensztajn, (1987) reported that, unloaded TAG from chylomicrons and very low density lipoproteins (VLDL) are hydrolyzed by LPL in capillary endothelial surface. Fatty acids are liberated by LPL are available for the tissues as energy sources especially in the muscle tissue.

Ong *et al.*, (1988) reported that, LPL gene expression may play an important role in the correlation between the

content of mRNA for LPL and protein synthesis rate for LPL (ribosomal RNA) in the fat cells.

Eckel *et al.*, (1992) reported that, LPL is often regulated inversely in adipose tissue and muscle. So either increase in adipose LPL or decrease in muscle LPL may play an important role in lipid partitioning towards adipose tissue storage and development of obesity.

Peter *et al.*, (1993) stated that, lipid in the diet is represented mainly by triacylglycerol, after digestion it form monoacylglycerols and fatty acids. These particles are recombined in the intestinal wall and linked with protein and secreted initially into lymphatic system and reach to circulation as lipoproteins (Chylomicrons). All TAG of chylomicrons are not taken up directly by the liver, it is firstly metabolized by extra hepatic tissues which possess LPL that hydrolyzes the triacylglycerol and releasing fatty acids. The released fatty acids are incorporated into tissue lipids and/ or oxidized as fuel. FFA enter lipogensis in both fat cells and liver cells. Triacylglycerol represent the main fuel reserve of the body and subsequent TAG is hydrolyzed through process called (lipolysis). The resulting fatty acids released into circulation as free fatty acids, which are taken up by most of tissues except brain and erythrocytes. In the liver cells, FFA has two pathways firstly, triacylglycerol that arising from lipogensis is

secreted into circulation as very low density lipoprotein (VLDL) and ended as chylomicrons. The second pathway occurs as partial oxidation of free fatty acids forming ketone bodies (Ketogenesis) which passes to extra hepatic tissues and act as another major fuel source.

Arner *et al.*, (1991) reported that, LPL activity and lipoprotein lipase mRNA levels were significantly higher in women than men. In men, the enzyme activity was higher in abdominal than gluteal adipose tissue. In both sexes lipoprotein lipase mRNA levels were three folds high in abdominal adipose tissue as compared to gluteal site in non obese men & women.

Brunzell *et al.*, (1973) found that, LPL acts as the primary enzyme required for CMS catabolism. The key for this observation was the use of CMS to assess lipolytic activity. Subsequent studies using artificial emulsions of lipid, showed that, LPL deficient patients had significant TAG lipolytic activity in their post heparin plasma. This lead to the discovery of hepatic lipase. Patients with elevated TAG levels, showed elevations of VLDL resulted in chylomicronemia, and this observation correctly attributed to saturation of LPL activity.

Eckel (1989) and Goldberg (1996) reported that, the physiological actions of LPL in catabolism of chylomicrons

and VLDL and in the production of much of the lipids and apolipoproteins that form HDL have been appreciated for more than a decade. The evolving role of LPL as a molecule that can anchor atherogenic lipoproteins to matrix molecule within the artery wall was first noted *in vitro* **(Saxena *et al.*, 1992)** and appears under some circumstances to modulate atherosclerosis in mice **(Babaev *et al.*, 1999).**

3. Organ-specific LPL action:

Animal studies have illustrated metabolic functions of LPL exclusive of control of plasma lipoproteins **(Zechner, 1997).** The major tissues control the circulating levels of plasma lipoproteins are the adipose tissues and muscle. However LPL is expressed in other sites including the nervous system, heart, liver, adrenals, macrophages, proximal tubules of the kidneys, pancreatic islet cells, and lungs **(Merkel *et al.*, 2002).** In these organs, LPL may have specialized functions. Islet cells of the pancreas express LPL and this activity has been related to insulin secretion and lipotoxicity (**Cruz *et al.*, 2001**). In the lung LPL mediated lipolysis may be important for surfactant production (**Hamosh *et al.*, 1976**).

3.1. LPL in the liver:

More than two decades, a careful study in aging rats showed that, the LPL activity in the liver and peripheral tissues

is reciprocally related **(Chajek *et al.*, 1977).** LPL is normally not synthesized and expressed in adult liver, however it is expressed in the liver of new born animals. Either because the pups ingest milk or because of a developmental genetic program, LPL activity increases in heart, skeletal muscle and in adipose tissue, at the same time LPL activity in the liver is extinguished **(Merkel *et al.*, 2002).** On the other hand, LPL is expressed in the liver in response to tumor necrosis factor (TNF) **(Enerback *et al.*, 1988)** and after tumor implantation **(Masuno *et al.*, 1984)**. i.e. during cachexia, LPL was expressed solely in the liver. The neonatal death of knockout mice, LPL was averted **(Merkel *et al.*, 1998)**, Probably because of sever hypoglycemia found in completely LPL deficient mice was prevented. Liver LPL targeted circulating TAGS to the liver and increased ketone bodies production. Ketone bodies can be used as energy as an alternative to glucose. However, many pups died after several weeks suffering from fatty liver **(Merkel *et al.*, 2002)**. Another intervention that induces LPL in the liver is the use of peroxisome proliferator activator receptor (PPAR) agonist drugs **(Fruchart, 1999)**. Although PPAR-α drugs increase liver oxidation of fatty acids (FAs), drugs with predominantly (PPARγ) activity are associated with fatty liver in some rodents **(Boelsterli and Bedoucha, 2002)**. Presumably they cause an increase in triglyceride synthesis and/or uptake

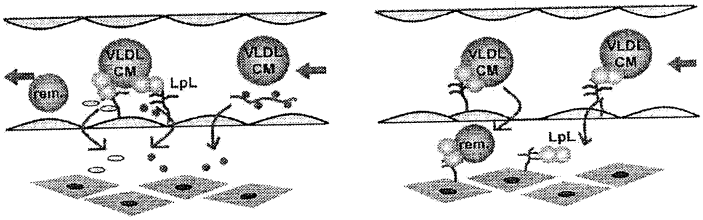
similar to that occurs in peripheral tissues like adipose tissue. The oxysterol responsive transcription factor (LXR) also increased LPL expression in the liver (**Zhang *et al.*, 2001**) and caused fatty liver.

3.2. LPL in the heart:

Although the heart is a major site of LPL synthesis. The role of cardiac LPL in both the energy balance of this tissue and in the regulation of plasma lipoproteins is under renewed investigation **(Merkel *et al.*, 2002).** Fatty acids are an important source of energy for heart and skeletal muscle, providing over 70% of the energy needs for cardiac function. LPL is likely to be the central enzyme in cardiac fatty acid uptake. Although fatty acids can be delivered to the heart while circulating on albumin, their molar concentration is (~10 fold) less than that of lipoprotein TAG. Therefore, if only 10% of the TAG is hydrolyzed during its passage through the heart vasculature, this would create an equivalent amount of fatty acids. Cardiac muscle is the tissue with the greatest expression of LPL **(Enerback and Gimble, 1993)** and it is likely, especially in the postprandial period, that a large amount of dietary TAG is converted to fatty acid (FA) within the heart.

The heart is not generally viewed as a "major player" in the regulation of plasma lipoprotein levels. Mice without

cardiac LPL survive **(Merkel *et al.*, 1998)**. Although their heart function has not been examined in detail. In contrast, heart only LPL-expressing mice maintained normal levels of plasma TAG and HDL despite the lack of skeletal muscle and adipose LPL and a reduced amount of post heparin LPL (**Levak-Franks *et al.*, 1999)**. An additional role of LPL in the heart may be to provide it with nonhydrolyzable core lipids. Studies several decades ago by **Goodman *et al.*, (1983)** showed that, peripheral tissues, including the heart, internalize core lipids from CMS, not all core lipids or remnants are destined to end their circulatory life in the liver. Uptake of retinyl esters, correlates with LPL over expression in the heart and skeletal muscles **(Van Bennekum *et al.*, 1999)**. Using labeled chylomicrons and emulsions, **(Hultin *et al.*, 1995)** increased the uptake of core lipids by peripheral tissues. A similar observation was recently made by **(Qi *et al.*, 2002)** who noted that, uptake of cholesteryl esters was modified by lipid composition of emulsion and this explained by one options that capillaries become leaky and allow large TAG-rich lipoproteins to enter the subendothelial space. Fatty acids disrupt endothelial monolayers *in vitro* (**Hennig *et al.*, 1984**) and LPL-mediated hydrolysis of VLDL also makes arteries more permeable (**Rutledge *et al.*, 1997).** and this figure indicated action of LPL **(Blanchette-Mackeie *et al.*, 1989)**.



