HORMONAL REGULATION OF LIPOPROTEIN LIPASE IN ADIPOSE TISSUE

(STUDIES IN THE RAT AND IN HUMANS)

HORMONALE REGULATIE VAN LIPOPROTEINE LIPASE IN VETWEEFSEL

(STUDIES IN DE RAT EN BIJ DE MENS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. A.H.G. RINNOOY KAN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
VRIJDAG 26 FEBRUARI 1988 TE 15.45 UUR

DOOR

MARINUS GERARDUS ADRIANUS BAGGEN

GEBOREN TE LEUR

1988
Offsetdrukkerij Kanters B.V.,
Alblasserdam

PROMOTIECOMMISSIE:

Promotor:	Prof. Dr. J.C. Birkenhäger
Overige leden:	Prof. Dr. D.W. Erkelens
	Prof. Dr. W.C. Hülsmann
	Prof. Dr. S.W.J. Lamberts
Co-promotor:	Dr. H. Jansen

Ter nagedachtenis aan mijn vader,
voor mijn moeder.

CONTENTS

VOORWOORD 7
LIST OF ABBREVIATIONS 8

CHAPTER I: INTRODUCTION. 9
I.1. Lipoprotein lipase: general. 10
I.2. The characteristics of lipoprotein lipase. 10
I.3. Regulation of lipoprotein lipase. 12
I.4. Nutritional aspects on lipoprotein lipase. 12
I.5. Substrate affinity. 14
I.6. Hormonal regulation. 14
I.7. Aims of the study. 19
I.8. References. 21

CHAPTER II.
Lipoprotein lipase activity and triglyceride metabolism in the perfused epididymal fat pad. 33

CHAPTER III.
The effect of Synacthen administration on lipoprotein lipase activity in the epididymal fat pad of the rat. 53

CHAPTER IV.
Effects of Synacthen on lipid metabolism in the perfused epididymal fat pad of the rat. 65

CHAPTER V.
Disparate effects of ACTH (1-24) and corticosterone on lipoprotein lipase in rat adipose tissue. 79

CHAPTER VI.
Effects of doxazosin and propranolol administration on lipoprotein lipases in cholesterol-fed rats. 89

CHAPTER VII.
Effects of prazosin and propranolol treatment on plasma lipoproteins and post-heparin plasma lipase activities in mildly hypertensive males after coronary bypass grafting. 99

CHAPTER VIII. : GENERAL DISCUSSION. 114

SUMMARY 129

SAMENVATTING 133

CURRICULUM VITAE 137

LIST OF ABBREVIATIONS

AA	Amino acids
CABG	Coronary artery bypass grafting
FA	Fatty acids
FFA	Free fatty acids
gww	Gram wet weight
HDL	High-density lipoproteins
HL	Hepatic lipase
LDL	Low-density lipoproteins
LPL	Lipoprotein lipase
mU	milli Units
SD	Standard deviation
SEM	Standard error of the mean
TG	Triglycerides
TG-FA	Triglyceride fatty acids
VLDL	Very low-density Lipoproteins

VOORWOORD

Bij het voltooien van dit proefschrift wil ik graag iedereen bedanken die, op directe of indirecte wijze, een bijdrage heeft geleverd in de totstandkoming hiervan.
- Mijn ouders, die mij door de opvoeding die ze me gegeven hebben in staat hebben gesteld te gaan studeren.
- Mijn co-promotor Hans Jansen, die door zijn stimulerende discussies over het werk en zijn grote hulpvaardigheid bij moeilijkheden het onderzoek tot een succes heeft gemaakt.
- Mijn promotor, Jan Birkenhäger, die door zijn bezielende leiding, zeer grote kennis en inzicht mij steeds opnieuw weer enthousiast wist te maken als er tegenvallende resultaten leken te zijn. Tevens voor de perfecte opleiding tot internist, die ik van hem in de academie heb mogen ontvangen.
- Ronald Lammers, de man met de gouden handen, zonder wie de perfusie techniek nooit ontwikkeld zou zijn.
Tevens, Ronald, bedankt voor alle hulp bij verzorgen en spuiten van de proefdieren,
- Louis Verschoor, voor zijn bijdrage aan de discussies over de resultaten van de experimenten, waarvan ik bij de opstelling van nieuwe experimenten dankbaar gebruik heb gemaakt.
- Dieneke van Wessem, voor de manier waarop ze het merendeel van mijn manuscripten tot een leesbaar geheel maakte op de word-processor.
- Joke van Vuure, voor de fantastische hulp bij het uitzoeken van de literatuur.
- Tot slot, alle collega's en vrienden, die niet met name genoemd zijn en die op allerlei manieren hebben geholpen om deze zware bevalling tot een goed eind te brengen.

CHAPTER I

INTRODUCTION

I.1. Lipoprotein lipase: general

Long chain fatty acids are essential to all mammalian tissues, both as constituents of complex lipids necessary for the structural and functional integrity of cells and as important energy source (1,2). Long chain fatty acids are carried in the circulation as free fatty acids (FFA) bound to albumin (3), as triglyceride-fatty acids (TG-FA) within chylomicrons and very low-density lipoproteins (VLDL), as phospholipids and esterified cholesterol. The uptake of FFA by individual tissues is largely a function of their concentration in the blood and of the rate of the blood flow through the tissue (1,2).TG-FA however, are contained in the core of large particles which cannot cross the capillary endothelium. Their removal from the blood depends therefore on prior hydrolysis at the capillary lining, a reaction catalyzed by the enzyme lipoprotein lipase (LPL) (4,5,6,7). The extent to which circulating lipoprotein TG-FA are taken up by individual tissues will depend therefore on the LPL activity of each tissue relative to the activity in other tissues (6). Since the activity of LPL in various tissues can change rapidly under different physiological conditions, the enzyme has a directive role in the distribution of plasma TG-FA. Thus LPL is important in not only determining the rate, but in addition the pattern of uptake of TG-FA from the blood.The ability to change the supply of TG-FA according to the specific demands of each tissue is of particular importance, since there is essentially no mechanism for controlling the distribution of circulating FFA to individual tissues (1,2,7). LPL is thus not only the key enzyme in the removal of TG from the circulation, but in addition, its activity provides a means to channel long chain fatty acids to individual tissues according to the specific metabolic needs of the latter.

I.2. The characteristics of lipoprotein lipase

Hahn was the first to report clearing of severe alimentary lipemia in dogs after transfusion of heparin containing blood (8). Injection of heparin alone could also lead to clearing of blood TG in dogs. In addition to these in vivo studies (9), it

was soon thereafter established that in vitro mixing of lipidemic plasma with plasma separated from the blood of an animal that had received heparin, leads to rapid hydrolysis of the lipid (10). These studies suggested that a factor, released into the circulation after heparin administration, is able to clear alimentary lipidemia under in vivo and in vitro conditions. This factor initially called "clearing factor", was named lipoprotein lipase by Korn (11) who showed that the enzyme hydrolyzes chylomicron TG. It was established that the enzyme was present in several tissues, and located close to the capillary wall (14,46) and that there is a direct relationship between the level of tissue LPL activity and the rate of lipoprotein TG-FA uptake (12). The earliest and most extensive studies have been carried out with adipose tissue , which takes up and stores most of the circulating TG-FA (6,7,12-15). The highest levels of LPL activity are found in heart (16-22), lactating mammary gland (23-27) and milk (26,28-30). Other tissues in which LPL has been described are lung (31-34), skeletal muscle (35-38), aorta (39,40), brain (41), corpus luteum (42,43) and placenta (44,45). Organs which do not have LPL cannot utilize circulating lipoprotein TG-FA and use for energy FFA and glucose.

The enzyme as isolated from different sources, is a glycoprotein with a molecular weight of 57 kD (101) and a carbohydrate content of 3-10%; the only exception seems to be a smaller molecular weight species isolated from the heart (18,99) and from post heparin plasma (54). The enzyme hydrolyzes lipoprotein TG to FFA and monoglycerides (at pH 7,5-9.0), at the luminal surface of the capillary endothelium (6,7,67,68), where it is bound to heparan sulphate.

The hydrophobic portion of the heparan sulphate proteoglycan is anchored in the phospholipid monolayer of the outer leaflet of the endothelial cell plasma membrane. The glycosaminoglycan chains of the heparan sulphate proteoglycan exist as part of the extracellular endothelial glycocalyx and the strong negative charge present on these sulphated polymers provides the basis of the electrostatic interaction between the surface of the endothelium and the binding site on the LPL protein. In addition to

the heparan sulphate glycosaminoglycan, the enzyme also binds apolipoprotein C-II, an essential physiological activator of the enzyme, which is present in the surface film of VLDL and chylomicrons, via a specific binding site. Two other specific sites of the enzyme exist: the catalytical active site and a lipid-binding site, to which the phospholipids in the surface of the lipoprotein particle bind. The lipoprotein may also interact with the endothelial glycosaminoglycan via apolipoprotein E (69,70).
LPL activity in vitro has a pH optimum of 8.4 (98) and is highly inhibited by 1 M. salt and protamine sulphate (21). In vitro LPL can be identified (or is characterized) by the dependency upon a specific apoprotein C-II for optimal activity. These three characteristics -alkine pH optimum, activation by serum (apoprotein C-II), and inhibition by high concentrations of sodium chloride - have been used to differentiate between LPL and other lipases in various tissues.

I.3. Regulation of lipoprotein lipase activity: general.
In order to effectively channel circulating TG-FA to various organs according to their specific needs, the enzyme has to fulfill two demands: 1. its level has to adjust rapidly and specifically to the temporary needs of each organ; 2. its location in the tissues should maximize the contact between circulating lipoproteins and enzyme.
Indeed the location of the enzyme at the luminal surface of the capillary endothelium (from which it can be rapidly released by heparin), was one of the first reported characteristics of LPL (15).
Studies during the last 30 years have shown that LPL activity levels change rapidly under a variety of physiological conditions.

