Original article:

THE ROLE OF LIVER IN LEPTIN METABOLISM IN EXPERIMENTAL NEPHROTIC SYNDROME

Dawoud Fakhry Habib[1*], Abdelgawad Ali Fahmi[2], Naglaa Mohamed Kholousy[1**], Ahmed Ibrahim Amin[2], Mostafa Shalaby[3], Mohamed Mahmoud Ahmed[1***], Asem Metwally Abo Shanab[1**]

[1] National Research Centre in Cairo
  * Medical Biochemistry; ** Immunogenetic; *** Internal medicine Departments
[2] Chemistry Department - Faculty of Science, Cairo University
[3] Faculty of Veterinary - Cairo University

* corresponding author: Dawood Fakhry Habib, e-mail:dfakhry45@hotmail.com

ABSTRACT

Leptin is a hormone influencing food intake, energy expenditure and body weight. It is produced by adipocytes, exerts its effects on brain, endocrine pancreas and other organs by activating trans-membrane receptors and is cleared from plasma mainly by the kidneys. Several studies have suggested that leptin's effects on metabolism are mediated by the liver. Our aim was to evaluate the role of the liver in the metabolism of leptin by comparing the serum leptin level in the portal vein with that in inferior vena cava and to study the relationship between leptin and lipoprotein levels in healthy and nephrotic rats. Experimental nephrotic syndrome was conducted in rats by intraperitoneal injection of the supernatant from the kidney suspension obtained by previous unilateral nephrectomy of the same rat and complete Freund's adjuvant. There was a highly significant rise in leptin and lipid profile levels in the nephrotic rats compared with the normal rats. A highly significant increase in leptin in the inferior vena cava was detected compared with the level in the portal veins of nephrotic rats, while insignificant difference was observed in normal rats. This work has stressed the role of liver in leptin and lipid metabolism in nephrotic rats.

Keywords: Leptin, nephrotic syndrome, liver, lipids, lipoproteins, apolipoproteins, rats

INTRODUCTION

Leptin is a hormone influencing food intake, energy expenditure and body weight. This hormone is produced by adipocytes, exerts its effects on brain, endocrine pancreas and other organs by activating trans-membrane receptors and is cleared from plasma mainly by the kidneys (Chabova et al., 1999). The kidney plays a key role in the systemic elimination of circulating leptin (Cumin et al., 1997). Huang et al. (2006) established a role for leptin in the acute regulation of liver triglyceride (TG) levels in vivo. Leptin-induced decreases in triglyceride secretion from the liver also suggest a role for leptin in determining the triglyceride content of very low-density lipoprotein (VLDL), a critical determinant of the plasma lipoprotein profile. The mechanisms responsible for decreased intrahepatic triglyceride stores and triglyceride secretion probably involve nutrient partitioning away from storage, because leptin increases the activity of fat oxidation pathways in liver. Several studies have suggested that leptin's effects on metabolism

are mediated by the liver. This possibility has been suggested based on the liver's integral role in lipid metabolism and its significant energetic demands of approximately 20 % of standard metabolic rate (SMR) in the rat (Porter and Brand, 1993). and leptin's ability to suppress stearoyl-CoA desaturase 1 (SCD-1) in liver (Cohen et al., 2002). And leptin's reduction of the oxidation system in liver mitochondria of ob/ob mice could either be a result of normalizing leptin levels or secondary to its ability to correct hepatic steatosis (Singh et al., 2009). Singh et al. (2009) suggested that the reduced substrate oxidation and marked increase in VLDL secretion by the liver seen after leptin treatment suggest that the hormone causes the liver to reduce its own metabolic activity and shunt lipids to other tissues where they are metabolized. If, as the data suggest the liver is a conduit for nutrients that are metabolized elsewhere, where then these exported hepatic lipid stores being oxidized. The results showed that leptin treatment decreases coupled respiration in both skeletal muscle and the heart, thus causing a decrease in the replication-competent retrovirus (RCR) of these tissues. Thus the mechanism by which leptin increases energy expenditure is not clear and could include effects on mitochondrial coupling as well as non-mitochondrial respiration, such as metabolic futile cycles in heart and muscle and/or effects in other tissues, such as white adipose tissue (Wang et al., 1999). Leptin mediates hepatic stellate cells (HSC) activation and liver fibrosis through indirect effects on Kupffer cells (KC); these effects are partly mediated by TGF-beta1 (Wang et al., 2009). The liver has an integral role in lipid metabolism and also has significant energetic demands; therefore, studies have suggested that leptin's effect on metabolism may be mediated by the liver. Leptin-deficient mice have significant abnormalities in macronutrient metabolism, which can be corrected by leptin administration. A recent study has shown that acute leptin infusion rapidly reverses hepatic steatosis and plasma dyslipidemia induced by a high sucrose diet in rats, and the preservation of hepatic leptin action after a high sucrose diet is associated with the maintenance of low adiposity and plasma leptin concentrations (Sharma et al., 2010; Huang et al., 2007).