A. Lipolysis during lipoprotein transit through the capillary

B. LPL action within the subendothelial space

This figure indicate that, LPL mediated tissue-uptake of lipids. LPL appears to mediate uptake of lipolyzed lipids (Fatty acids) and core lipids, such as triglycerides (TAGS), cholesterol esters and retinyl esters. Several pathways allow organ uptake of lipids. Fatty acid associated with albumin can cross the endothelial barrier: lipolysis of VLDL or chylomicions (CMS) release F. A. in addition, as is known to occur for transfer of lipids and apolipoprotein from TAG rich lipoprotein to HDL, surface lipid, apolipoproteins and some core lipids may dissociate from particle as a complex (lipolysis product). Although lipolysis of nascent TAG- rich lipoproteins probably requires initial hydrolysis within the circulation; lipolysis may continue within the subendothelial space either because the smaller lipoproteins are able to cross the capillary endothelial barrier or because the barrier (leaky lipolysis itself will cause capillary leakage. LPL, present on the surface of parenchymal cells such as adipocytes and myocytes, could interact with these particles.

Perhaps the pool of LPL that is present on the mycocyte surface **(Blanchette-Mackie *et al.*, 1989)**, as well as that on the endothelial lumen, participates in tissue lipid uptake. The *in vitro* observations that LPL will anchor lipoproteins to cell surfaces and then augment their uptake either via receptors or internalization of proteoglycans may be operative *in vivo*. The pathway can also be mediated by enzymatically inactive LPL, which, in the presence of active LPL, appears to capture lipoproteins and increase the efficiency of lipolysis in tissues without active LPL, the inactive LPL can still increase tissue cholesterol uptake **(Merkel *et al.*, 2002).**

LPL mediated uptake of lipid in excess of that required for tissue energetics may be harmful. Adipose tissue has been developed as a site of storage of fat derived calories, this occurs even in LPL deficiency. Several models of dilated cardiomycopathy are associated with excess lipid accumulation in the heart. Thus although FAS are preferred fuel for cardiac muscle, too much fat, excess oxidation of fatty acids or accumulation of other lipids may lead to dysfunction. The other potential complication of excess LPL mediated fat uptake may be insulin resistance, as reported by **Ferreira *et al.*, (2001) and Kim *et al.*, (2001)**. However, **Voshol *et al.*, (2001)** reported the reverse.

Transcription of LPL gene increases during adipocyte differentiation **(Semenkovich *et al.*, 1989)** and decreases in mature adipocytes after treatment with TNF-α **(Zechner *et al.*, 1988 and Morin *et al.*, 1995)** and agents that increases CAMP **(Raynolds *et al.*, 1990 and Antras *et al.*, 1991)**. Reduction in LPL gene expression in the liver following birth, may be conferred by ascilencer, identified in the -263 to -241 location of the chicken LPL promoter **(Zhang and Bensadoun, 1999).** PPAR-α agonists may be able to override, this silencing in adult animals **(Schoonjans *et al.*, 2000).** Transcription of the LPL gene in the rat heart increases about 10 folds in 10-20 day rate pups **(Singh-Bist, *et al.*, 1994)**

4. LPL and Hepatic lipase:

Lipoprotein lipase (LPL) and hepatic lipase (HL) are distinct enzymes that hydrolyze lipoprotein tricyclglycerols. LPL is essential for normal chylomicron and very low density lipoprotein catabolism and transfer of cholesterol, phospholipids and apolipoproteins between lipoprotein particles **(Havel *et al.*, 1973 and Goldberg, *et al.*, 1988).** Hepatic lipase (HL) may also be important in lipoprotein and phospholipid metabolism **(Goldberg *et al.*, 1982, and Landin *et al.*, 1984)** and through its effects on high density lipoproteins, may mediate the delivery of cholesterol from

peripheral tissues to the liver (**Jansen and Hulsmann, 1980, Bambergev *et al.*, 1983, and Bamberger *et al.*, 1985**). LPL regulation is complex and show tissue specificity e.g. heart and adipose tissue respond differently to the same physiologic and hormonal signals **(Cryer *et al.*, 1981 and Garfinkel and Schotz, 1987).**

Lipoprotein lipase (LPL) belongs to mammalian lipase family that includes pancreatic lipase (PL), hepatic lipase (HL), gastric lipase, and endothelial lipase **(Persson, *et al.*, 1989 and Rader and Jaye, 2000).** The primary function of LPL is triglyceride hydrolysis in triglyceride rich lipoproteins, such as chylomicron and very low density lipoprotein (VLDL) particles, which are converted to remnants **(Santamarina-Fojo *et al.*, 1994)**. LPL is secreted from a variety of tissue such as adipocyte, macrophage, and muscle cells, and is bound to capillary bed of endothelium. Via cellular surface heparin sulfate proteoglycans (HSPG), a function. reflected in LPL'S strong affinity for heparin **(Gerdes *et al.*, 1997)** and LPL deficiencies in humans are manifested as sever hypertriglycerdemia. **(NordesTAGaard *et al.*, 1997)** and also arteriosclerosis **(Benlian *et al.*, 1996).** Genetically engineered mice lacking LPL also showed as hyper triglyceridemia **(Olivecrona *et al.*, 1993)**. LPL functions as aligand for

lipoprotein receptors, such as low density lipoprotein (LDL) receptor, LDL receptors, related protein (LRP) and VLDL receptor **(Beisiegel *et al.*, 1991, Argraves *et al.*, 1995, Strickland *et al.*, 1995 and Takahashi *et al.*, 1995).**

A model structure of LPL based on the crystal structure of human pancreatic lipase (PL) as template was recorded by **Van Tilbeurgh *et al.*, (1994)**. The model structure exhibited two domains a large N-terminal domain (1-312 amino acid residues) and small C-terminal domain (313-448 residues). The sequences of PL and LPL are identical at 31% of their residues in the N-terminal domain (40% similarity) and are 28% identical in the C-terminal domain (38% similarity). The catalytic efficiency and heparin-binding functions of the N-terminal domain extensively studied by **Emmerich *et al.*, (1992) and Hata *et al.*, (1993).** Achimeric enzyme with the N-terminal domain of LPL and the C-terminal domain of HL (LPL/HL) exhibited the characteristic catalytic activity of LPL, as well as other LPL-specific functions, such as activation by APOC-II and inhibition by NaCl (**Davis *et al.*, 1992).** Horse PL **(Bourne *et al.*, 1994),** human PL **(Winkler, *et al.*, 1990)**, and complexes of human PL and procolipase **(Van Tibeurgh *et al.*, 1993 and Vant Tilbeurgh *et al.*, 1992)** have been crystallized. These studies demonstrated that the active site in the N-terminal domain has two conformation an active open

and inactive closed conformation **(Van Tilbeurgh *et al.*, 1993).** A surface loop functions as alid and governs the interaction of the lipid substrate with the enzyme's catalytic site **(Dugi *et al.*, 1992).**

On the protein surface at a site opposite to the lid, a cluster of basic amino acid (Arg279, Lys280, Arg 282) that constitutes a high affinity heparin binding site is present by **Hata *et al.*, (1993).** The function of the C-terminal domain has also been addressed with achimeric enzyme (LPL/HL), which exhibits an affinity for heparin similar to that of native LPL **(Hill *et al.*, 1998),** suggesting that the major heparin-binding site occurs in LPL's N-terminal domain. Recently, however several lines of evidence have demonstrated that, the C-terminal domain is also important in heparin binding. **(Yoko Kobayash *et al.*, 2002)**. In which the Arg 405, Arg 407 and Lys409 residues of avian LPL, which correspond to the Lys403, Arg 405 and Lys407 residues, respectively, in the C-terminal domain of human LPL, have been demonstrated to be responsible for heparin binding **(Sendak and Bensadam, 1998)**. In another study with transgenic mice expressing a human LPL with residues substituted for basic amino acids at positions 403, 405 and 407 and the mutant LPL has normal

enzyme activity but with a reduced affinity for heparin **(Lutz *et al.*, 2001).**