I.4. Nutritional effects on lipoprotein lipase.
In the fed animal, a large amount of circulating TG-FA is taken up by adipose tissue, reësterified and stored within adipocytes. After feeding the activity of LPL is, therefore, high in this

tissue. During fasting, LPL activity and thus the uptake of extracellular liberated FA decreases greatly. Hormone sensitive lipase, that hydrolyzes intracellularly stored TG, becomes active in the tissue, leading to the release of FFA into the blood (47). Another tissue that takes up a major part of the circulating TG-FA is skeletal muscle (6,48,49). In that tissue fatty acids are used as an important energy source. This is especially the case in muscles containing a large proportion of slow twitch, red fibers, such as the soleus. Indeed LPL activity is much higher in red, than in white skeletal muscle (37,38, 50,51). Whereas LPL activity does not change in white muscle during fasting, it increases in red muscle, enabling the red muscles to use VLDL-TG during this period. On a wet weight basis, LPL activity is much higher in cardiac, than in skeletal muscle: also this activity rises markedly after fasting, indicating that the efficiency of utilisation of plasma TG-FA from VLDL increases under fasting conditions. In the rat the lung maintains a constant level of LPL activity that seems independent of the nutritional status (33), In the mouse, an increase in the LPL activity in the lung after a 24 hour fast has been reported (32). The normal or high activity of LPL in lung, heart and red skeletal muscle during fasting, coupled with the low activity in adipose tissue, results in an effective channelling of circulating VLDL-TG to tissues which have to be supplied with long chain fatty acids for their normal metabolic function. Since tissues compete for circulating FFA, the tissues which maintain or increase their LPL activity have, in addition, the advantage of access to circulating TG-FA during fasting.

The composition of the diet has an effect on LPL activity (83,85,86). LPL activity in adipose tissue is lower in rats fed on diets high in fat content, in contrast to diets high in carbohydrate. LPL activity is also lower in rats fed on sucrose rather than on a starch diet. By contrast, this activity in heart and skeletal muscle was greater in rats fed on the high fat compared with those fed on the high carbohydrate diets. It has been suggested (83) that changes in hormonal balance (exaggerated glucocorticoid response to high fat diets (87,88)) and

sensitivity (insulin insensitivity on high fat diets (87,89)) could be responsible for the relative changes in heart and adipose tissue LPL activities. Differences in hormonal status could also account for why feeding a diet rich in rapeseed oil increased LPL activity in adipose tissue (90). However, refeeding could at the best in part be responsible as the rats used in the latter experiments were starved for 1 day, and then refed for 4 days on a diet in which erucic acid comprised 43% of the fatty acids. Feeding with a diet rich in corn oil, rather than beef thallow, has also been reported to increase LPL activity in adipose tissue (91).

I.5. Substrate affinity

The delivery of circulating TG-FA to specific tissues could also be affected by differences in affinity between LPL and lipoproteins in various tissues. A comparison between the kinetics of TG removal by the isolated perfused rat heart and adipose tissue has shown that LPL in heart endothelium has a high substrate affinity (K_m 0.07 mM TG), whereas the enzyme in the endothelium of adipose tissue has a low substrate affinity (K_m 0.70 mM TG) (52,53). These tissue specific lipases differ not only in kinetic characteristics, but also structurally (53,54). It has been proposed therefore, that the activity of high and low affinity LPL, in heart and adipose tissue respectively, may have an important physiological role in the regulation of local TG clearance (55).

I.6. Hormonal regulation

Present evidence indicates that LPL is synthesized within parenchymal cells in the endoplasmatic reticulum as an inactive precursor and its activation take place before it reaches the trans golgi cisternae (102,103). It is released from these cells and transported to the luminal surface of the capillary endothelium. Very little is known about the mechanism of release from the cells, its transport through interstitium and basement membrane to the endothelial cell and the mechanism by which it reaches

the luminal surface of the endothelium (and the - possible- regulation of these processes).

On the basis of earlier studies it has been proposed that in adipose tissue, LPL exists in two forms - an intracellular precursor form (LPL-b) and an extracellular form (LPL-a), representing about 5-10% of total tissue enzyme (56). The transformation of LPL-b to LPL-a would be independent of protein synthesis, occurring just prior to the release from the cell and could therefore represent activation of the precursor enzyme (56).

These investigators suggested that the high activity of LPL in adipose tissue of fed rats results from an increase in enzyme protein (57).

Ashby et al. have proposed a scheme for the synthesis and release of the enzyme from the adipocyte (58,59). The synthesis of a proenzyme is regulated by insulin and corticosteroids (59); the next step is also hormone and energy dependent, but requires glucose and probably involves the glycosylation of the proenzyme (60,61). Inhibition of glycosylation by tunicamycin completely inhibits enzyme secretion (5,62). The "complete" active enzyme is released from the cell by microtubular transport, a mechanism suggested by the inhibition of enzyme secretion by colchicine (63,64).

In the first step, glucocorticoids probably enhance LPL activity by regulation of proenzyme synthesis, (20,34,59,65,66), possibly at the level of RNA synthesis (59). The second step, conversion of proenzyme to "complete" enzyme is essential for enzyme secretion and most probably involves glycosylation of the proenzyme protein (5,60-62). Insulin is supposed to act on (pro)-enzyme synthesis as well as on the activation.

A possible additional site of regulation of enzyme activity is the inactivation of the "complete" enzyme just prior to its release from the cell by adrenalin (58,59), which can rapidly alter the functional activity at the endothelial surface. It has been suggested (58) that such a rapid change induced by adrenergic stimuli would act to stop TG-FA uptake more rapidly than changing the rate of enzyme synthesis could do.

Gonadal hormones and nutritional factors are known to influence the distribution of human fat. Estrogen administration markedly lowers adipose tissue LPL in the rat (94-96) but not in man (97). Ovariectomy increases LPL activity in the rat (94). Progesterone and testosterone do not affect the enzyme activity in adipose tissue of either male or ovariectomized rats. The decrease of rat adipose tissue LPL after estrogen treatment most likely is not caused by inadequate food intake since the rats in the different experimental groups had a similar weight gain. It is not yet known whether the low adipose tissue LPL activity is caused directly by the hormone or whether it is mediated through the secretion of prolactin.

The most striking example of hormonal regulation of LPL activity occurs just before parturition and during lactation in rats. The non-lactating mammary gland is completely devoid of LPL; however, shortly before parturition, there is a dramatic rise in enzyme activity, while concomittently there is an almost complete loss of enzyme activity from the adipose tissue (23). Throughout lactation, LPL remains low in all the fat depots, while the levels stay high in the mammary gland until weaning. Studies in lactating rats, hypophysectomized on the fifth day after parturition, have shown that these dramatic changes in enzyme activity are regulated by pituitary prolactin (27), which throughout lactation completely depresses LPL activity in adipose tissue, while at the same time, maintaining a very high enzyme level in the mammary gland.

The role of glucagon in the regulation of LPL remains uncertain. Glucagon levels are increased by fasting, and glucagon hastens the decrease in vitro of adipose tissue LPL activity (81); still the in vivo activity of the enzyme in adipose tissue is increased in fed or fasting rats injected with glucagon (92). Speake et al. suggested that the glucagon preparation may have contained some insulin. Furthermore, one cannot exclude that high glucagon levels lead to hyperinsulinemia.

Hormones other than insulin, glucagon and catecholamines have also been implicated in the control of the enzyme's activity in adipose tissue. Of these, the glucocorticoids are of particular

interest with respect to regulation on a longer time scale. The activity of the enzyme in adipose tissue has been shown to be increased after glucocorticoid injection in vivo (71). Male rats injected with hydrocortisone-hemisuccinate (8 mg) corticosterone (8 mg) or dexamethasone (0.2 mg) intraperitoneally. Adipose tisue LPL released by a low dose of heparin and heart LPL were raised in activity 2 to 4 hours after the injection, in fed animals. In adrenalectomized rats, sacrificed 3-4 days after the operation, adipose tissue LPL activity (acetone-ether powder) was decreased both in fed and fasted animals. This effect could be abolished by dexamethasone, injected 3 hours before sacrifice, only in fasted rats. Ashby et al. (59) measured the LPL activity of epididymal fat bodies from rats, starved for 24 hours during incubation, without heparin, at 37°C in vitro. Protein-synthesis-dependent increases in the activity of the enzyme occur in the presence of insulin (0.2-2.0 mU/ml) and are markedly potentiated by addition of dexamethasone (400 nM) to the incubation medium. The magnitude of the rise in LPL activity was much greater than in the presence of insulin alone, while dexamethasone alone only caused a small increase of the activity. In these experiments, he used several corticosteroids and found that all of these were effective in enhancing the response to insulin, although the synthetic compounds, dexamethasone and triamcinolone acetonide, are the most potent. Adrenalin (10 μM) antagonizes the increase in the activity of the enzyme in the tissue. Ashby et al. (59) came to the conclusion that the combined effects of changes in plasma insulin and glucocorticoid concentrations are responsible for the changes in adipose tissue LPL activity that occur with changes in nutritional status in vivo. In earlier experiments De Gasquet and Pequignot found that the normally observed changes in LPL activity over a 24-hour period were correlated with the corticosteroid levels: high levels were associated with low LPL activity and vice versa. Krotkiewski (72), using male Sprague-Dawly rats treated the rats with dexamethasone (0.02 mg i.p.) for 28 days. In spite of high insulin levels (from 43 μU/ml to 77 μU/ml) the LPL activity in the epididymal fat pad decreased to less than 50% from the

initial value in these fasted animals.

Krausz (73), using male albino rats treated with triamcinolone (0.1 and 2.5 mg) for five days and Bagdade (74) who used male Sprague-Dawly rats treated with dexamethasone (0.025 mg) for two weeks, both found a decrease in adipose tissue LPL from fed animals. Bagdade used the heparin-elutable method to measure LPL activity (75) and Krausz the acetone-ether powder method (76).