Nephrotic syndrome remains a serious clinical condition characterized by marked proteinurea, hypoproteinemia and hypercholesterolemia, usually accompanied by the presence of oedema. It could be presumed that leptin plays an important role in the complex metabolic processes occurring in patients with nephrotic syndrome (Merta et al., 2003). In children with nephrotic kidney, urinary leptin excretion is increased but its level is unchanged in serum; serum leptin level correlated with lipid parameters (Wasilewska et al., 2005). Serum leptin levels were slightly increased in the active phase of the nephrotic syndrome (Ece et al., 2004). Nicola et al. (1996) demonstrated that the nephrotic kidney might participate in the de novo synthesis or interconversion of the lipoproteins were higher in the active phase of the nephrotic syndrome (Ece et al., 2004).

The nephrotic liver secretes more lipoproteins than the normal liver: It secreted twice as much VLDL and HDL2 and 30 % more LDL and HDL1 than the normal liver; this was accompanied by an increased secretion of both apolipoprotein A-I and apolipoprotein E (Vaziri and Liang, 2004). Apolipoprotein A-I is persistently found in LDL of nephrotic rats but not in LDL of the controls, in HDL1 of nephrotic rats apolipoprotein A-I replaces apolipoprotein E as the major constituent peptide and the relative content of apolipoprotein A-I in HDL2 of nephrotic rats is enhanced (Vaziri and Liang, 2004; Calandra et al., 1981; Gherardi and Calandra, 1980).

The aim of the present work was to evaluate the role of the liver in the metabolism of leptin by comparing the serum leptin level in the portal vein with that of the inferior vena cava and to study the relationship between leptin and lipoprotein levels in healthy and nephrotic rats.

MATERIALS AND METHODS

Experimental animals: Thirty male albino rats weighed 160-190 gm, from the Animal House of the National Research Centre, were used. Rats were fed on a stock diet composed of whole wheat meal, skimmed milk powder, fish flour, dry yeast, stabilized vitamin A, D and NDP energy of 12 % (Morcos, 1980). Diet was given in plenty to the rats throughout the experimentation period (for 3 months). Animals were provided with water ad-libitum. The animal experiments were done in accordance with the regulations of the Ethical Committee of the National Research Centre.

The rats were divided into two equal groups (each group consisted of 15 rats):

Group I: saline-treated group served as healthy controls.

Group II: had their kidneys made nephrotic by injecting them intraperitoneally with 0.2 cc low speed supernatant (3000-4000 rpm for 1-1.5 hours) of 1:2 blood free own kidney in saline obtained by previous unilateral nephrectomy with 0.3 Freund's complete adjuvant (is an antigen emulsified in mineral oil, used to stimulate production of tumour necrosis factor). Each rat was injected six whole injections one each 14 days) after the technique of Heymann et al. (1959).