LPL controls TAG partitioning between adispose tissues and muscles thereby increasing fattening or providing energy in the form of fatty acids for muscle growth **(Hocquette *et al.*, 1998)**. In sheep fed maintenance ration, it was found that about 55-60% of the total amount of free fatty acids originate from hydrolysis of circulating TAG by LPL and the skeletal muscle mass and the heart together could utilize approximately 40% of the non-estrified fatty acids **(Pethick and Dunshea, 1993).** For this reason it seems to us very important to study LPL activity not only in adipose tissues but also in muscles and heart to get a better knowledge of the control of TAG partitioning between these tissues in meat producing cattle **(Hocquette *et al.*, 1998).** Few studies concerning LPL in muscles of ruminant species, despite numerous studies in adipose tissue **(Bonnet *et al.*, 1998)** or milk **(Olivecrona *et al.*, 1992).** However, the presence of LPL activity which is (8-17) fold lower in beef heart compared to that in rat heart **(Cryer and Jones, 1979 and LaDu *et al.*, 1991)**. In the rat and bovine LPL activity is expected to be lower in skeletal muscle than in heart **(LaDu *et al.*, 1991).**

LPL mRNA level were higher in growing calves than in foetuses as recorded in rat **(Kirchgessner *et al.*, 1989)**, in mouse and human **(Kirchgessner *et al.*, 1987)** and rat tissues **(Kirchgessner *et al.*, 1989 and LaDu *et al.*, 1991)** and levels of LPL transcripts were higher in bovine heart and oxidative skeletal muscles which use fatty acids as energy source than in muscles composed of fast twitch white fibers which used glucose as fuel **(LaDu *et al.*, 1991).** Because there are great differences in metabolism among adipose sites have been previously reported in pigs **(Anderson *et al.*, 1972)** and in calves (**Hocquette *et al.*, 1997)**, in cows, LPL activity may also differ among adipose, tissue from various anatomical sites **(Chilliard and Robelin, 1985)** and these differences are mainly related to differences in LPL mRNA levels among adipose site. The higher LPL activity are the higher adipocyte size as described in (**Hocquette *et al.*, 1998 and Eckel, 1987).** The level of LPL transcripts are positively related to LPL activity in bovine tissue including muscle & adispose tissue **(Hocquette *et al.*, 1998)**.

Dietary triglycerides (TAGS) are hydrolyzed to monoglyceride and two free fatty acids (FFAS) for absorption in the digestive tract. The absorbed FFAS are re-estrified to TAGS and incorporated in plasma lipoproteins. TAGS present

in plasma lipoproteins are hydroyzed by various kinds of endogenous lipase to provide FFAs for storage in adipose tissue or for oxidation in other tissues **(Oku *et al.*, 2006).**

Lipoprotein lipase (LPL), hepatic lipase (HL), pancreatic lipase (PL) and endothelial lipase (EL) belong to lipase gene family **(Hide *et al.*, 1992 and Wang and Schotz, 2002)**. These lipases share with high degrees of structural similarity with each other but play different roles in lipid metabolism.

LPL participates in the cellular uptake of plasma chylomicron and very low density lipoprotein in various kinds of extrahepatic tissues **(Nilsson-Ehle *et al.*, 1980 and Mead *et al.*, 2002).** HL is primary synthesized in the liver and involved in chylomicron-remnant and high density lipoprotein metabolism **(Santamarina-Fojo *et al.*, 1998)**. PL is a digestive enzyme which plays a central role in dietary triglycerides (TAGS) digestion. Endothelial lipase (EL) shows as a phospholipase A1-like activity and share in high density lipoprotein metabolism **(Hirata *et al.*, 1999 and Jaye *et al.*, 1999).** The activities of these lipases are regulated in part at a transcriptional level in response to physiological state of animals **(Semb and Olivecrona, 1989, Staels *et al.*, 1990, Jaye *et al.*, 1999, Wicker and Puigserver, 1990, Benhizia *et al.*, 1994, Bonnet *et al.*, 1999 and Ruge *et al.*, 2004).**

Studies performed on fish reported that rainbow lipoprotein lipase and salt-resistant lipase (HL) activity are high in ovaries and decrease in adipose tissue in the months of spawning **(Black and Skinner, 1987).** Also in rainbow, LPL gene is expressed in ovary during oogenesis but not in embryos **(Kwon *et al.*, 2001).** Furthermore, nutritional regulation of LPL has been studied in rainbow trout and found that, LPL activity in adispose tissue is increased during post prandial period and decreases with fasting, and the activity is regulated by insulin **(Albatat *et al.*, 2006).**

LPL is functional at the surface of endothelial cells, but it is not clear which cells synthesize the enzyme and what its distribution within tissues and vessels. The previous studies reported that, the major LPL producing tissues are muscles, adipose tissue and mammary gland as recorded by **Camps *et al.*, (1991).** The enzyme is synthesized by scattered cells such as macrophage in lung, spleen and kupffer cells in liver. In endothelial cells can not synthesize the enzyme, and this indicates that, the endothelial LPL originate in other cells. In the liver, strong immune reaction was detected in the sinusoid in contrast to low level of mRNA expression suggesting that liver takes up circulating LPL from blood **(Camps, *et al.*, 1991).**

A several studies have shown that the liver extracts LPL from the circulating blood, suggesting that the liver may be a major site of degradation for LPL. perfusion studies have shown that LPL bound in the liver can initially be released again by heparin **(Vilaro *et al.*, 1988).** It is known that the liver has a high-sulfate type of heparin sulfate **(Graham *et al.*, 1988 and Stow *et al.*, 1985)**, which should bind the enzyme tightly and according to this view, initial binding of LPL to heparin sensitive sites in the liver would be similar to the binding of the enzyme in other tissues **(Camps *et al.*, 1991)**.

Previous studies showed that, exogenous LPL bound by the liver is functional and can engage and hydrolyze lipoproteins **(Vilaro *et al.*, 1988).** However, LPL bound in the liver soon loses its catalytic activity and can no long be released by heparin, and is degraded. There are two possible sequences of events for this, one is that LPL is internalized bound to heparin sulfated proteoglycans and so inactivation would then be protected by lowered. pH in endosomes **(Bengtsson and Olivecrona, 1985)**. The other possible process is that the enzyme, firstly transfers to other types of binding sites which mediate the internalization. It has been reported that there are substantial amounts of inactive LPL protein in blood and it is possible that most of the transport of LPL from peripheral tissues to the liver occur after dissociation

of the enzyme into catalytically inactive monomers with decreased affinity for heparin-sulfate **(Kern *et al.*, 1990),** This process has been referred to as a built in the mechanisms for self destruction in the active, dimeric LPL molecule **(Osborne, *et al.*, 1985).**

LPL activity was high and it was detected in adispose tissue, heart and lung of guinea pig and liver and spleen presented about (8. 6 and 6. 9%) respectively of the epididymal adipose tissue activity. Lower activities were present in muscle and in kidney (1. 6% of epididymal adipose tissue activity), and LPL activity was inhibited by anti LPL-serum and highest inhibition were detected in adipose tissue, heart and lung while lower inhibition was detected. In other tissue and this leading to suggesting the presence of other lipolytic activities **(Camps *et al.*, 1991).**

5. LPL during infection and inflammation:

As a response to infection or cancer, dramatic changes in lipid and energy metabolism are seen. Most of which are hypertriglyceridemia in combination with increased VLDL production and increased adipose tissue lipolysis and weight loss (cachexia) in theses situations, adipose tissue LPL decreases as a result of cytokine action **(Tracey and Cerami, 1992 and Hardardettir, *et al.*, 1994).** While liver LPL

expression can be induced in adult animal by cytokines. After a single dose of TNF (tumor necrosis factor) LPL mRNA and LPL activity was found in livers of several rodent species **(Chajek-Shaul *et al.*, 1989; Enerback *et al.*, 1988 and Chajck-Shaul *et al.*, 1989).** In addition, a markedly increased liver LPL activity has been shown in mice after tumor implantation **(Masuno *et al.*, 1984).**

Triacylglycerols constitute a major component of circulating lipoproteins particles. While the role of triacylglycerol in the pathogenesis of atheroscerlosis is uncertain, the recent epidemiologic evidence suggests that elevated triacyl glycerol may mediated myocardial infarction risk through effects on fibrinolytic activity **(Aberg *et al.*, 1985, and Hamsten *et al.*, 1985).**

There is a relationship between kidney disease and derangement of lipoprotein metabolism **(Crook *et al.*, 2003),** in which there is evidence that hyperlipidema accelerates the progression of glomerulo-sclerosis **(Kamanna, 2002).** There is evidence that the kidney disease causes disturbances of lipoprotein metabolism **(Crook *et al.*, 2003).** They stated that, lipoproteins bind to glomerular mesangial cells and induce proliferation and cytokine expression and dysregulation of these processes is part of pathogenesis of chronic renal

disease. **Stevenson *et al.*, (2001)** found that, LPL enhanced the binding of very low density lipoproteins (VLDL) to mesangial cells by as much as 200 folds amplified VLDL-driven mesangial cell proliferation and increased VLDL-induced platelet-derived growth factor (PDGF) expression.