Cigolini and Smith (82) studied the influence of pharmacological (3 μM) concentrations of hydrocortisone on biopsies of human adipose tissue, maintained in culture for one week. Hydrocortisone decreased the acetone-ether LPL activity at high physiological (0.3 μM) and pharmacological (3 μM) concentrations. The presence of insulin (1000 μU/ml) alone tended to increase the LPL activity, although the difference did not reach statistical significance. The combination of insulin and glucocorticoids caused an increase in LPL activity. The enhancing effect seems to be influenced by of the hydrocortisone/insulin ratio. Increasing the glucocorticoid concentration tenfold, while maintainig the insulin concentration constant, let to a lower effect on the LPL activity. However, increasing the insulin concentrations at a given cortisol concentration caused a further increase of LPL activity. In the same study the authors found that pharmacological hydrocortisone concentrations induce a fall of the number of insulin receptors on adipocytes. Recently Speake et al. (92) reported that the incorporation of ^{3}H-leucine into LPL during incubations of rat epididymal fat bodies in vitro is significantly stimulated by dexamethasone (400 nM), whereas total protein synthesis was unaffected. The stimulation by dexamethasone required the presence of insulin (2000 μU/ml). Taskinen et al. (100) studied the lipoprotein patterns and lipolytic activities in patients with Cushing's syndrome. They could not find any difference between controls and patients in LPL activity in biopsies of peripheral adipose tissue and in plasma after an intravenous injection of heparin. In contrast to the adipocytes of the rat (78), these cells do not have corticotropin receptors in humans (79).

In adrenalectomized rats (71), a situation with high levels of corticotropin, as well as in hypercorticistic rats by treatment with glucocorticoids (20,72-74), a situation with suppressed corticotropin secretion, LPL activity is decreased in adipose tissue. From older, in vitro studies, it is known that corticotropin, just as glucagon and catecholamines, counteract the stimulatory effect of insulin on adipose tissue LPL (80,81). In in vivo experiments, by Lawson et al. (83), using male rats, fed on starch and corn-oil diets for three weeks, a corticotropin injection (0.2 U) induced a rapid fall. A fall of 30-35% one hour after the injection and lasting for at least six hours of adipose tissue LPL activity in winter, but not in the period between march and september.
Chernick et al. (84) did not observe any effect of ACTH on LPL activity in the cultured 3T3-L1 cell, although it did stimulate intracellular lipolysis. Effects of lipolytic agents on the activity of LPL were variable in these cells and concentration dependent. LPL activity was decreased only by concentrations of adrenalin greater than those inducing maximal intracellular lipolysis and the decrease in activity occurred about 30 minutes after the increase in glycerol release.

I.7. Aims of the study.

Current data strongly suggest the most important role for insulin in the hormonal regulation of adipose tissue LPL activity. It is not clear from the literature what the role is of glucocorticoids in the regulation of the enzyme. Stress hormones as ACTH and adrenalin for example seem to decrease LPL activity or to have no effect at all on the enzyme. One of the reasons for difficulties in interpreting the data obtained so far is the fact that different methods were used to measure LPL activity. Another point is, that biological enzyme activities are measured in vitro, so that one is not informed on what really happens in vivo.
We have focussed our attention to the influence of physiologically circulating glucocorticoids, ACTH and insulin on LPL activity in rats. Aims of the study were: - which role have steroid

and peptide hormones in the regulation of adipose tissue LPL? - to what degree reflects the adipose tissue LPL activity measured in vitro that prevailing in situ? - what is the balance of in- and efflux of fatty acids in/from adipose tissue under various conditions? - What is the influence of α- and ß-adrenergic antagonists on adipose tissue LPL and plasma lipoproteins? In chapter VIII the effects of antagonists of catecholamines on lipoproteins and lipolytic enzymes in post-heparin plasma of humans is shown and discussed. To study the effects of changes in LPL activity in situ on triglyceride metabolism in rats (in different states) the epididymal fat pad was selectively perfused with labeled chylomicrons and VLDL.

I.8. REFERENCES

1. Frederickson, D.S., Gordon, R.S. Jr. Transport of fatty acids. Physiol. Rev. 38: 585-630, 1958.
2. Scow, R.O., Chernick, S.S. Mobilization, transport and utilization of free fatty acids. In: Comprehensive Biochemistry. vol. 18, edited by M. Florkin and E.H. Stotz., Elsevier, Amsterdam, 1970, pp. 19-49.
3. Spector, A.A., John, K and Fletcher, J.E. Binding of longchain fatty acids to bovine serum albumin. J. Lipid Res. 10: 56-67, 1969.
4. Borensztajn, J. Lipoprotein Lipase. In: Biochemistry of Atherosclerosis, edited by A.M. Scanu, R.W. Wissler and G.S. Getz. Biochemistry of disease. Vol: 7, edited by E.Farber and H.C. Pitot, New York: Marcel Dekker, pp. 231-245, 1979.
5. Cryer, A. Tissue Lipoprotein Lipase activity and its action in lipoprotein metabolism. Int. J. Biochem. 13: 525-541, 1981.
6. Robinson, D.S. The function of the plasma Triglycerides in fatty acid transport. In: Comprehensive Biochemistry, vol.18, edited by M. Florkin and E.H. Stotz. Elsevier, Amsterdam, pp. 51-116, 1970.
7. Scow, R.O., Hamosh, M., Blanchette-Mackie, E.J. and Evans, A.J. Uptake of blood triglyceride by various tissues. Lipids, 7: 497-505, 1972.
8. Hahn, P.F. Abolishment of alimentary lipemia following injection of heparin. Science, 98: 19-20, 1943.
9. Weld, C.B. Alimentary lipaedemia and heparin. Can. Med. Ass. J. 51: 578, 1944.
10. Anderson, N.G. and Fawcett, B. An antichylomicronemic substance produced by heparin injection. Proc. Soc. Exp. Biol. Med., 74: 768-771, 1950.
11. Korn, E.D. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart.

J. Biol. Chem. 215: 1-14, 1955.
II. Substrate specificity and activation of coconut oil. J. Biol. Chem. 215: 15-26, 1955.

12. Bezman, A., Felts, J.M., and Havel, R.J. Relation between incorporation of triglyceride fatty acids and heparin-released lipoprotein lipase from adipose tissue slices. J. Lipid Res. 3: 427-431, 1962.

13. Korn, E.D., Quigley, T.W. Jr. Lipoprotein Lipase of chicken adipose tissue.
J. Biol. Chem. 226: 833-839, 1957.

14. Robinson, D.S. The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues. Adv. Lipid Res. 1: 133-182, 1963.

15. Robinson, D.S. and Harris, P.M. The production of lipolytic activity in the circulation of the hind limb in response to heparin.
Q. J. Exp. Physiol. 44: 80-90, 1959.

16. Borensztajn, J., Otway, S. and Robinson, D.S. Effect of fasting on the clearing factor lipase (lipoprotein lipase) activity of fresh and defatted preparations of rat heart muscle. J. Lipid Res. 11: 102-110, 1970.

17. Borensztajn, J.and Robinson, D.S. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. J. Lipid Res. 11: 111-117, 1970.

18. Chung, J. and Scanu, A.M. Isolation, molecular properties and kinetic characterization of lipoprotein lipase from rat heart. J. Biol. Chem. 252: 4202-4209, 1977.

19. De Gasquet, P. and Pequignot, E. Lipoprotein lipase activities in adipose tissues, heart and diaphragm of the genetically obese mouse (ob/ob).
Biochem. J. 127: 445-447, 1972.

20. De Gasquet, P., Pequignot-Planche, E., Tonnu, N.T. and Diaby, F.A. Effect of glucocorticoids on lipoprotein lipase activity in rat heart and adipose tissue.
Horm. Metab. Res. 7: 152-157, 1975.

21. Ehnholm, C., Kinnunen, P.K.J., Huttunen, J.K., Nikkilä, E.A. and Ohta, M. Purification and characterization of lipoprotein lipase from pig myocardium. Biochem. J. 149: 649-655, 1975.

22. Jansen, H., Stam, H., Kalkman, C. and Hülsmann, W.C. On the dual localization of lipoprotein lipase in rat heart studies with a modified perfusion technique. Biochem. Biophys. Res. Comm. 92: 411-416, 1980.

23. Hamosh, M., Clary, T.R., Chernick, S.S. and Scow. R.O. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim. Biophys. Acta, 210: 473-482, 1970.

24. Mc.Bride, O.W. and Korn, E.D. The Lipoprotein lipase of mammary gland and the correlation of its activity to lactation. J. Lipid Res. 4: 17-20, 1963.

25. Otway, S. and Robinson, D.S. The significance of changes in tissue clearing-factor lipase activity in relation to the lipaemia of pregnancy. Biochem. J. 106: 677-682, 1968.

26. Robinson, D.S. Changes in the lipolytic activity of the guinea pig mammary gland at parturition. J. Lipid Res. 4: 21-23, 1963.

27. Zinder, O., Hamosh, M.,Clary Fleck, T.R. and Scow, R.O. Effect of prolactin on lipoprotein lipase in mammary gland and adipose tissue of rats. Am. J. Physiol. 226: 744-748, 1974.

28. Egelrud, T. and Olivecrona, T. The purification of a lipoprotein lipase from bovine skim milk. J. Biol. Chem. 247: 6212-6217, 1972.

29. Hamosh, M. and Scow, R.O. Lipoprotein lipase activity in guinea pig and rat milk. Biochim. Biophys. Acta. 231: 283-289, 1971.

30. Hernell, O. and Olivecrona, T. Human milk lipases. I. Serum-stimulated lipase. J. Lipid Res. 15: 367-374, 1974.

31. Brady, M. and Higgins, J.A. The properties of the lipoprotein lipases of rat heart, lung and adipose tissue. Biochim. Biophys. Acta. 137: 140-146, 1967.

32. Cryer, A. and Jones, H.M. The distribution of lipoprotein lipase (clearing factor lipase) activity in the adiposal, muscular and lung tissues of ten animal species. Comp. Biochem. Physiol. 63B: 501-505, 1979.

33. Hamosh, M. and Hamosh, P. Lipoprotein lipase in rat lung. The effect of fasting.
Biochim. Biophys. Acta. 380: 132-140, 1975.

34. Hamosh, M., Yeager, H.Jr., Shechter, Y and Hamosh, P. Lipoprotein lipase in rat lung. Effect of dexamethasone. Biochim. Biophys. Acta. 431: 519-525, 1976.