Unilateral nephrectomy

General anaesthesia of rats was done through inhalation of Di-ethyl ether, the hair at the site of operation was clipped and shaved then washed by soap and water, and disinfected by alcohol 70 %, followed by Tincture iodine 4 %. A longtidunal incision in the skin and muscles was made, and by blunt forceps the wound was widened till appearance of the kidney. Strong ligation to the renal artery and renal vein was made separately followed by excision of kidney. After nephrectomy, coaptation of the wound occurred by lemberet's sutures. Intraperitoneal injections of operated rats had been done after complete recovery for about four weeks.

Biochemical analysis

Blood samples were obtained from the portal vein and inferior vena cava under ether anaesthesia. Blood was collected by using syringe into dry clean test tubes, then allowed to clot and centrifuged at 4000 rpm for 10 minutes to separate the serum. Serum was collected into dry clean test tubes and low density lipoprotein (LDL-Cholesterol) was determined immediately. The rest of serum was frozen at -20 °C for the subsequent estimation of the other parameters.

Estimation of serum leptin

Leptin was estimated by an immunoenzymometric assay for the quantitative measurement of human leptin in serum and plasma "The Biosource Leptin Enzyme Amplified Sensitivity Immuno assay (EASIA)". The kit was supplied by BioSource Europe S.A., rue de l' Industrie 8, B-1400 Nivelles, Belgium.

Determination of serum total cholesterol

Total cholesterol was determined using the enzymatic method of Allain et al., (1974). Using the total cholesterol kit of Bio-Merieux Laboratory Reagents and Products. Bio-Merieux 69280 Marcy-1, Etoile, France.

Determination of serum triglycerides

Serum triglyceride was determined by the quantitative enzymatic calorimetric determination of triglycerides according to Wahlefeld (1974) by using Stanbio Enzymatic 2930 East Houston street, San Antonio, Texas 78202 USA.

Determination of serum phospholipids

Serum phospholipid was determined by enzymatic method according to Trinder (1969) using phospholipids kit of Quimica Clinica Aplicada 43870 Amposta/Tarragona, Espana. HDL-cholesterol was estimated according to Glatter equation (1984) where

Serum HDL-Cholesterol =

Total cholesterol $\dfrac{Triglyceride}{5}$ + LDL-cholesterol

Determination of serum LDL-cholesterol

Serum LDL-cholesterol was determined according to Steinberg (1981) by using LDL cholesterol/phospholipids kit of Bio Merieux Laboratory Reagents and Products Bio Merieux 69280 Marcy-1, Etoile, France

Determination of serum VLDL-cholesterol

VLDL-cholesterol was estimated according to Glatter equation (1984).

Determination of serum apolipoprotein A-1 and B (Apo A-1, B)

Apolipoprotein A-1 and B were estimated by immunoprecipitin analysis method according to Finley et al. (1976). Apo A-1 kits obtained from Atlantic Antibodies, An INCSTAR Company INCSTAR Corporation-Stillwater, Minnesota, U.S.A.

Statistical analysis of data

Data are expressed as mean ± S.E. Statistical significance of the difference was analyzed using one way-ANOVA and post-hoc Duncan test for multiple group comparison. P values of <0.05 were considered statistically significant. The correlation coefficient (r) which is a measure of the degree of closeness of the linear relationship between two variables (X and Y) was determined, r always lies between -1 and +1 .

RESULTS

There was a nonsignificant rise in leptin, apolipoprotein A-I and apolipoprotein B levels in the inferior vena cava compared with the portal venous blood of normal rats. However, a significant rise in leptin, apolipoprotein A-I and apolipoprotein B was detected in the inferior vena cava compared with its level in the portal venous blood of nephrotic rats (Table 1). Also there was a high serum leptin, apolipoprotein A-I and apolipoprotein B levels in the nephrotic

rats compared with the normal controls (Table 1). There was an insignificant change in lipid profile in the inferior vena cava compared with the portal venous blood of normal rats (Table 2) and a significant rise in lipid profile in the inferior vena cava compared with its level in the portal venous blood of nephrotic rats (Table 2). There was high serum all lipid profile levels in the nephrotic rats compared with the normal controls (Table 2). There was no correlation between portal venous serum leptin and serum lipids in normal control as in nephrotic rats except for triglycerides and HDL (+ve) and apolipoprotein A-1 and apolipoprotein B (-ve). Also there was no correlation between portal venous serum leptin and serum lipids in nephrotic rats except for apolipoprotein A-1 and apolipoprotein B (-ve) (Tables 3, 4). There was no correlation between inferior vena cava serum leptin and serum lipids in normal control and nephrotic rats except for apolipoprotein A-1 (-ve) (Tables 3, 4).