The implication in that is the presence of increased amount of LPL in this location might contribute to disease initiation and/or progression. There are many reports that, LPL activity is suppressed in chronic renal disease in human **(Marsh, 2002),** but the mechanisms for this not fully understood and these patients often had increased VLDL triglycerides and decreased levels of HDL cholesterol and it has been suggested that the decreased LPL activity contributes to the dyslipidemia. Experimental chronic renal failure in animals is associated with decreased LPL expression in several tissues **(Sato *et al.*, 2002),** but it is not known whether decreased production of LPL in the kidney itself It is an important factor for the overall decrease in the body LPL. In view of strong connection between LPL and the kidney disease, it is suprising that there are no direct studies on LPL in kidney. This may be, at least in part, because it has been reported that LPL activity is low in the kidney of rats **(Kirchgessner *et al.*, 1989 and Semenkovich *et al.*, 1989),** and in guinea pigs **(Camps *et al.*,1990)**

6. Effect of age:

Hepatic lipase (HL) mRNA can be detected in liver only but not in other tissues including adrenal and ovary and detected by Northern blot gel analysis and was presented as a single 1. 87 kb and a single 4. 0 kb LPL mRNA species was detected in epididymal fat, heart, psoas muscle, lactating mammary gland, adrenal, lung, ovary, but not in adult kidney, liver, intestine and brain. Quantitative blot hybridization analysis demonstrated the following relative amounts of LPL mRNA in rat tissues: adipose tissue (100%), heart (94%), adrenal (6. 6%), muscle (3. 8%), lung (3. 0%), kidney (0%) and adult liver (0%).

The same quantitative analysis was used to study lipase mRNA levels during development. There was little postnatal variation in LPL mRNA in adipose tissue, maximal levels were detected at the earliest time points studied for both inguinal and epideidymal fat and heart, however LPL mRNA was detected at low 6 days before birth and increased 278 folds as the animals grow to adulthood. Levels of LPL mRNA in lung, psoas muscle, adrenal gland and HL mRNA in liver showed the same biphasic pattern during development: a 2. 4 to 11. 3

fold increase around the time of birth followed by a 2. 3 to 19. 9 fold increase at weaning thus developmental regulation of the genes for two different lipases, HL (in liver) and LPL (in several tissues) may be similar **(Semenkovich *et al.*, 1989).**

7. Lipoprotein lipase activity:

Peter, (1993) reported that, phospholipids and apolipoprotein C-II (APOC-II) are required as cofactor for lipoprotein. Lipase activity in which APOC-II is contain on a specific phospholipid bind site. Through which it is attached to lipoproteins. Thus chylomicrons & VLDL provide the enzyme for their metabolism with both it's substrate and co factor. Hydrolysis occur while the lipoproteins are attached to the enzyme on the endothelium. Heart lipoprotein lipase has low km value for triacyl glycerol whereas the km value of the enzyme in the adipose tissue is greater 10 time as the concentration of plasma triacylglycerol decrease in transition from the fed to starved condition. So the heart enzyme remain saturated with substrate but saturation of the enzyme in the adipose tissue is diminishes. Thus redirecting uptake from adipose tissue to heart and a similar redirection occur during the lactation in which the activity of (LPL) in a dipose tissue is diminishes and increase in mammary gland activity and this

allowing uptake of lipoprotein triacyl glycerol long chain fatty acid for milk fat synthesis.

Lithell *et al.*, (1977) reported that, there is no difference in LPL activity between fresh adipose tissue & adipose tissue that has been frozen and stored at -70°C.

Hirsch *et al.*, (1968) LPL activity was expressed either per (g) or per 10^7 fat cell, the number of fat cells was obtained by dividing the total lipid weight of the sample by the mean cellular lipid weight; enzyme activity is determined by one n mol of fatty acid released per minute was equal to 1 mu. enzyme activity.

Ong *et al.*, (1994) LPL mRNA were quantiated by both Northern blotting and reverse transcriptase polymerase chain reaction (RTPCR).

Eckel, (1987) recorded that there are many of studies have demonstrated that (LPL) activity is increase in a dispose tissue with hypothyroidism in rat.

Saffari *et al.*, (1992) recorded that thyroid hormone decrease translation of LPL and so decrease it's activity.

Arner *et al.*, (1991) reported that there was no significant relationship between mRNA and (LPL) activity whether LPL

activity was expressed per cell or per g lipid weight and was (r = 0. 0-0. 5).

Rebuffe-Scrive *et al.*, (1987) found that there is no difference in lipoprotein lipase activity (subcutaneous in femoral and gluteal tissue in none obese men.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Animals and samples:

Tissue samples (spleen, liver, kidney, adrenal gland, heart, peri-renal adipose tissue and testis) were collected from 3 male Friesian cows immediately after slaughtering in Desouk abattoir. All animals were apparently healthy. Their ages ranged from 12 to 18 months and their body weights ranged from 150 to 200 kg.

Samples were collected under sterile condition in autoclaved eppendorff tubes and rapidly immersed in liquid nitrogen until RNA extraction and PCR analysis.

2. Preparation and composition of different buffers and solutions:

2.1. Preparation of 2% Agarose gel:

Agarose	2 gm
Ethidium bromide	10 100ml
50X TAE buffer	1ml/50ml
Deionized water	100 ml

Two gram of agarose were dissolved by boiling in 100 ml bidistilled water. Then 10 of ethidium bromide and 1 ml

50X TAE buffer were added and mixed well. The mixture was poured in electrophoresis plate and left until solidified.

2.2. 50X TAE buffer (stock solution):

Tris base	242 gm
Acetic acid	57.1 ml
0.5M EDTA (PH 8.0)	100 ml

In 600 mls of bi distilled water, 242 g of tris base were dissolved. Then 100 ml of 0.5M EDTA pH 8.0 and 57.1 ml of acetic acid were added and the mixture completed to one liter with bidistilled water.

Working solution (1X TAE):

Twenty mls of stock solution were added to 980 mls bi distilled water and mixed well.

2.3. EDTA 0.5M PH 8.0:

In 300 mls of bi distilled water, 93.06g EDTA were completely dissolved using few pellets of NaOH. The pH was adjusted to 8.0 using pH meter and the mixture completed to 500 ml with bidisitilled water.

2.4. Running buffer (1X TAE):

Ten mls of 50X TAE buffer were completely mixed with 500 mls bi distilled water.

2.5. Loading buffer:

The loading buffer was composed as shown in the following table.

Glycerol	5 ml
Bromophenol Blue (BPB)	0.0416 g
Xylen cyanol (XC)	0.0416 g
Distilled water	10 ml

Bromophenol blue (0.0416 g) and Xylen cyanol (0.0416 g) were dissolved in 5 mls glycerol and completed to 10 ml with bi distilled water.

2.6. RNase-free water:

Equal volumes of Diethyl pyrocarbonate (DEPC) 0.01% v/v and bi distilled water were mixed and left at room temperature for 24 hours then autoclaved.

3. RNA extraction:

RNA extraction was performed using biozole kit (Bioflux co.) according to **Chomcryunski and Sachi (1987)**.

Tissue samples about 25 mg. were transferred from liquid nitrogen to eppendorff tube containing 500 l o f biozol reagent and homogenized completely using electric homogenizer. The

homogenates were incubated at -20°C for 15 minutes to allow complete lysis of tissue and the homogenate became clear. Then 100
sample at a ratio of 1: 5 Biozol, mixture was vigorously shaked and then incubated for 15 minutes at room temperature.

The mixture was centrifuged at 12,000 rpm for 15 minutes at 4°C. The mixture separates into a lower phase, an inter phase and upper aqueous phase. The supernatant was transferred to a new autoclaved eppendorff tube.

Equal volume of cold isopropyl alcohol and the supernatant were mixed well and incubated at -20°C for 20 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes.

After centrifugation the supernatant was discarded and the formed pellets washed with cold 75% ethanol (equall Biozol) then centrifuged at 12,000 rpm for another 5 minutes.