35. Cryer, A., Riley, S.E., Williams, E.R. and Robinson, D.S. Effect of nutritional status on rat adipose tissue, muscle and post-heparin plasma clearing factor lipase activities: their relationship to triglyceride fatty acid uptake by fat-cells and to plasma insulin concentrations.
Clin. Sci. Molec. Med. 50: 213-221, 1976.

36. Enser, M.. Clearing-factor lipase in obese hyperglycaemic mice (ob/ob). Biochem. J. 129: 447-453, 1972.

37. Linder, C., Chernick, S.S., Fleck, T.R. and Scow, R.O. Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats.
Am. J. Physiol. 231: 860-864, 1976.

38. Lithell, H. and Boberg, J. Determination of lipoprotein-lipase activity in human skeletal muscle tissue. Biochim. Biophys. Acta 528: 58-68, 1978.

39. Dicorleto, P.E. and Zilversmit, D.B. Lipoprotein lipase activity in bovine aorta.
Proc. Soc. Exp. Biol. Med. 148: 1101-1105, 1975.

40. Henson, L.C. and Schotz, M.C. Detection and partial characterization of lipoprotein lipase in bovine aorta. Biochim. Biophys. Acta. 409: 360-366, 1975.

41. Chajek, T., Stein, O. and Stein, Y. Pre- and post-natal development of lipoprotein lipase and hepatic

triglyceride hydrolase activity in rat tissues. Atherosclerosis 26: 549-561, 1977.

42. Benson, J.D., Bensadoun, A. and Cohen,D. Lipoprotein lipase of ovarian follicles in the domestic chicken (Gallus Domesticus). Proc. Soc. Exp. Biol. Med. 148: 347-350, 1975.

43. Shemesh, M., Bensadoun, A. and Hansel, W. Lipoprotein lipase activity in the bovine corpus luteum during the estrous cycle and early pregnancy. Proc. Soc. Exp. Biol. Med. 151: 667-669, 1976.

44. Elphick, M.C. and Hull, D. Rabbit placental clearing-factor lipase and transfer to the foetus of fatty acids derived from triglycerides injected into the mother. J. Physiol. 273: 475-487, 1977.

45. Mallov, S. and Alousi, A.A. Lipoprotein lipase activity of rat and human placenta. Proc. Soc. Exp. Biol. Med. 119: 301-306, 1965.

46. Robinson, D.S. and French, J.E. Heparin, the clearing factor lipase and fat transport. Pharm. Rev. 12: 241-260, 1960.

47. Khoo, J.C., Steinberg, D., Thompson, B. and Mayer, S.E. Hormonal regulation of adipocyte enzymes. The effects of epinephrine and insulin on the control of lipase, phosphorylase kinase, phosphorylase and glycogen synthase. J. Biol. Chem. 248: 3823-3830, 1973.

48. Bragdon, J.H. and Gordon, R.S.Jr. Tissue distribution of ^{14}C after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. J. Clin. Invest. 37: 574-579, 1958.

49. Jones, N.L. and Havel, R.J. Metabolism of free fatty acids and chylomicron triglycerides during exercise in rats. Am. J. Physiol. 213: 824-828, 1967.

50. Borensztajn, J., Rone, M.S., Babirak, S.P., Mc.Garr, J.A. and Oscai, L.B. Effect of exercise on lipoprotein lipase activity in rat heart and skeletal muscle. Am. J. Physiol. 229: 394-397, 1975.

51. Tan, M.H., Sata, T., Havel, R.J. The significance of lipoprotein lipase in rat skeletal muscles. J. Lipid Res. 18: 363-370, 1977.

52. Fielding, C.J. Lipoprotein lipase: evidence for high- and low-affinity enzyme sites. Biochemistry 15: 879-884, 1976.

53. Fielding, P.E., Shore, V.G. and Fielding, C.J. Lipoprotein lipase. Isolation and characterization of a second enzyme species from postheparin plasma. Biochemistry 16: 1896-1900, 1977.

54. Fielding, P.E., Shore,V.G. and Fielding, C.J. Lipoprotein lipase: properties of the enzyme isolated from post-heparin plasma. Biochemistry 13: 4318-4323, 1974.

55. Fielding, C.J. and Havel, R.J. Lipoprotein lipase. Arch. Pathol. Lab. Med. 101: 225-229, 1977.

56. Nilsson-Ehle, P., Garfinkel, A.S. and Schotz, M.C. Intra- and extracellular forms of lipoprotein lipase in adipose tissue. Biochim. Biophys. Acta. 431: 147-156, 1976.

57. Jansen, H., Garfinkel, A.S., Twu, J.S., Nikazy, J. and Schotz, M.C. Regulation of lipoprotein lipase, immunological study of adipose tissue. Biochim. Biophys. Acta. 531: 109-114, 1978.

58. Ashby, P., Bennett, D.P., Spencer, I.M. and Robinson, D.S. Post-translational regulation of lipoprotein lipase activity in adipose tissue. Biochem. J. 176: 865-872, 1978.

59. Ashby, P. and Robinson, D.S. Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipase. Biochem. J. 188: 185-192, 1980.

60. Parkin, S.M., Walker, K., Ashby, P. and Robinson, D.S. Effects of glucose and insulin on the activation of lipoprotein lipase and on protein synthesis in rat adipose tissue. Biochem. J. 188: 193-199, 1980.

61. Spooner, P.M., Chernick, S.S., Garrison, M.M. and Scow, R.O. Insulin regulation of lipoprotein lipase activity and release in 3T3-L1 adipocytes. J. Biol. Chem. 254: 10021-10029, 1979.

62. Jensen, G.L., Baly, D.L., Brannon, P.M. and Bensadoun, A. Synthesis and secretion of lipolytic enzymes by cultured chicken hepatocytes. J. Biol. Chem. 255: 11141-11148, 1980

63. Chajek, T., Stein, O. and Stein, Y. Interference with the transport of heparin-releasable lipoprotein lipase in the perfused rat heart by colchicine and vinblastine. Biochim. Biophys. Acta. 388: 260-267, 1975.

64. Cryer, A., Mc.Donald, A., Williams, E.R. and Robinson D.S. Colchicine inhibition of the heparin-stimulated release of clearing-factor lipase from isolated fat-cells. Biochem. J. 152: 717-720, 1975.

65. Blasquez, E.,Lipshaw, L.A., Blasquez, M. and Foa, P.P. The synthesis and release of insulin in fetal, nursing and young adult rats: Studies in vivo and in vitro. Pediat. Res. 9: 17-25, 1975.

66. Blasquez, E., Sugase, T., Blasquez, M. and Foa, P.P. Neonatal changes in the concentration of rat liver cyclic AMP and of serum glucose, free fatty acids, insulin, pancreatic, and total glucagon in man and in the rat. J. Lab. Clin. Med. 83: 957-967, 1974.

67. Smith, L.C. and Scow, R.O. Chylomicrons. Mechanism of transfer of lipolytic products to cells. Prog. Biochem. Pharmacol. 15: 109-138, 1979.

68. Stein, O. and Stein, Y. Catabolism of serum lipoproteins. Prog. Biochem. Pharmacol. 15: 216-237, 1979.

69. Mc.Kinnon, N.O. and Cryer, A. A comparison of the composition and catabolism in vitro of porcine very low density lipoprotein subfractions prepared by gel exclusion and heparin-affinity chromatography. Int. J. Biochem. 16: 213-218, 1984.

70. Williams, M.P., Streeter, H.B., Wusteman, F.S. and Cryer,

A. Heparan sulphate and the binding of lipoprotein lipase to porcine thoracic aorta endothelium. Biochim. Biophys. Acta. 756: 83-91, 1983.

71. de Gasquet, P. and Pequignot, E. Changes in adipose tissue and heart lipoprotein lipase activities and in serum glucose, insulin and corticosterone concentrations in rats adapted to a daily meal. Horm. Metab. Res. 5: 440-443, 1973.

72. Krotkiewski, M., Björntorp, P. and Smith, U. The effect of long-term dexamethasone treatment on lipoprotein lipase activity in rat fat cells. Horm. Metab. Res. 8: 245-246, 1976.

73. Krausz, Y., Bar-On,H. and Shafrir, E. Origin and pattern of glucocorticoid-induced hyperlipidemia in rats. Biochim. Biophys. Acta 663: 69-82, 1981.

74. Bagdade, J.D., Yee, E., Albers, J. and Pykälistö, O.J. Glucocorticoids and triglyceride transport: effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat. Metabolism 25: 533-542, 1976.

75. Pykälistö, O.J., Vogel, W.C., and Bierman, E.L. The tissue distribution of triacylglycerol lipase, monoacylglycerol lipase and phospholipase A in fed and fasted rats. Biochim. Biophys. Acta. 369: 254-263, 1974.

76. Nilsson-Ehle, P., Tornquist, H., and Belfrage, P. Rapid determination of lipoprotein lipase activity in human adipose tissue. Clin. Chim. Acta 42: 383-390, 1972.

77. Oelofsen, W., and Ramachandran, J. Studies of corticotropin receptors on rat adipocytes. Arch. Biochem. Biophys. 225: 414-421, 1983.

78. Bray, G.A., Trygstad, O. Lipolysis in human adipose tissue: comparison of human pituitary hormones with other lipolytic agents. Acta Endocrinologica 70: 1-20, 1972.

79. Mendelson, C.R., Smith, M.E., Cleland, W.H., and Simpson,

E.R. Regulation of aromatase activity of cultured adipose stromal cells by catecholamines and adrenocorticotropin.
Mol. Cell. Endo. 37: 61-72, 1984.

80. Wing, D.R., Salaman, M.R., Robinson, D.S. Clearing-factor lipase in adipose tissue: factors influencing the increase in enzyme activity produced on incubation of tissue from starved rats in vitro.
Biochem. J. 99: 648-656, 1966.

81. Nestel, P.J., Austin, W. Relationship between adipose lipoprotein lipase activity and compounds which affect intracellular lipolysis.
Life. Sci. 8, part II: 157-164, 1969.