Table 1: Serum leptin, apolipoprotein A-1 and apolipoprotein B levels in portal vein and inferior vena cava blood in different experimental groups

Group	Leptin	Apolipo-protein A-1	Apolipo-protein B
portal vein – Control **(n=9)**	2.36±0.04	47.02±0.43	34.34±0.41
inferior vena cava – Control (n=9)	2.4±0.045	46.54±0.31	33.93±0.38
portal vein – Nephrotic **(n=9)**	3.53*±0.048	104.58*±0.33	85.15*±0.37
inferior vena cava – Nephrotic (n=9)	3.69^{**+}±0.054	114.13^{**+}±0.27	93.15^{**+}±0.95

Statistical differences were observed between:
p < 0.05: portal vein – Control and portal vein – Nephrotic*
p < 0.05: inferior vena cava – Control and inferior vena cava – Nephrotic **
p < 0.05: portal vein – Nephrotic and inferior vena cava – Nephrotic+
n: number of rats/groups

Table 2: Serum lipid fractions in portal vein and inferior vena cava blood in different experimental groups

Group	Total cholesterol	Triglycerides	Phospholipids	Low density lipoproteins	High density lipoproteins	Very low density lipoproteins
portal vein – Control **n=9**	77.54±1.87	66.72±1.51	131.12±2.25	27.52±1.56	36.68±0.85	13.35±0.3
inferior vena cava – Control **n=9**	76.79±1.77	64.82±1.52	129.81±2.07	27.94±1.89	35.73±1.04	12.97±0.3
portal vein – Nephrotic **n=8**	129.47[*]±.36	185.74[*]±1.48	205.94[*]±1.1	34.92[*]±1.11	57.39[*]±0.13	37.15[*]±0.3
inferior vena cava – Nephrotic **n=8**	156.08[**]±1.48	235.37[**+]±2.17	244.55[**+]±2.53	34.59[**]±1.13	74.49[**+]±0.48	46.99[**+]±0.46

Statistical differences were observed between:
p < 0.05: portal vein – Control and portal vein – Nephrotic*
p < 0.05: inferior vena cava – Control and inferior vena cava – Nephrotic **
p < 0.05: portal vein – Nephrotic and inferior vena cava – Nephrotic+
n: number of rats/groups

Table 3: Correlation between leptin and serum lipid profile in portal vein of normal control and nephrotic rats

Group		Total cholesterol	Trigly cerides	Phospho-lipids	Low density lipo-proteins	High density lipo-proteins	Very low density lipoproteins	Apo-lipoprotein A-1	Apolipoprotein B
portal vein – Control	r	-0.339	0.945	-0.313	-0.246	0.971	-0.159	-.412	-.403
	P-value	NS	< 0.01	NS	NS	< 0.01	NS	< 0.05	<0.05
portal vein – Nephrotic	r	0.129	0.150	0.021	0.276	0.013	0.151	0.717	0.696
	P-value	NS	NS	NS	NS	NS	NS	< 0.05	< 0.05

NS = Not significant

Table 4: Correlation between leptin and serum lipids in inferior vena cava of normal control and nephrotic rats

Group		Total cholesterol	Trigly cerides	Phospho-lipids	Low density lipo-proteins	High density lipo-proteins	Very low density lipoproteins	Apo-lipoprotein A-1	Apolipoprotein B
inferior vena cava – Control	r	-0.334	0.937	-0.257	-0.311	0.934	-0.210	-0.457	-0.583
	P-value	NS	< 0.01	NS	NS	< 0.01	NS	< 0.05	<0.05
inferior vena cava – Nephrotic	r	0.192	0.396	0.581	0.182	0.025	0.364	0.624	0.139
	P-value	NS	NS	< 0.05	NS	NS	NS	< 0.05	NS

NS = Not significant

DISCUSSION

Many authors have demonstrated that leptin is synthesized in the liver in addition to many other tissues including the stomach, ovary, and placenta (Soukas et al., 1999; Margetic et al., 2002).