The supernatant was discarded and RNA pellet was air dried in order to prevent complete dryness of RNA pellet for avoiding RNA degradation.

The RNA pellets were dissolved with 50
free water. The RNA quantity and quality were estimated

spectrophotometrically measuring at wave length 260 and 280 nm.

4. RT-PCR:

All equipments and glasses were autoclaved to avoid RNase contamination. One microgram (1 µg) RNA was used for RT-PCR as follows: one microgram (1g) extracted RNA from different tissues under study was mixed with 0.5 final volume to of 4.5 The mixture denaturated at 72°C for 10 minutes. In PCR machine (HYBAID) reverse transcriptase was made by using 4.5 5.5 the mixture to reach the final volume of 10 mixture was formed from 2 5X buffer, 2 nucleotide triphosphate (dNTPs), 1 50 pmol, Gibco company) and 0.5 (RTase) (100 unit,Gibco company).

The samples were incubated at 20 °C for 10 minutes and in 37°C for 1 hour and then heated at 94°C for 5 minutes for inactivation of enzyme. The temperature of the apparatus was declined to 4°C for obtaining cDNA.

4. PCR reaction:

PCR reaction was conducted on RT PCR sample in PCR machine (HYBAID), for a final volume of 25

- 25
- cDNA (template) 1
- 10x PCR buffer 2.5
- dNTPs 2
- MgCl 2
- forward primer 0.1
- reverse primer 0.1
- Taq. polymerase 0.125
- DEPC 17.175

Taq. polymerase (perkin-Elmer, Fostercity CA. USA) forward primer of LPL 5′ GATGATG-3′ is 5′ GGTAAATGTCCAC-3′ 25 µL.

PCR was conducted for 40 cycles for LPL expression each cycle consisted of denaturation at 94°C for 4 minutes and 94°C for 30 seconds and annealing at 58°C for 1 hour and extension at 72°C for 1 hour and 72°C for 7 minutes, then cooled till 4°C.

For bovine glyceraldehyde-3-phosphate dehydrogenase (G3PDH), PCR condition was made as follows: the annealing temperature was 59 °C for one hour the forward primer: 5'-ACCACTGTCCACGCCATCAC-3' and the reverse primer: 5'-TCCACCACCCTGTTTGCTGTA-3' (Sigma). The reaction was carried out for 35 cycles. After electrophoresis in 2% agarose gel, the PCR products were stained with ethidium bromide and visaualized under UV lamb. Intensities of RT-PCR bands were measured densitometrically and compared to that of internal standard (G3PDH) using NIH Image program.

RESULTS

RESULTS

Tissue distribution of bovine lipoprotein lipase (LPL) mRNA

For determination of mRNA expression of bovine LPL , RT-PCR analysis was performed for various tissues of Friesian cows.

Table (1) and Figure (2): showed that, the highest LPL mRNA expression was encountered in Testis (0.71), whereas in liver, heart, adipose tissue, adrenal gland, and kidney were 0.66, 0.58, 0.45, 0.40, and 0.39 respectively. The lowest LPL mRNA expression was present in spleen (0.26).

Table (2) and Figure (4) showed that, the highest LPL mRNA expression was found in Testis (0.98), whereas in heart, adrenal gland, liver, spleen, and kidney were 0.78, 0.74, 0.73, 0.69, and 0.61 respectively. The lowest LPL in mRNA was detected in adipose tissue (0.54).

Table(3) and Figure (6) showed that, the highest LPL mRNA expression was present in Testis (1.42) whereas in heart, kidney, spleen, adrenal gland, and adipose tissue were 1.41,

1.27, 1.14, 1.04, and 0.93 respectively. The lowest LPL mRNA expression was encountered in liver (0.91).

Figure (7) showed that, the highest LPL mRNA expression was present in testis (1.03) whereas in heart, liver, kidney, adrenal gland, spleen were 0.92, 0.77, 0.76, 0.73 and 0.70, respectively. The lowest expression was found in adipose tissue (0.64).

Cow 1

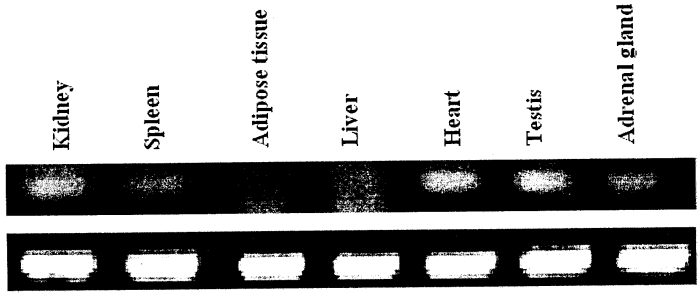


Fig. 1: Electrophoresis of PCR sample of Cow 1.

LPL mRNA expression in bovine tissues, total RNA was extracted from bovine tissues, 1 µg RNA was reverse transcribed, amplified by PCR and separated by 2% agarose gel with ethidium bromide.

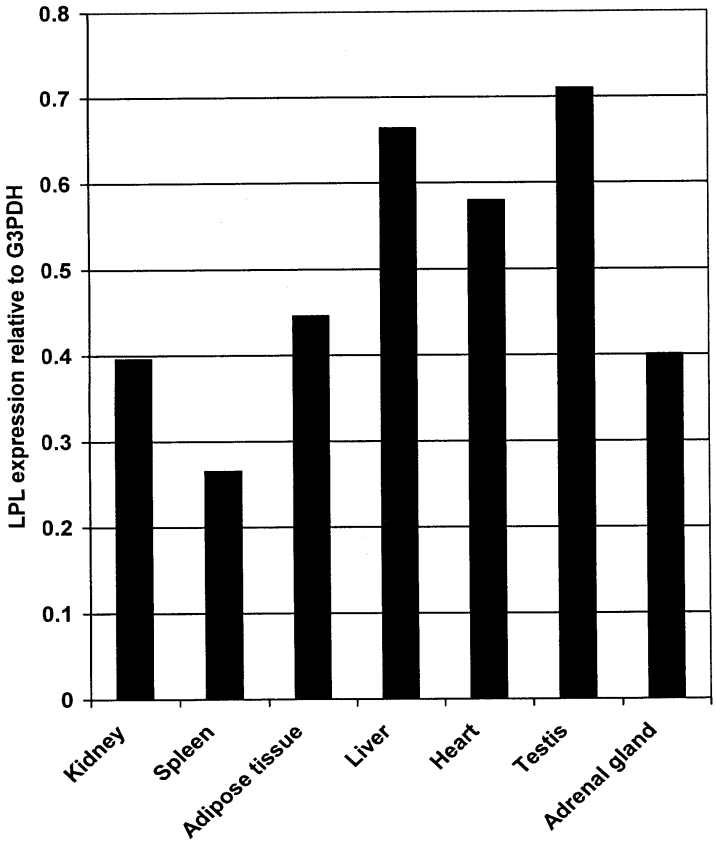


Fig. 2: Tissue distribution of bovine LPL in cow 1.

Table (1): Densiotometric analysis (Band intensity)

Cow I

Criteria / Organ	LPL	G3PDH	LPL/G3
Kidney	2331	5891	0.395688
Spleen	1620	6111	0.265096
Adipose tissue	2543	5702	0.445984
Liver	3622	5457	0.663735
Heart	3305	5698	0.580028
Testis	4305	6061	0.710279
Adrenal gland	2333	5826	0.400446

Cow 2

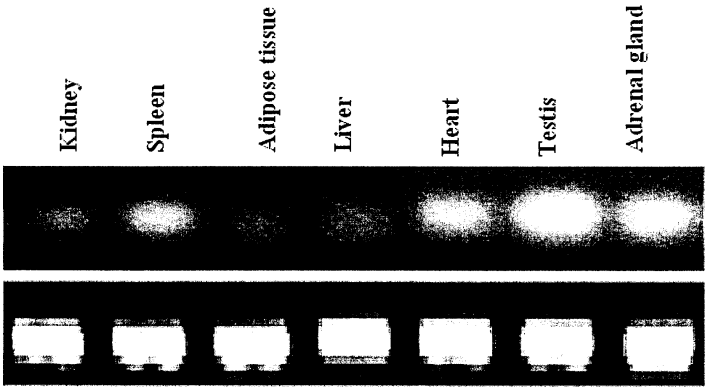


Fig. 3: Electrophoresis of PCR sample of Cow 2.