82. Cigolini, M., Smith, U. Human adipose tissue in culture. VIII. Studies on the insulin-antagonistic effect of glucocorticoids. Metabolism 28: 502-510, 1979.

83. Lawson, N., Pollard, A.D., Jennings, R.J., Gurr, M.I. and Brindley, D.N. The activities of lipoprotein lipase and of enzymes involved in triacylglycerol synthesis in rat adipose tissue.
Biochem. J. 200: 285-294, 1981.

84. Chernick, S.S., Spooner, P.M., Garrison, M.M. and Scow, R.O. Effect of epinephrine and other lipolytic agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 adipocytes.
J. Lipid Res. 27: 286-294, 1986.

85. Weisenburg-Delorme, C.L., Harris, K.L. Effects of diet on lipoprotein lipase activity in the rat.
J. Nutr. 105: 447-451, 1975.

86. Childs, M.T., Tollefson, J., Knopp, R.H. and Applebaum-Bowden D. Lipid metabolism in pregnancy. VIII Effects of dietary fat versus carbohydrate on lipoprotein and hepatic lipids and tissue triglyceride lipases.
Metab. Clin. Exp. 30: 27-35, 1981.

87. Brindley, D.N. Regulation of hepatic triacylglycerol synthesis and lipoprotein metabolism by glucocorticoids.
Clin. Sci. 61: 129-133, 1981.

88. Lenz, P.H., Wien, G.H. and Fleischman, A.I. Corticoid release and gluconeogenesis following triglyceride ingestion in the rat. Lipids 5: 524-530, 1970.

89. Hallfrisch, J.,Lazar, F.L. and Reiser, S. Epididymal fat metabolism in rats fed sucrose or starch. Nutr. Rep. Int. 18: 359-367, 1978.

90. Hülsmann, W.C., Geelhoed-Mieras, M.M., Jansen, H., and Houtsmüller, U.M.T. Alteration of the lipase activities of muscle, adipose tissue and liver by rapeseed oil feeding of rats. Biochim. Biophys. Acta 572: 183-187, 1979.

91. Cryer, A., Kirtland, J.,Jones, H.M. and Gurr, M.I. Lipoprotein lipase activity in the tissues of guinea pigs exposed to different dietary fats from conception to three months of age. Biochem. J. 170: 169-172, 1978.

92. Speake, B.K.,Parkin, S.M. and Robinson, D.S. Regulation of the synthesis of lipoprotein lipase in adipose tissue by dexamethasone. Biochim. Biophys. Acta. 881: 155-157, 1986.

93. Borensztajn, J., Keig, P. and Rubenstein, A.H. The role of glucagon in the regulation of myocardial lipoprotein lipase activity. Biochem. Biophys. Res. Com. 53: 603-608, 1973.

94. Hamosh, M and Hamosh, P. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. J. Clin. Invest. 55: 1132-1135, 1975.

95. Kim, H.J. and Kalkhoff, R.K. Sex steroid influence on triglyceride metabolism. J. Clin. Invest. 56: 888-896, 1975.

96. Wilson, D.E., Flowers, C.M., Carlile, S.I. and Udall, K.S. Estrogen treatment and gonadal function in the regulation of lipoprotein lipase. Atherosclerosis 24: 491-499 ,1976.

97. Applebaum, D.M., Goldberg, A.P., Pykälistö, O.J., Bruzell, J.D. and Hazzard, W.R. Effect of estrogen on post-heparin lipolytic activity. J. Clin. Invest. 59: 601-608, 1977.

98. Huttunen, J.K., Ehnholm, C., Kinnunen, P.K.J. and Nikkilä, E.A. An immunochemical method for the selective measurement of two triglyceride lipases in human postheparin plasma.
Clin. Chim. Acta. 63: 335-347, 1975.

99. Stam, S. and Hülsmann, W.C. Effects of hormones, amino acids and specific inhibitors on rat heart heparin-releasable lipoprotein lipase and tissue neutral lipase activities during long-term perfusion.
Biochim. Biophys. Acta 794: 72-82, 1984.

100. Taskinen, M-R., Nikkilä, E., Pelkonen, R. and Sane, T. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome.
J. Endocrinol. Metab. 57: 619-626, 1983.

101. Amri, E-Z., Vannier, C., Etienne, J. and Ailhaud, G. Maturation and secretion of lipoprotein lipase in cultured adipose cells. II. Effects of tunucamycin on activation and secretion of the enzyme.
Biochim. Biophys. Acta 875: 334-343, 1986.

102. Vannier, C., Amri, E., Etienne, J., Négrel, R. and Ailhaud, G. Maturation and secretion of lipoprotein lipase in cultured cells. I. Intracellular activation of the enzyme.
J. Biol. Chem. 260: 4424-4431, 1985.

103. Vannier, C., Etienne, J. and Ailhaud, G. Intracellular localization of lipoprotein-lipase in adipose tissue.
Biochim. Biophys. Acta 875: 344-354, 1986.

CHAPTER II

LIPOPROTEIN LIPASE ACTIVITY AND TRIGLYCERIDE METABOLISM IN THE PERFUSED FAT PAD.

SUMMARY.

The in vitro perfused epididymal fat pad of the rat was used to study several aspects of the relationship between lipoprotein lipase (LPL) activity, hydrolysis rate of lipoprotein triglycerides (TG) and the uptake of liberated fatty acids (FA) by the tissue.

1. In fasted animals 2.0 ± 0.8% and in fed rats 14.4 ± 1.5% of the perfused chylomicron-TG was hydrolyzed. For VLDL-TG these figures were 3.6 ± 1.6% and 4.8 ± 1.2% respectively. Of the perfused chylomicron-TG 1.0 ± 0.2% in fasted and 2.7 ± 0.3% in fed animals was taken up in the tissue. For VLDL-TG these figures were 1.2 ± 0.2% and 0.8 ± 0.2% respectively.

The hydrolysis rate of chylomicron-TG and the uptake of liberated fatty acids was positively correlated in fasted ($r = 0.60$, $p < 0.05$) and fed ($r = 0.53$, $p < 0.05$) animals. Therefore chylomicron-TG seems to be a better substrate for adipose tissue LPL than VLDL-TG in fed animals.

2. Heparin-releasable LPL activity and chylomicron-TG hydrolysis were strongly correlated in fasted ($r = 0.96$; $p < 0.0005$) and fed ($r = 0.94$; $p < 0.002$) animals: no statistically significant correlation was found between heparin-releasable LPL activity and uptake of FA ($r = 0.71$ respectively $r = 0.54$). During all the perfusions a spontaneous LPL-release occurred (about 1 o/oo of the tissue activity per min). The sum of the in 60 min. released LPL activity and the remaining LPL activity in the tissue, was equal to the LPL activity in the non-perfused fat pad.

3. If FA-free albumin was present in the perfusion medium, no hydrolysis of chylomicron-TG or uptake of FA occurred.

4. When the perfusion flow rate was increased from 25 µl/min to 100 µl/min the percentage of the perfused chylomicron-TG hydrolyzed, decreased from 40% to 12%, so that the absolute amount of FA liberated, remained the same.

INTRODUCTION.

The most important function of adipose tissue is to store and liberate fatty acids (FA) (10,13,16,26). The FA are derived from de novo synthesis in the adipocyte and from triglyceride (TG)-rich lipoproteins. Lipoproteine lipase (LPL;EC 3.1.1.34) is the enzyme responsible for the hydrolysis of plasma lipoprotein-TG and can in this way control the removal of TG-FA from the circulation (17,22,23). The substrates for this enzyme are chylomicrons and VLDL. After feeding, when chylomicrons appear in the blood, adipose tissue LPL rises, while skeletal muscle LPL does not; under fasting conditions, when VLDL is the most important TG bearing lipoprotein, LPL activity is low in adipose tissue and high in skeletal muscle, for which tissue FA is an important energy source. Therefore, chylomicron-TG probably represents the physiological substrate for adipose tissue LPL.

It has been demonstrated that LPL is an important enzyme for the hydrolysis of plasma triglycerides. LPL deficiency leads to gross accumulation of chylomicrons in the plasma, while upon specific inhibition of the enzyme activity with antibody either in intact laboratory animals or in the in vitro perfused rat heart hydrolysis of extracellular triglycerides becomes completely impaired (6,12,24). Less clear is the direct relationship between LPL-activity and the rate at which perfused triglycerides are hydrolyzed. Chen et al. (9) have suggested that besides the enzymatic activity of LPL, additional factors may help to regulate the lipoprotein-TG hydrolysis rate. It is also not clear whether a relationship exists between the rate of hydrolysis of lipoprotein-TG in the vascular compartment and uptake of the hydrolysis products in the tissues. Therefore we set out to study this relationship and factors that might influence it. To this purpose we used an in situ epididymal fat pad perfusion model described by Ho and Meng in 1964 (15). In this model physiological conditions are difficult to maintain, and swelling of the tissue may easily occur. Therefore we first established the conditions under which the viability of the tissue was maintained.

MATERIALS & METHODS

Animals.

Male rats of the Wistar strain with a weight between 180 to 220 gram were used. The rats had free access to standard lab chow and tap water unless noted otherwise. Lights were on from 09.30 to 21.30. The fasted groups were deprived of all caloric intake 16 h before use.

Perfusion model.

The perfusion was carried out in principle as described by Ho and Meng (15). The left epididymal fat pad was removed, and used as the non-perfused control for LPL activity. The testicular branch of the right spermatic artery was ligated. The fat pad was placed in a gaze wetted with saline (NaCl 0.9% w/v). Temperature was kept at 37°C. After cannulation of the aorta just below the right spermatic artery (and after ligation of lumbar arteries and the aorta proximally), perfusion was started with a buffered medium (see below) at a flow rate of 100 µl/min. The time between ligation of the aorta and the start of the perfusion was less than one minute. The inferior caval vein was cannulated just below the inflow of the spermatic vein. Large veins entering the vein in this segment were ligated as well as the inferior caval vein proximally.