On the other hand, the liver, spleen, and kidney may participate in leptin clearance (Kochan et al., 2006; Zivna et al., 2002; Makoto et al., 2001). However, the mechanism of leptin degradation/clearance has not been well established in humans; liver is proposed to be the most likely organ which degrades circulating leptin in vivo (Van Heek et al., 1996).

In the present work, there was a non-significant rise in leptin in the inferior vena cava compared with the portal venous blood of normal rats. Other studies supported our work suggesting that there was parallel synthesis and clearance of leptin by liver (Kochan et al., 2006; Margetic et al., 2002; Zivna et al., 2002; Makoto et al., 2001; Shimizu et al., 1998). In contrast, we found a significant rise in leptin in the inferior vena cava compared with its level in the portal venous blood of nephrotic rats (Table 1). Makoto et al. (2001) suggested that leptin is cleared in part by the portosystemic circulation through the liver while the kidneys play a principal role in the elimination of this peptide (Henmi et al., 2008).

Henmi et al. (2008) have confirmed increased soluble leptin receptor (sOB-R) concentration in nephrotic patients, as compared with the healthy subjects, and this soluble form of receptor (Ob-Re, also known as sOb-R), which lacks a transmembrane domain, is directly released into the circulation, whereas by binding leptin, it controls the amount of free leptin and the rate of leptin clearance (Zastrow et al. 2003).

Cohen et al. (2005) demonstrated that the liver is a major source of plasma-soluble leptin receptor (SLR) expression in some states (negative energy balance) and indicated a novel role for the liver in modulating leptin action. Thus their study revealed an unexpected role of the liver in

modulating total circulating leptin levels and possibly its biological activity which is in accordance with our findings.

Nephrotic syndrome is accompanied by enlargement of the liver due to accumulation of neutral fats (triglycerides) in the hepatocytes. Many authors have demonstrated that serum leptin levels were also significantly higher in subjects with fatty liver and that the concentration of circulating leptin correlates with fatty liver caused by accumulation of visceral fat (Tobe et al., 1999) which support our finding in their study. Huang et al. (2001) demonstrated that high levels of leptin can be caused by the delayed clearance of leptin from the circulation due to binding to its soluble receptor. The authors concluded that sOB-R is up-regulated and an over-expression of plasma-soluble leptin receptor (sOB-R) results in an increase of circulating leptin in nephrotic syndrome. In addition Cohen et al. (2005) indicated that the liver is a major source of plasma-soluble leptin receptor (SLR) expression, this illustrates the rise of leptin level in inferior vena cava. Moreover the rise in the level of leptin in the inferior vena cava of nephrotic rats might reflect decreased clearance of the hormone by the liver because of the fatty changes in this disease.

In the present work, there was a high serum leptin level in the nephrotic rats compared with the normal controls (Table 1). However many authors demonstrated no significant difference in plasma leptin concentrations between the healthy subjects and nephrotic patients (Henmi et al., 2008; Wasilewska et al., 2005; Ozata et al., 2002; Tobe et al., 1999).