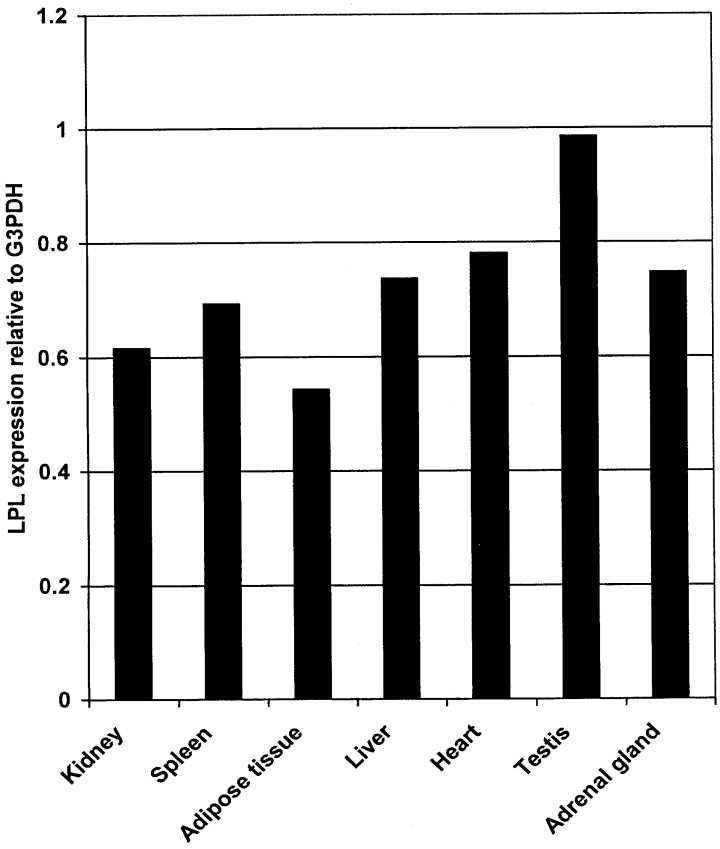


Fig. 4: Tissue distribution of bovine LPL in cow 2.

Table (2): Densiotometric analysis (Band intensity)

Cow 2

Criteria / Organ	LPL	G3PDH	LPL/G3
Kidney	2427	3944	0.615365
Spleen	3031	4378	0.692325
Adipose tissue	2650	4879	0.543144
Liver	2929	3979	0.736115
Heart	3729	4775	0.780942
Testis	4935	5007	0.98562
Adrenal gland	3729	4995	0.746547

Cow 3

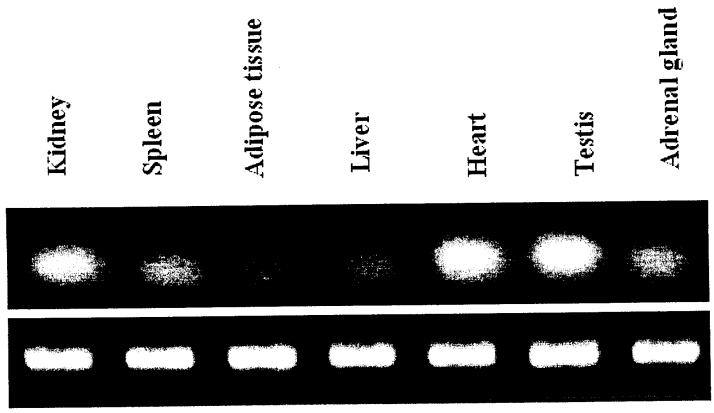


Fig. 5: Electrophoresis of PCR sample of Cow 3.

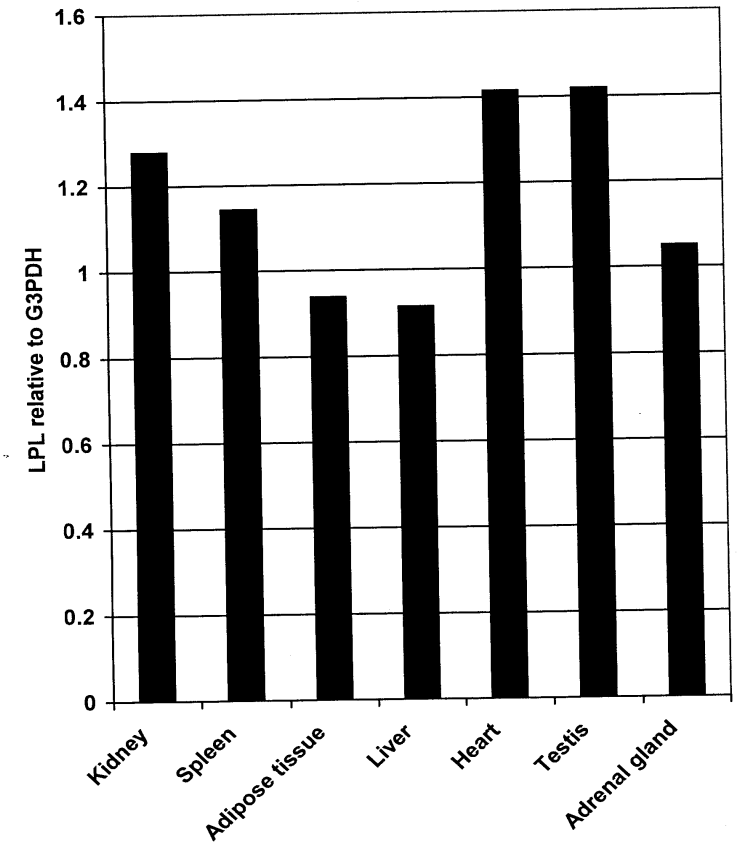


Fig. 6: Tissue distribution of bovine LPL in cow 3.

Table (3): Densiotometric analysis (Band intensity)

Cow III

Criteria / Organ	LPL	G3PDH	LPL/G3
Kidney	**5900**	**4613**	**1.278994**
Spleen	**5417**	**4734**	**1.144275**
Adipose tissue	**4425**	**4715**	**0.938494**
Liver	**4424**	**4835**	**0.914995**
Heart	**6915**	**4884**	**1.415848**
Testis	**6878**	**4846**	**1.419315**
Adrenal gland	**4789**	**4561**	**1.049989**

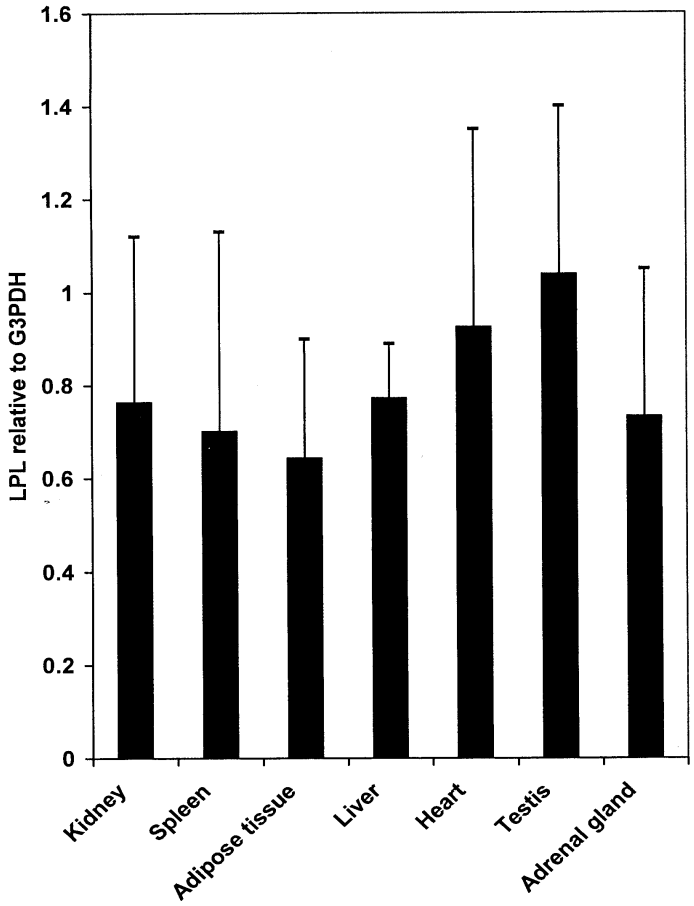


Fig. (7): PCR analysis of LPL mRNA in various bovine tissues.