The fat pad was one way perfused in situ without recirculation. The perfusion medium consisted of minimal essential medium (MEM; Gibco, Paisley, Scotland) supplemented with L-glutamine, non-essential amino acids (Gibco, Paisley, Scotland), $NaHCO_3$ (final concentration 26 mM), albumin (Sigma fraction V, St. Louis, USA; 5% w/v), glucose (5 mM) and insulin (Actrapid^R 40 U/ml, Novo, Denmark; final concentration 100 µU/ml). When indicated, fatty acid-free albumin (Fluka AG, Buchs, Switserland) was used. Washed red blood cells were added as oxygen carrier (haematocrit of the medium: 0.20). When indicated, heparin 50 IU/ml (Tromboliquine, Organon, Oss, the Netherlands) was added to the perfusion medium. The medium was equilibrated with 95% O_2 - 5% CO_2 by gentle continuous bubbling. The vial with medium (6 ml at start) was placed in a small dewar vessel which was circulated with water of 40°C. The temperature of the medium, entering the aorta

was 37°C. The flow rate was governed at the inflow site by a peristaltic pump at 100µl/min, unless noted otherwise. After perfusion for 150 minutes, the ATP content (60 ± 24 nmoles/g) and energy charge (0.91 ± 0.01) of the perfused adipose tissue were similar to that in the non-perfused pad (60 ± 19 nmoles/g resp. 0.93 ± 0.01). The pH of the perfusate remained in the physiological range (7.35 - 7.45). Lactate nor nucleosides were detectable in the perfusate. With tritium labeled leucine, added to the perfusion medium, we found incorporation of aminoacids in the protein fraction of the fat pad, which demonstrated that de novo protein synthesis occurred under the above described circumstances. If the perfusions were carried out with flow rates higher than 200 µl/min, the fat pads became edematous and the ATP level and energy charge dropped to 50% of the control values. Fractions of the perfusate (mostly 10 min.-fractions) were collected in ice-cooled tubes. After perfusion with TG-rich substrates, the perfusion was continued for ten minutes with 0.9% NaCl (w/v) to remove all the intravascular lipid.
In experiments using ^{131}I-labeled albumin in the medium it was found that no label remained in the tissue after ending the perfusion. Therefore no (labeled) chylomicrons remained in the vascular compartment after the wash-out with 0.9% NaCl.

Preparation of TG-rich particles.

Chylomicrons were obtained in principle following the procedure of Bolman et al.(5). Through a tube leading into the jejunum a solution containing 0.6% NaCl, 0.03% KCl and 0.05% Nembutal (Abbott S.A.,Saint Demy sur Avre, France) was infused at a rate of 1 to 2 ml per hour. After 1 - 2 hours this solution was replaced by a mixture of 0.5 ml Intralipid 20% (Kabi Vitrum, Stockholm, Sweden) and 1.5 ml containing 15 µCi 1-^{14}C palmitic acid (Radioactive materials, Amersham, U.K.) complexed to 20% albumin (3). After the fat emulsion was given, infusion was continued with the NaCl, KCl and Nembutal solution. When the lymph became milky in appearance it was collected for 3 hours. From the radioactive label 40% was recovered in this period, 87 to 93% of which was found in the TG fraction. The TG concentration in the lymph was between 25 and 30 mM. The lymph was

centrifuged at 500 g during ten minutes to remove clots. The lymph was added to the perfusion medium at a final TG concentration of 1 mM unless stated otherwise.

With chylomicrons prepared from donor rats on a normal rat chow diet instead of intralipid, similar results were obtained. This indicates that neither the fatty acid composition of the chylomicron-TG nor the way of preparation largely affects the results.

Overnight fasted rats were used as donor for VLDL. One ml of a solution containing 1-^{14}C-palmitic acid (10 µCi), complexed to albumin 20% was injected into the tail vein. After thirty minutes the animals were anaesthesized with ether. Blood was collected from the aorta in tubes containing Na_2 EDTA (final concentration 5 mM) and centrifugated for 15 min at 2000 g Plasma from three rats was pooled. The plasma (9 ml) was transferred into an ultracentrifuge tube (Beckman, polyallomere ultracentrifugation tube) and overlayered with 2 ml NaCl 0.9% (w/v). The VLDL-fraction was isolated by centrifugation at 200.000 g in a swinging bucket rotor (Beckman SW 41) for 20 hours. The top fraction containing VLDL was collected by tube-slicing. Recovery of radioactivity in the VLDL-fraction was 1 - 2% of the injected dose; chemically measured TG concentration in the VLDL containing top fraction was 3 - 4 mM. VLDL fraction was added to the perfusion medium at a final TG concentration of 1 mM.

By determining the FA composition of chylomicron-TG and medium, before and after perfusion, using gas chromatography, we observed that adipose tissue LPL has a low substrate specificity and is unable to discriminate between long chain saturated and unsaturated fatty acids. This is in accordance with the in vitro data of Morly et al. using milk-lipoprotein lipase (19) and with those of Abumrad et al. (1), who found that oleate, stearate, linoleate and palmitate are all good substrates for uptake by adipocytes.

Analytical methods.

Lipid analysis. 100 µl of the perfusion medium and of each perfusate fraction was extracted according to Bligh and Dyer

(4). For the calculation of the recovery of fatty acids and triglycerides an internal standard was added prior to the extraction procedure (5000 dpm ^{3}H-palmitic acid and 5000 dpm tripalmitylglycerol labeled with ^{3}H in the palmitic acid moiety. The organic phase was collected and evaporated under a nitrogen stream. Lipids were dissolved in 100 µl chloroform and quantitatively transferred to a thin layer silicagel plate (0.25 mm; Merck, Darmstadt, BRD). The internal standard samples were brought on the silicagel, to determine the place of fatty acids and triglycerides after development. The plates were developed with a heptane-ether-acetic acid (60:40:1, v:v:v) mixture. The place of internal standards was determined and the areas of the different lipid fractions (fatty acids = FA; monoglycerides = MG; diglycerides = DG; triglycerides = TG) were separately scraped off and radioactivity in ^{3}H and ^{14}C was determined by liquid scintillation counting. The hydrolysis of triglycerides during the perfusion experiments was expressed as the quotient of the increment in radioactive free fatty acids divided by the radioactivity in the TG-fraction at the start of the perfusion. Radioactivity was for 87 to 93% present in TG. The increase in radioactivity in the MG and DG fractions after perfusion was always negligibly. After the perfusion procedure the fat pad was weighed. Part of it was used for lipid extraction (4).
For the estimation of the recovery, ^{3}H-palmitic acid and ^{3}H-triglyceride were added before extraction. The chloroform phase was evaporated under a nitrogen stream and radioactivity counted by liquid scintillation. The uptake of fatty acids in the perfused fat pad was expressed as the quotient of radioactivity that remained in the fat pad after the perfusion and the total radioactivity, in the perfusion-medium at the start, which had passed the fat pad during the perfusion-period.

Lipoprotein lipase measurement.

The fat pads (perfused and the non-perfused controls) were defatted with acetone and diethyl-ether as described by Garfinkel et al. (14). Acetone ether powder LPL was extracted in Tris-ethylene glycol buffer (50 mmoles Tris in 1 M ethylene glycol, pH:8.0 ; 2 - 5 mg powder/ml buffer). From the extract

and the fractions of the perfusate 100 µl was taken to measure LPL. The substrate of the LPL-assay consisted of a glycerol stabilized glycerol-tri 9,10 (n)-^{3}H-oleate phospholipid emulsion as described in detail by Nilsson-Ehle et al. (20). The activity is expressed as milli units (= nmoles free fatty acid released per minute). Serum of overnight fasted male rats served as LPL activator. To study if the above described medium served as a good LPL stabilizer, in vitro experiments were carried out, using the above described perfusion medium and Krebs-Ringer solution (pH:8.3, supplemented with albumin, 5% w/v and glucose, 5 mM). As LPL source, we used the heparin-elutable LPL activity from adipose tissue after an incubation for 40 minutes at 37°C as described by Taskinen et al.(25). The LPL activity measured in this medium was said to be 100%. One ml of this medium was then added to 10 ml of the above described perfusion and Krebs Ringer medium, and held at 37°C for another twenty minutes. The LPL activity fell in this period from 100% to 92% using the perfusion medium and to 78% of the LPL activity at the start using Krebs-Ringer solution. Therefore, the medium we used in our perfusion experiments stabilized LPL activity much better than the Krebs-Ringer solution did.

Other methods.

Triglycerides (Merck-diagnostica, Darmstadt, BRD) and lactate (Boehringer Mannheim, BRD) were determined with an enzymatic method, free fatty acids according to Laurell (18) and nucleosides by High Performance Liquid Chromatography. The statistical significance of differences between groups was calculated, using the unpaired Student's t-test.

RESULTS.

Effect of different conditions on lipoprotein lipase activity.