But Huang et al. (2001) demonstrated that high levels of leptin in nephrosis can be caused by the delayed clearance of leptin from the circulation due to binding to its soluble receptor. They concluded that sOB-R is up-regulated and an over-expression of sOB-R resulted in an increase of circulating leptin in nephrotic syndrome (Henmi et al., 2008). These results are in accordance with the results of the present work. We add that

our method of induction of nephrotic syndrome used unilateral nephrectomized rats so the amount of clearance and elimination of leptin by one kidney is less than that by two kidneys. This leads to increase of leptin in our experimental circumstances. This explanation is in line with the findings of Toblli et al. (2002) who suggested that adriamycin-induced nephrotic rats had fatty livers and they demonstrated that a number of clinical entities, including nephrotic syndrome, present light to moderate enlargement of the liver due to accumulation of neutral fats (triglycerides) in the hepatocytes. Even though the outcome of hepatic steatosis does not seem to be harmful, when an additional inflammatory component is present, a variable degree of hepatic fibrosis and chronic liver disease could occur. Other authors demonstrated that serum leptin levels were also significantly higher in subjects with fatty liver that the concentration of circulating leptin correlates with fatty liver caused by accumulation of visceral fat (Tobe et al., 1999). Leptin is secreted by hepatic stellate cells and it acts locally in the liver and causes fibrosis (Crespo et al., 2002).

The present finding indicated significant rises in the serum lipids in the inferior vena cava blood compared with in the portal veins of nephrotic rats (Table 2). This excludes intestinal absorption of fats from being a source of increased serum lipids. Several workers had previously shown the increased hepatic apo A-I secretion in their nephrotic models (Medyńska et al., 2008; Sparks et al., 1981).

Sparks et al. (1981) reported that the high levels of apo A-I in the plasma of nephrotic rats was due to increased hepatic synthesis which resulted in an expansion of the pool size and saturation of the catabolic pathways. They were in the opinion that small increases in apo A-I synthesis lead to large increases in its concentration in the plasma and saturation of catabolic pathways.

Studies also showed that the relationship between leptin and lipid levels in patients with chronic glomerulonephritis is limited (Wu and Windmueller, 2001).

Wu and Windmueller (2001) demonstrated a positive correlation between serum leptin and triglyceride and LDL levels in healthy children, and a negative correlation between serum leptin and HDL; this coordinates with our finding in the control rats. It was suggested that there is a relationship between leptin concentration and certain markers of the metabolic syndrome, including cholesterol, triglycerides and apolipoproteins (Fujimaki et al., 2001; Tamer et al., 2002). A substantial disturbance of lipid metabolism occurs in children with idiopathic nephrotic syndrome (Delvin et al., 2003; Vaziri, 2003).

Haluzík et al. (2000) concluded that serum leptin levels in most types of hyperlipidemia simply reflect the degree of adiposity expressed by BMI and are not clearly related to serum lipid and/or lipoprotein concentrations. Our results are in agreement with Wasilewska et al. (2005) who reported a positive correlation of leptin with total cholesterol, its LDL fraction and HDL fraction and a negative correlation with and apo A and apolipoprotein B (apo B) in nephrotic children(Tables 3, 4). In conclusion, the present study suggests that the liver plays an important role in leptin and lipids profile metabolism in nephrotic rats.

REFERENCES

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-5.

Calandra S, Gherardi E, Fainaru M, Guaitani A, Bartosek I. Secretion of lipoproteins, apolipoprotein A-I and apolipoprotein E by isolated and perfused liver of rat with experimental nephrotic syndrome. Biochem Biophys Acta 1981;665:331-8.

Chabova V, Tesar V, Perusicova J, Zima T, Zabka Rychlik I et al. Plasma leptin levels in patients with kidney diseases of various etiologies. Cas Lek Cesk 1999;138:465-8.

Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA et al. Role for stearoyl-CoA desaturase-1 in leptin mediated weight loss. Science 2002; 297:240–3.

Cohen P, Yang G, Yu X, Soukas AA, Wolfish CS, Friedman JM et al. Induction of leptin receptor expression in the liver by leptin and food deprivation. J Biol Chem 2005;280:10034-9.

Crespo J, Rivero M, Fábrega E, Cayón A, Amado JA, García-Unzeta MT et al. Plasma leptin and TNF-alpha levels in chronic hepatitis C patients and their relationship to hepatic fibrosis. Dig Dis Sci 2002;47:1604–10.

Cumin F, Baum HP, Levens N. Mechanism of leptin removal from the circulation by the Kidney. J Endocrinol 1997;155:577–85.