Table (4): Lipoprotein lipase expression in different cows.

Criteria / Organ	Cow 1	Cow 2	Cow 3	Mean	Sd
Kidney	0.395688	0.615365	1.278994	0.763349	0.459871
Spleen	0.265096	0.692325	1.144275	0.700565	0.439648
Adipose tissue	0.445984	0.543144	0.938494	0.642541	0.260867
Liver	0.663735	0.736115	0.914995	0.771615	0.129337
Heart	0.580028	0.780942	1.415848	0.925606	0.436285
Testis	0.710279	0.98562	1.419315	1.038405	0.357453
Adrenal gland	0.400446	0.746547	1.049989	0.732327	0.325005

DISCUSSION

DISCUSSION

Lipoprotein lipase (LPL) is an essential enzyme for lipid metabolism. In addition to it's role in regulating plasma lipoprotein, LPL provides free fatty acid (FFA) substrate for the further metabolic reactions. In adipose tissue (LPL) hydrolyses circulating triacyl glycerol into FFA which are resterified for subsequent lipid storage **(Eckel. 1987).** While in muscle tissue LPL deliver FFA which is catabolized in muscle tissue to provide energy **(Borensztajn.1987)**.

Much evidence point to relationship between kidney disease and dearrangement of lipoprotein metabolism **(Crook *et al.*, 2003).** This relation seems to go both ways. There is evidence that hyperlipidemia accelerates the progression of glomerulosclerosis **(Kamanna, 2002),** and there is evidence that kidney disease causes disturbance of lipoprotein metabolism. In both of this case the enzyme LPL has been discussed as an important factor.

There are many reports that LPL activity is suppressed in chronic kidney disease in human. Although of this relationship between kidney and LPL. It is surprising that there are no direct

studies on LPL in kidney. It has been reported that, LPL activity is low in kidney of rat **(Kirchgessnar *et al.*, 1989),** and in guinea pig **(Camps *et al.*, 1990)**. On the other hand there are reports of substantial amounts of LPL mRNA in mouse kidney **(Kirchgessenr *et al.*, 1989)**, and mink kidney **(Lindberg *et al.*, 1998)**. This suggested that there are large differences in the expression of LPL in kidney between animal species. LPL activity in kidney response to nutritional state where there is marked reduction about 50% on fasting and the activity return to fed level within few hours after refeeding, this response was also in adipose tissue **(Ruge *et al.*, 2004)**. Moreover, LPL mRNA were rarely detectable in kidney using Northern blot analysis **(Hocquette *et al.*, 1998)**.

In the present study, our results revealed that there was detectable expression of LPL mRNA. The discrepancy between our results and the results reported by **Hocquette *et al.*, (1998)** could be attributed to breed difference and/or feeding state at the time of sampling.

There is substantial hydrolysis of chylomicrons (CMS) triacyl glycerol on perfusion through rat spleen indicating the role of LPL in lipoproteins metabolism **(Minham and Mayas. 1989)**. The previously mentioned findings agreed with our studies

which revealed that, LPL mRNA was expressed in spleen of bovine.

However, **Hocquette *et al.*, (1998)** reported that, the expression of LPL mRNA exhibited low levels in spleen of Montbliarde male calves. Moreover, **Camps *et al.*, (1991)** studied the immunohistochemical profile of liver and spleen in rat for tracing the distribution of LPL in the different areas of these organs. They found that, LPL mRNA was expressed in white and red pulp of spleen and in the periportal areas of liver.

Vilaro *et al.*, (1988) attributed the presence of LPL in liver extracts to circulating blood, they also added that the main site of LPL degradation is the liver. This finding came in accordance to the studies of **Camps *et al.*, (1991)** who showed that there was strong immunoreactions over the lumen of hepatic artery and portal vein whereas the reaction was weak over hepatic vein.

Although some of these cells showed LPL activity, they did not showed LPL mRNA expression.

Hocquette *et al.*, (1998) reported that, LPL mRNA was rarely detectable in liver of Montbeliard male calves by using

Northern blot analysis. Our results demonstrated detectable expression of LPL mRNA in bovine liver. These results are in accordance with those reported by **Camps *et al.*, (1991)** who reported that, by using immunohistochemistry, the greatest LPL activity was encountered in periportal area more than other areas in the liver. They attributed their findings to the expression of LPL mRNA in some areas of the liver. They added that, the presence of LPL activity might be extracted from the blood. This fact indicates that LPL mRNA depend on the area of sample as well as the quantity depend on feeding state.

Efficient system for delivery of lipid (fatty acids, phospholipids and cholesterol) to tissues are essential in mammal's reproduction. Endothelial lipase (EL) is a discovered member of triglyceride lipase family are all capable of releasing free fatty acids (FFA) from TG and/or phospholipids for cellular uptake, LPL and EL can also convey cellular cholesterol uptake **(Eisenbperg, 1992, and Rumsey *et al.*, 1992).**

Our results concerning testis demonstrated high mRNA expression which could be play a major role for fatty acid production required for spermatogenesis. The high expression of LPL was also reported in rat by **Marie *et al.*, (2005)** who found that endothelial lipase and LPL were highly expressed in testis.

The previous finding agreed **Oku *et al.*, (2006)** who showed that, LPL1 and LPL2 were highly expressed in testis of red sea bream pagrus major.

Lipoprotein lipase (LPL) is synthesized within the fat cell and transported to its site of action on the capillary endothelium of adipose tissues. The activity of adipose tissue LPL can be regulated at multiple levels including transcription, translation, glycosylation and release from fat cells **(Eckel, 1989).** Gene expression may be of importance in this respect since there is a correlation between the content of mRNA for LPL in rat fat cells and protein synthesis rate for LPL in these cells **(Ong *et al.*, 1988).**

Lipoprotein lipase mRNA level were significantly higher in women than men in abdominal and gluteal subcutaneous adipose tissue **(Arner *et al.*, 1991)** and in both sexes the LPL mRNA levels were three fold higher in abdominal as compared to the gluteal site.

There are great differences in metabolism among adipose sites have been reported in pig **(Anderson *et al.*, 1972)** and in calves **(Hocquette *et al.*, 1998)**. In the cow LPL activity may also differ among adipose tissues in the various anatomical sites

(Chilliard and Robelin, 1985) these differences are mainly related to differences in LPL mRNA levels among adipose sites. The differences in LPL activity among a variety of bovine tissues are mainly reflected by differences in LPL mRNA levels **(Hocquette *et al.*, 1998)**. It is worthy note that LPL activity did not always parallel to mRNA levels among tissues in rats **(LaDu *et al.*, 1991)**.

The decrement in expression of LPL in adipose tissue observed in our present study compared to the findings cited by the other workers in this respect realized that expression is not correlated to the activity.

Our results concerning this point were in close agreement to the findings of **LaDu *et al.* (1991)** who reported that, LPL activity did not always parallel to mRNA level among tissue in rat. Further the low expression of LPL in adipose tissue encountered in our study may be accompanied with long half life of this enzyme.

Although in this study our result revealed that low expression of LPL in adipose tissue, this result may explained as follow. The level of expression is not correlated to activity, this is accordance to the finding of (**LaDu *et al.*, 1991**) who reported

that LPL activity did not always parallel to mRNA level among tissue in rat. Further the low expression of LPL in adipose tissue encountered in our study could be attributed to long half life of this enzyme. Moreover, **Rug *et al.*, (2004)** stated that feeding state affect the expression of mRNA.

Our present study demonstrated that expression of LPL was detectable in these results are in accordance with that of **Hocquette *et al.*, (1998)** who reported that, LPL mRNA was low in adrenal gland of bovine. We could concluded that, expression mRNA follows the needs of this gland for cholesterol and the role of LPL in triacyl glycerol degradation and production of free fatty acid for oxidation and energy production as well as acetyl COA for cholesterol synthesis.

SUMMARY

SUMMARY

Lipoprotein lipase (LPL) is the enzyme responsible for hydrolysis of triacyl glycerol of plasma lipoprotein to fatty acids that are available for tissues as energy sources especially in muscle or for storage in the form of triacylglycerol especially in adipose tissue. Since LPL controls triacylglycerol partitioning between adipose tissues and muscle, so the LPL is important enzyme for fattening of animal.

The present work was planned to using the polymerase chain reaction (PCR) for detection of LPL mRNA Expression in different bovine tissues.

1- Experiment

The experiment was carried on three male cow (Ox) their ages were ranged from 12 to 18 months, and their body weights were ranged from 150 to 200 Kg. All animals were apparently healthy.