LPL activity was measured in the perfusate and in the acetone ether powder of the epididymal fat pads. Without heparin addition to the medium a spontaneous release of LPL was detected during the whole perfusion period (Fig.1). The activity was 5-times higher in the fed compared to the fasted animals. Addition of heparin 60 minutes after the the initiation of the

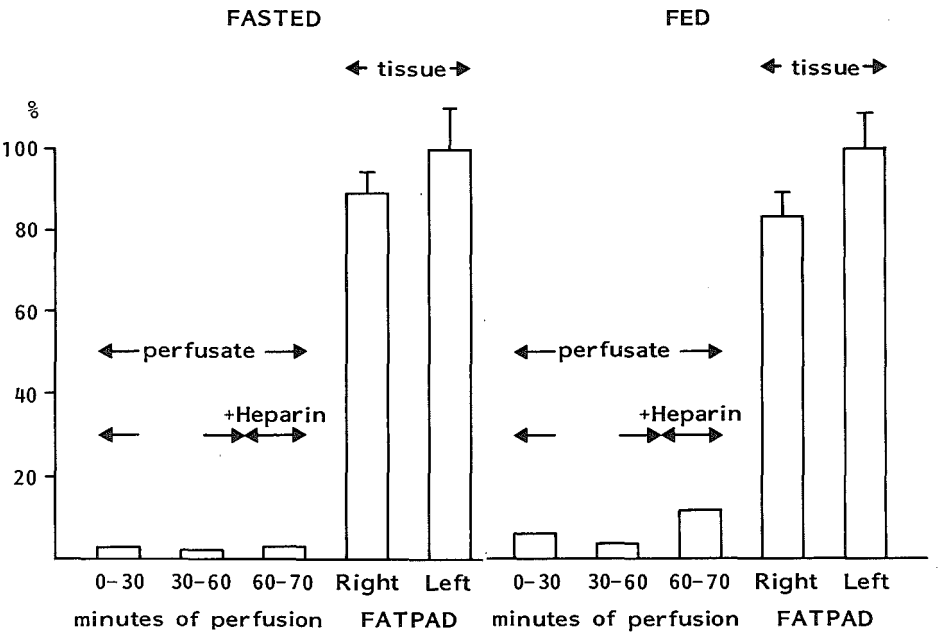


Fig. 1

LPL activity in perfusion medium (= perfusate) after a 70 minutes lasting perfusion in fed and fasted animals (each group consisted of 7 animals). During the last 10 minutes of the perfusion, heparin (50 IU/ml) was added. The LPL activities are expressed as percentage of the tissue LPL activity of the non-perfused left pads (mean ± SEM); the mean of the latter was taken as 100%.

perfusion increased the release of enzyme activity especially in the pads of fed rats. The percentage of the heparin-releasable pool of the LPL activity of the non-perfused fat pad, was in both conditions the same. After the perfusion of fat pads of fed animals with heparin, the LPL activity remaining in the perfused right pad was 83 ± 6% (n = 7) of that in the non perfused left pad (100 ± 9%). In fasted animals this decrease by heparin was in absolute numbers less clear but percentually the same (89 ± 5% vs. 100 ± 10% n = 7), when the LPL activity in the non-perfused pad is taken as 100% (Fig.1). The difference in LPL activity between the perfused and non-perfused fat pad is accounted for by the LPL activity in the heparin-releasable fraction (11 ± 1% in fasted and 17 ± 2% in fed animals).

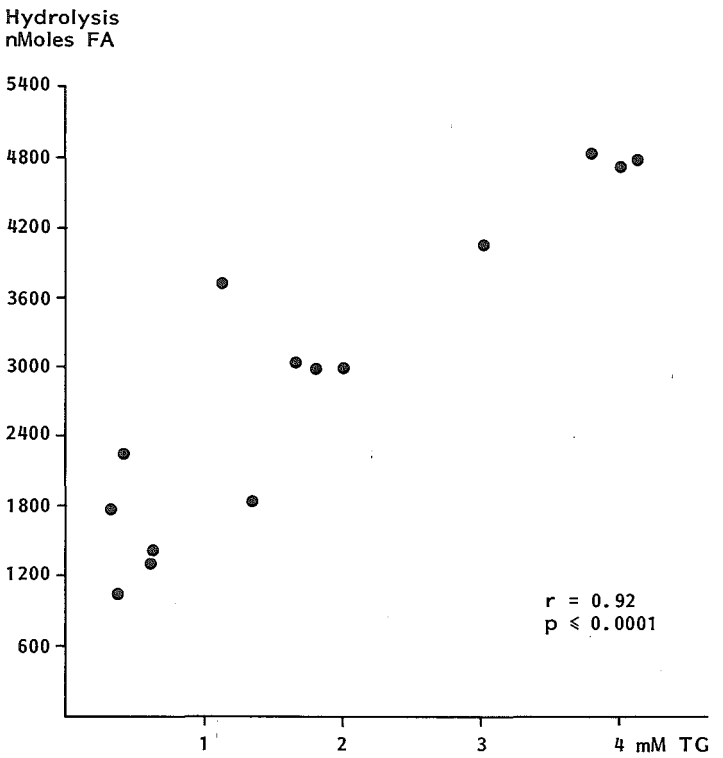


Fig. 2

Influence of various chylomicron-TG concentrations (mM) in the perfusion medium on the hydrolysis rate (expressed as nmoles FA) using the epididymal fat pad of fed animals.

Hydrolysis and uptake of TG-rich lipoproteins in the perfused fat pad.

During a sixty minutes lasting perfusion of the right epididymal fat pad of fed animals with chylomicrons, a positive correlation was found between TG concentration and hydrolysis rate ($r = 0.92$ $p < 0.0001$) using TG concentrations up to 4 mM (Fig.2). The uptake of liberated FA was positively correlated ($r = 0.85$; $p < 0.0003$) with the TG concentration in the perfusion medium (Fig.3).

Hydrolysis rate and uptake of liberated FA were in these experiments correlated ($r = 0.86$; $p < 0.0002$).

The uptake of FA during different time intervals of the perfusion period was measured in four experiments each.

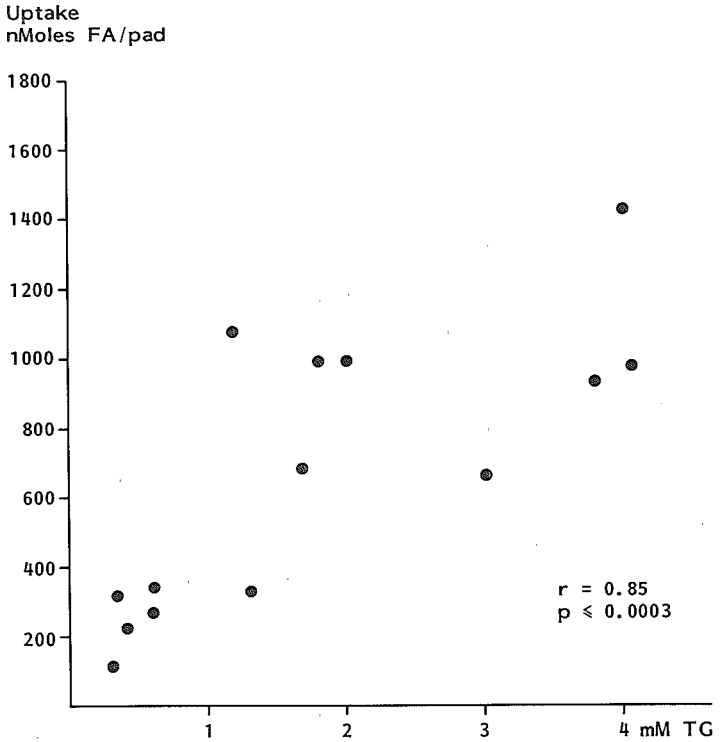


Fig. 3

Influence of various chylomicron-TG concentrations (mM) in the perfusion medium on uptake of liberated fatty acids (nmoles/pad) by the perfused adipose tissue of fed animals.

Perfusion of pads from fasted animals were carried out starting with a medium containing labeled chylomicrons during twenty minutes or after preperfusion of the fat pad for fourty-five minutes with unlabeled chylomicrons followed by perfusion with labeled chylomicrons. The uptake of ^{14}C-FA was 1.6 ± 0.3% and 1.4 ± 0.2% respectively. So, preferential uptake of label was not found, and triglyceride removal by the perfused adipose tissue remained essentially constant during the perfusion period.

The effect of different FA concentrations in the perfusion medium, leading to different FA/albumin ratios on TG-hydrolysis of chylomicrons were studied (Table I). An increase in the concentration of FFA in the perfusion medium (^{3}H-labeled palmitic acid complexed to albumin), had no effect on TG-hydrolysis or on the uptake of ^{14}C-FA, liberated from TG.

Table I

The effect of the fatty acid concentration on chylomicron-triglyceride hydrolysis and uptake of fatty acids in the perfused fat pad°.

ratio FA/albumin mol/mol	hydrolysis %	uptake ^{14}-FA* nmol/pad	uptake ^{3}H-FA+ nmol/pad
1:1	19 ± 3	515 ± 98	404 ± 62
2:1	12 ± 2	830 ± 293	896 ± 289
4:1	17 ± 3	844 ± 176	1745 ± 324**

° Results are expressed as mean ± SEM of three separate experiments. In every experiment ^{3}H-labeled palmitic acid complexed to albumin as well as chylomicrons were added to the perfusion medium.
* Uptake of hydrolyzed ^{14}C-labeled fatty acids from chylomicrons.
\+ Uptake of ^{3}H-labeled palmitic acid complexed to albumin in nmoles per pad.
** Significantly different from the group with ratio FA/albumin 1 : 1 ($p < 0.02$).

In the absence of FA (FA-free albumin) hydrolysis of TG could not be detected. The uptake in the fat pad of ^{3}H-palmitic acid, complexed with albumin, was related to the concentration and thus to the ratio of FFA/albumin in the perfusion medium (Table I). This indicates that uptake of free fatty acids (bound to albumin) is concentration dependent, and independent from the uptake of fatty acids, released into the vascular compartment during hydrolysis of chylomicron-TG.

If the flow rate was changed from 100 µl to 25 µl/min the percentage of the perfused chylomicron-TG that was hydrolyzed increased with a factor four. However, the uptake of liberated fatty acids in the tissue did not change (Table II).

The rate of TG-hydrolysis in chylomicrons was low in fat pads of fasted animals and significantly higher when fed animals were used. Hydrolysis of VLDL-TG in fasted animals showed a greater variation but was not significantly different from that found with chylomicrons (Table III). In fed animals the hydrolysis of chylomicron-TG was much higher than that of VLDL-TG.

From the fatty acids liberated from chylomicron-TG during perfusion of pads of fasted animals about 50% was taken up in the tissue corresponding with 1.0 ± 0.2% of the total amount of chylomicron-TG that passed the pads during the perfusion period (Table III). The uptake of chylomicron-FA by the perfused adipose tissue after feeding was 2 - 3 times higher than in

Table II

Effect of flow rate on the hydrolysis of chylomicron triglyceride and uptake of fatty acids in the perfused fat pad*

flow rate	hydrolysis %	uptake ^{14}C-FA° nmoles/pad	uptake ^{3}H-FA+ nmoles/pad
25 ul/min	40 ± 6**	669 ± 142	453 ± 64
50 ul/min	18 ± 1	1060 ± 333	494 ± 103
100 ul/min	12 ± 2	830 ± 293	896 ± 289

* Results are given as mean ± SEM of three experiments, molar ratio FA/ albumin was 2:1 In every experiment ^{3}H-labeled palmitic acid complexed to albumin as well as chylomicrons were added to the perfusion medium.