Delvin EE, Merouani A, Levy E. Dyslipidemia in nephrotic syndrome: causes revised. Clin Biochem 2003;36:95–101.

Ece A, Atamer Y, Gurkan F, Bilici M, Kocyirit Y. Anti-oxidant status in relation to lipoproteins, leptin and pro-inflammatory cytokines in children with steroid-sensitive nephritic syndrome. Nephrology 2004;9: 366-73.

Finley PR, Williams JR, Bayers JM. Immunochemical determination of human immunoglobulins with a centrifugal analyzer. Clin Chem 1976;22:1037-141.

Fujimaki S, Kanda T, Fujta K, Tamura J, Kobayashi I. The significance of measuring plasma leptin in acute myocardial infarction. J Int Med Res 2001;29:108–13.

Gherardi E, Calandra S. Experimental nephrotic syndrome induced in the rat by puromycin aminonucleoside: Hepatic synthesis of neutral lipids and phospholipids from 3H water and 3H palmitate. Lipids 1980;15:108-12.

Glatter TR. Hyperlipidemia. What is 'normal,' who should be treated and how. Postgrad Med 1984;76:49-55, 58-9.

Haluzík M, Fiedler J, Nedvídková J, Ceska R. Serum leptin levels in patients with hyperlipidemias. Nutrition 2000;16:429-33.

Henmi K, Yoshida M, Yoshikawa N, Nakabayashi I, Hirano T. Relationship between plasma leptin or soluble cleaved leptin-receptor concentrations and glucocorticoid sensitivity of peripheral blood mononuclear cells in patients with nephrotic syndrome. Int Immunopharmacol 2008; 8: 1703–6.

Heymann W, Hachel DB, Harwood S, Wilson SGF, Hunter JLP. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. Proc Soc Exp Biol 1959;100:660-4.

Huang L, Wang Z, Li C. Modulation of circulating leptin levels by its soluble receptor. J Biol Chem 2001;276:6343–9.

Huang W, Dedousis N, Bandi A, Lopaschuk GD, O'Doherty RM. Liver triglyceride secretion and lipid oxidative metabolism are rapidly altered by leptin in vivo. Endocrinology 2006;147:1480–7.

Huang W, Dedousis N, O'Doherty RM. Hepatic steatosis and plasma dyslipidemia induced by a high-sucrose diet are corrected by an acute leptin infusion. J Appl Physiol 2007;102:2260–5.

Kochan Z, Karbowska J, Meissner W. Leptin is synthesized in the liver and adipose tissue of the dunlin (Calidris alpina). Gen Comp Endocrinol 2006;148:336–9.

Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord 2002;26:1407-33.

Medyńska A, Kilis-Pstrusinska K, Zwolinska D, Chorazyczewski A. Serum leptin levels in children with chronic glomerulonephritis. Adv Clin Exp Med 2008;17: 137–40.

Merta M, Rysavá R, Ríhová Z, Kmentová D, Remes O, Tesar V. Leptin in patients with nephrotic syndrome. Sb Lek 2003;104: 279-84.

Morcos SR. Personal communication. National Research Centre. El-Tahrir Street, Dokki, Cairo, 1980.

Nakamuta M, Tada S, Uchimura K, Enjoji M, Kinukawa N, Iwamoto H et al. Serum leptin levels in patients with nonalcoholic chronic liver disease. Hepatogastroenterology 2001;48:527-32.

Nicola WG, Mikhail TH, Girgis RB, Ibrahim KA, El Khayat Z, Habib DF. Role of the nephritic kidney in lipoprotein metabolism. Med J Cairo Univ 1996;64 (Suppl): 111-25.

Ozata M, Oktenli C, Gulec M, Ozgurtas T, Bulucu F, Caglar K et al. Increased fasting plasma acylation-stimulating protein concentrations in nephrotic syndrome. J Clin Endocrinol Metab 2002;87:853–8.

Porter R, Brand M. Body mass dependence of H+ leak in mitochondria and its relevance to metabolic rate. Nature 1993;362: 628–30.