Tissue samples from liver, spleen, kidney, adrenal gland, heart, perirenal adipose tissue, testis and were used in our experiment. Samples were obtained from Desouk abattoir, Kafr El-Sheikh Governorate and collected immediately after

slaughtering of animals. Samples were obtained under complete sterile conditions. The samples were placed in autoclaved Eppendorff tubes and rapidly immersed in liquid nitrogen until extraction of RNA and PCR analysis .

2- RNA extraction

RNA extraction was performed using Biozol kit for isolation of RNA.

3- RT-PCR

One microgram (1 g)of RNA extracted from tissue samples, and added to 0.5 oligdT and denaturated at 72 °C for one hour, then RT-PCR was conducted for one cycle consisted of 20 °C for 10 minutes, 37 °C for one hour and 94 °C for 5 minutes and finally reached to 4 °C .

4- PCR

Polymerase reaction (PCR) was conducted on RT-PCR sample for LPL, and used 1l of cDNA template in total volume reaction of 25 40 cycles for LPL expression each cycle consisted of denaturation at 94°C for 4 minutes and 94°C for 30 seconds and annealing at 58°C for 1 hour and extension at 72°C for 1 hour and 72°C for 7 minutes,

then cooled till 4°C. PCR for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) as standard expression in all tissues, PCR was conducted for 35 cycle each cycle composed from 94 °C for 30 sec., annealing at 59 °C for 1 hour, extension at 72 °C for 1 hour and 72 °C for 7 minutes, then cooled to 4 °C. Electrophoresis was made in 2% agarose gel. The PCR product was stained with ethidium bromide and visualized under UV lamb. Intensities of RT-PCR bands were analyzed densitometrically and compared to internal standard (G3PDH) using NIH image.

5- RESULT

This study revealed that, LPL mRNA was expressed in all tested tissues, and the highest expression was present in testis (1.03). The expression in heart, liver, kidney, adrenal gland, spleen were 0.92, 0.77, 0.76, 0.73, and 0.70 respectively and the lowest expression was in adipose tissue (0.64).

6- CONCLUSION

It could be concluded from the present study that, expression of LPL mRNA differs according to the type of tissue. The highest expression was in testis which may confirms the

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ARABIC SUMMARY

الملخص العربى

الليبوبروتين ليبيز هو الإنزيم المسئول عن تكسير الجلسريدات الثلاثية إلى الأحماض الدهنية والجليسرول. ونظرا للأهمية الكبرى للأحماض الدهنية كمصدر للطاقة فى الأنسجة وخصوصا العضلات وكذلك يمكن تخزينها فى هيئة جليسريدات ثلاثية خصوصا فى النسيج الدهنى. كما أنه (LPL) يتحكم فى تجزئة الجليسريدات الثلاثية بين النسيج الدهنى والعضلات لذلك تكمن أهمية هذا الإنزيم فى تسمين الحيوانات.

تهدف هذه الدراسة إلى استخدام تفاعل البلمرة المتسلسل لتحديد الحمض النووى الرسولى لإنزيم الليبوبروتين ليبيز. لذلك استخدم ثلاثة من ذكور الأبقار والتى يبلغ عمر كل منها (١٢-١٨ شهر) وتتراوح أوزانها ما بين ١٥٠ ، ٢٠٠ كيلو جرام تبدو عليها علامات الصحة.

العينات:

تم أخذ العينات من الكبد والقلب والطحال والكلى والخصية والنسيج الدهنى حول الكلى والغدة الكظرية. تم تجميع العينات بعد الذبح مباشرة من مجزر دسوق بمحافظة كفرالشيخ باستخدام أدوات معقمة ووضعت فى النيتروجين السائل مباشرة لحين تحديد الحمض النووى الرسولى للإنزيم وعمل تفاعل البلمرة المتسلسل.

١- استخلاص الحمض النووى الرسولى:

تم استخلاص الحمض النووى الرسولى (mRNA) باستخدام محلول البيزول (Biozol) لاستخلاص الحمض النووى الرسولى (mRNA).

٢- تفاعل البلمرة المتسلسل (Reverse Transcription-PCR):

واحد ميكروجرام من الحمض النووى الرسولى المستخلص من الأنسجة المختلفة ، ٠,٥ ميكرولتر من oligodT وتم تغيير طبيعتهم عند ٧٢°م لمدة ساعة. ثم تم عمل تفاعل البلمرة المتسلسل دورة واحدة عند ٢٠°م لمدة عشر دقائق ، ٣٧°م لمدة ساعة ، ٩٤°م لمدة خمسة دقائق إلى أن تصل درجة حرارة الجهاز إلى ٤°م.

٣- **تفاعل البلمرة المتسلسل:**

تم عمل تفاعل البلمرة المتسلسل على عينات RT-PCR حيث تم استخدام واحد ميكرولتر من الحمض النووى الدى أوكسى ريبوز المكمل حيث تم تشغيل تفاعل البلمرة المتسلسل أربعين دورة لمعرفة التعبير الجينى لليبوبروتين ليبيز كل دورة كانت درجة الحرارة ٩٤°م لمدة ثلاثون ثانية ودرجة الاتحاد ٥٨°م لمدة دقيقة ، ودرجة التمدد ٧٢°م لمدة دقيقة ، ٧٢°م لمدة سبع دقائق ثم درجة التبريد عند ٤°م.

٤- تم عمل تفاعل البلمرة للجليسرالدهيد-٣-فوسفات ديهيدروجينيز الجينى فى كل الأنسجة وتم عمل ٣٥ دورة كل دورة كانت تتكون من ٩٤°م لمدة ثلاثون ثانية ، ودرجة الاتحاد ٥٩°م لمدة دقيقة ودرجة التمدد ٧٢°م لمدة دقيقة ، ٧٢°م لمدة سبع دقائق وبردت إلى ٤°م. تم إجراء الهجرة الكهربائية للعينات فى ٢% أجروز جيل. تم صبغة عينات البلمرة المتسلسل فى كلتا الحالتين باستخدام أيثيديم بروميد وفحصت تحت لمبة الأشعة فوق البنفجسية. تم إجراء تحليل الحزمة الخاصة بـ RT-PCR تبعا لكثافتها ثم فحصها وقورنت بالثابت الداخلى (جليسرالدهيد ـ٣ـ فوسفات دى هيدروجينيز) باستخدام NIH image program.

النتائج:

أوضحت هذه الدراسة أن الحمض النووى الرسولى للتعبير الجينى لليبوبروتين ليبيز يوجد فى أنسجة أبقار الفريزيان المختلفة (الكبد والطحال والكلى والقلب والخصية والنسيج الدهنى حول الكلى والغدة الكظرية) وكان أعلى تعبير جينى فى الخصية (١,٠٣) يليها القلب والكبد والكلى والغدة الكظرية والطحال وكانت بنسبة (٠,٩٢ ، ٠,٧٧ ، ٠,٧٦ ، ٠,٧٣ ، ٠,٧٠) على التوالى. وكان أقل تعبير جينى فى النسيج الدهنى بنسبة (٠.٦٤).

الخلاصة:

مستوى التعبير الجينى للحمض النووى الرسولى لليبوبروتين ليبيز يختلف حسب نوع النسيج وكان أعلى تعبير جينى فى الخصية لاحتمال احتياج هذا الإنزيم فى عملية أيض الحيوانات المنوية.

جامعة كفرالشيخ
كلية الطب البيطري
قسم الفسيولوجيا والكيمياء الحيوية

قرار لجنة الحكم والمناقشة

قررت لجنة الحكم والمناقشة بجلستها المنعقدة في يوم الخميس الموافق ٢٠٠٧/٥/٣ م ترشيح السيد ط٠ب / طارق كمال محمود أبوزيد للحصول علي درجة الماجستير في العلوم الطبية البيطرية تخصص(الكيمياء الحيوية)
(تعبير الحمض النووى الرسولى لإنزيم الليبوبروتين ليبيز للانسجة المختلفة فى حيوانات المزرعة)

وتتكون لجنة الحكم والمناقشة من :- التوقيع

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تعبير الحمض النووى الرسولى لإنزيم الليبوبروتين ليبيز للأنسجة المختلفة فى حيوانات المزرعة

رسالة مقدمة من

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للحصول على
درجة الماجستير فى العلوم الطبية البيطرية
(الكيمياء الحيوية)

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