Sharma AM, Bartell SM, Baile CA, Chen B, Podolsky RH, McIndoe RA et al. Hepatic gene expression profiling reveals key pathways involved in leptin-mediated weight loss in ob/ob mice. PLoS One 2010; 5:e12147.

Shimizu H, Kakizaki S, Tsuchiya T, Nagamine T, Takagi H, Takayama H et al. An increase of circulating leptin in patients with liver cirrhosis. Int J Obesity 1998;22: 1234-8.

Singh A, Wirtz M, Parker N, Hogan M, Strahler J, Michailidis G et al. Leptin-mediated changes in hepatic mitochondrial metabolism, structure, and protein levels. Proc Natl Acad Sci U S A 2009;106:13100-5.

Soukas A, Cohen P, Friedman JM. Gene expression profile induced by leptin in white adipose tissue and liver. Nat Gen 1999;23:75.

Sparks CE, Tennenberg SD, Marsh JB. Catabolism of apo A-I of HDL in normal and nephritic rats. Metabolism1981;30:354-8.

Steinberg D. Metabolism of lipoproteins at the cellular level in relation to atherogenesis. In: Miller NE, Lewis B (Eds.): Lipoproteins, atherosclerosis, and coronary heart disease. Amsterdam: Elsevier North Holland, 1981 (pp 31-48).

Tamer L, Ercan B, Unlu A, Sucu N, Recdemir H, Eskandari G et al. The relationship between leptin and lipids in atherosclerosis. Indian Heart J 2002;54:692–6.

Tobe K, Ogura T, Tsukamoto C, Imai A, Matsuura K, Iwasaki Y et al. Relationship between serum leptin and fatty liver in Japanese male adolescent university students. Am J Gastroenterol 1999;94:3328-35.

Toblli JE, Ferder L, Stella I, Angerosa M, Inserra F. Enalapril prevents fatty liver in nephrotic rats. J Nephrol 2002;15:358-67.

Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:2427.

Van Heek M, Mullins DE, Wirth MA, Graziano MP, Fawzi AB, Compton DS et al. The relationship of tissue localization, distribution and turnover of feeding after intraperitoneal leptin administration to ob=ob and db=db mice. Horm Metab Res 1996;28: 653-8.

Vaziri ND. Molecular mechanisms of lipid disorders in nephrotic syndrome. Kidney Int 2003;63:1964–76.

Vaziri ND, Liang KH. Acyl-coenzyme A: cholesterol acyltransferase inhibition ameliorates proteinuria, hyperlipidemia, lecithin-cholesterol acyltransferase, SRB-1, and low-denisty lipoprotein receptor deficiencies in nephrotic syndrome. Circulation 2004;110:419-25.

Wahlefeld AW. Triglycerides determination after enzymatic hydrolysis. In: Bergmeyer HU (Ed.): Methods of enzymatic analysis, Vol. 5. New York: Academic Press, 1974 (pp 1831-5).

Wang M, Lee Y, Unger R. Novel form of lipolysis induced by letpin. J Biol Chem 1999;274:17541–4.

Wang J, Leclercq I, Brymora JM, Xu N, Ramezani-Moghadam M, London RM et al. Kupffer cells mediate leptin-induced liver fibrosis. Gastroenterology 2009;137:713-23.

Wasilewska A, Tomaszewska B, Zoch-Zwierz W, Biernacka A, Klewinowska K, Koput A. Serum and urine leptin concentration in children with nephrotic syndrome. Pediatr Nephrol 2005;20:597-602.

Wu AL, Windmueller HG. Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. J Biol Chem 1979;254:7316-22.

Zastrow O, Seidel B, Kiess W, Thiery J, Keller E, Böttner A et al. The soluble leptin receptor is crucial for leptin action: evidence from clinical and experimental data. Int J Obes Relat Metab Disord 2003;27: 1472-8.

Zivna H, Pavel Z, Vladimir P. Serum leptin concentrations after surgery in young rats. Nutrition 2002;18:643-6.