° Uptake of ^{14}C-labeled fatty acids liberated from chylomicrons, TG concentration 1 mM, expressed as nmoles per pad

\+ Uptake of ^{3}H-labeled palmitic acid complexed to albumin expressed as nmoles per pad

** Significantly different from both other groups ($p < 0.05$)

overnight fasted animals. In the fed condition 20% of the fatty acids liberated during chylomicron-TG hydrolysis is taken up by the adipose tissue.

In fasted and fed animals a strong correlation between heparin-releasable LPL activity and TG-hydrolysis of chylomicrons was found ($r = 0.96$, $p < 0.0005$ respectively $r = 0.94$, $p < 0.0002$, Table IV) and a much lower correlation existed between acetone ether powder LPL activity and chylomicron-TG hydrolysis ($r = 0.46$ respectively $r = 0.49$). In both conditions a low, and statistically not significant relationship was found between heparin-releasable LPL-activity and acetone-ether LPL activity ($r = 0.34$ in fasted respectively $r = 0.20$ in fed animals). In these experiments, hydrolysis rate of chylomicron-TG and uptake of the liberated fatty acids were significantly correlated with each other ($r = 0.60$ in fasted and $r = 0.54$ in fed animals, $p < 0.05$).

Table III

Hydrolysis of chylomicron-TG and uptake of liberated fatty acids by the perfused fat pad from fasted and fed animals.

		fasted rats		fed rats	
	n	hydrolysis %	uptake %	hydrolysis %	uptake %
chylomicrons	7	2.0 ± 0.8	1.0 ± 0.2	14.4 ± 1.5°	2.7 ± 0.3*
VLDL	7	3.6 ± 1.6	1.2 ± 0.2	4.8 ± 1.2**	0.8 ± 0.2**

Results are mean ± SEM
Hydrolysis and uptake are expressed as percentage as described in Materials and Methods

°	fed vs. fasted group	$p < 0.001$
*	fed vs. fasted group	$p < 0.001$
**	VLDL group vs. comparing chylo group	$p < 0.01$

Table IV

Relationship between heparin-releasable and acetone-ether LPL activity on the one hand and hydrolysis rate and uptake of liberated fatty acids on the other hand, in fasted and fed animals.

condition	n	TG° mM/l	heparin-releasable LPL activity (mU)	acetone-ether LPL activity* (mU/gww)	hydrolysis (nMoles FA/pad)	uptake (nMoles/pad)
fasted	7	0.88 ± 0.06	8 ± 1+	73 ± 7	564 ± 51●	130 ± 12
fed	7	0.93 ± 0.06	43 ± 5+	249 ± 25	2384 ± 186●	443 ± 67

Data are expressed as mean ± SEM.

° ^{14}C-labeled chylomicrons were used. End concentration of TG in the perfusion medium is given.

* The acetone-ether LPL activity was determined in the left, non-perfused fat pad

\+ Correlation between heparin-releasable LPL activity and hydrolysis rate in fasted ($r = 0.96$, $p < 0.0005$) and fed ($r = 0.94$, $p < 0.0002$) animals.

● Correlation between hydrolysis rate of chylomicron-TG and uptake of liberated FA in fasted ($r = 0.60$) and fed ($r = 0.53$) animals, $p < 0.05$.

DISCUSSION.

In the present study we applied some modifications to the perfusion technique, of the epididymal fat pad as described by Ho and Meng (15). Aims were to prevent swelling of the tissue and to maintain the adipose tissue in a good condition during the procedure. Judged by the ATP content and energy charge of the adipose tissue before and after perfusion, the tissue could

be kept in a metabolically healthy state for 150 minutes. This was accomplished using a flow rate of maximally 100 µl/min (in vivo the flow rate is 70 µl/g/min), and a medium containing albumin, glucose, insulin, amino acids and red blood cells as oxygen carrier. During all experiments we observed a spontaneous LPL-release in the perfusion medium. This is also reported by Scow et al. (22), but not by Fielding (11). The activity released into the medium was in fasted animals about 1 o/oo per minute of the total LPL activity in the tissue. In the fed state the spontaneous release of LPL activity was 4 - 5 times higher, but as a percentage of the LPL activity present in the fat pad the same as in fasted animals. Addition of heparin to the medium increased the release of the enzyme 20 to 25 times over the basal value. LPL activity in adipose tissue can be measured with different methods:in a homogenate of an acetone-ether powder or after elution of LPL from the adipose tissue by heparin. The latter method is believed to reflect the physiologically active extracellular enzyme activity.

Recently we described that in fed rats the enzyme activity after heparin elution in vitro was 10 times higher than in the fasted animals in contrast to a factor three found in acetone-ether powder extracts (2). In the perfusion model of the epididymal fat pad this factor was similar (3 to 5) for both the acetone ether powder LPL and the heparin-releasable LPL (Fig.1). Therefore with the in vitro heparin elution method not only the enzyme in the vascular compartment appears to be measured. However LPL is a labile enzyme and its activity rapidly decreases at 37°C. In vitro the elution temperature is 30°C, so the difference between LPL activity found with the in vitro elution method and the in situ perfusion method could also (partially) be explained by less loss of activity in vitro. Although the sum of the LPL activity released from the endothelium and the remaining LPL activity in the fat pad after the perfusion procedure, was equal to the activity extracted from the non-perfused fat pad, this does not exclude the possibility of an underestimation of LPL activity during the heparin perfusion.

The heparin-releasable pool measured by perfusion of the epididymal fat pad is 10%-20% of the total pool, in the fasted and fed state. This means that a considerable part of newly synthesized enzyme during feeding remains in the non-releasable pool. A strong correlation was found between the rate of chylomicron TG hydrolysis and the total amount of heparin-releasable LPL in the perfusion medium ($r=0.94$). This once more establish the conclusion that the LPL activity in the vascular compartment is the physiologically active fraction, especially since chylomicron-TG does not pass the endothelium (22,23).

In vitro we found that the LPL activity is influenced by the FA concentration: the enzyme activity is low using fatty acid-free albumin and increased to a maximum when the FA-concentration was increased leading to a FA/albumin ratio of 5 : 1. Albumin saturated with FA in the medium did inhibit the LPL activity. In the perfusion model of the fat pad with fatty acid-free albumin in the medium also no hydrolysis of chylomicrons could be detected. Using albumin with different amounts of fatty acids complexed to it, hydrolysis and uptake was present but did not change with different FA/albumin ratios. So in situ the relationship between FA-albumin and LPL activity appears less important than it seems to be in vitro. One of the possible explanations for this difference could be that in the perfusion situation the adipose tissue by itself is a FA acceptor too.

As to the influence of flow rate Scow et al. described a relationship with hormone sensitive FFA release from the fat pad, but he did not report on an influence of flow rate on chylomicron hydrolysis (22,23). We found a percentual increase of the chylomicron hydrolysis if the flow rate was lowered (Table II), but the uptake of liberated fatty acids did not increase. In fact not the hydrolysis of TG by LPL, but the uptake of FA by the adipose tissue appears to be the limiting factor.

The hydrolysis of chylomicrons and VLDL is low in the fasted state (Table III). In fed animals hydrolysis of chylomicron-TG had risen, but VLDL-TG hydrolysis did not change. These differences were also found for the uptake of the liberated TG-FA. This is in line with the general idea that chylomicron-TG is

preferentially taken up by adipose tissue and VLDL-TG by muscle tissue. However it should be kept in mind that in contrast to the chylomicrons used VLDL isolated from plasma has already been in contact with LPL. Therefore, the particles isolated may be regarded as a less good substrate for LPL. It is difficult to compare our results with data derived from in vivo studies on the uptake of liberated FA from chylomicron-TG by adipose tissue, since the liver is removing most of it after an intravenous injection (7,8). However, the uptake of chylomicron-TG-FA in perfused adipose tissue in our hands is comparable to that calculated by Scow et al. from their experiments with perfused parametrial fat (22,23). The uptake of liberated fatty acids is correlated with the chylomicron-TG concentration in the medium at least up to 4 mM TG; higher concentrations were not used.

In this study we perfused the epididymal fat pad of the rat under standardized, physiological conditions. It is demonstrated that for adipose tissue LPL, chylomicrons are a better substrate than VLDL. We confirm data from the literature that LPL activity in the heparin-releasable compartment strongly correlated with chylomicron-TG hydrolysis and less so with the uptake of liberated fatty acids. Only a relatively small part of the LPL activity after feeding (activated or synthesized?), is heparin releasable. Therefore with the methodology employed, the epididymal fat pad perfusion seems to be an outstanding model to study the regulation, synthesis and transport of lipoprotein lipase from the adipocyte to the endothelial compartment.

REFERENCES.

1. Abumrad, N.A., J.H. Park, and C.R. Park. Permeation of long chain fatty acid into adipocytes. J. Biol. Chem. 259: 8945-8953, 1984.

2. Baggen, M.G.A., R. Lammers, H. Jansen and J.C. Birkenhäger. The effect of synacthen administration on lipoprotein lipase activity in the epididymal fat pad of the rat. Metabolism 34: 1053-1056, 1985.

3. Birkenhäger, J.C. and T. Tjabbes. Turnover rate of plasma FFA and rate of esterification of plasma FFA to plasma triglycerides in obese humans before and after weight reduction. Metabolism 18: 18-32, 1969.

4. Bligh, E.G. and W.J. Dyer. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol 37: 911-917, 1959.

5. Bollman,J.L., J.C. Cain, J.H. Grindlay. Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. J. Lab. Clin. Med. 33: 1349-1353, 1948.

6. Borensztajn, J. and D.S. Robinson. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. J. Lipid Res. 11: 111-117, 1970.

7. Borgström, B. and P. Jordan. Metabolism of chylomicron glyceride as studied by ^{14}C-glycerol ^{14}C-palmiticacid labeled chylomicrons. Acta Soc. Med. Upsalien 64: 185-193, 1959.

8. Bragdon, J.H. and R.S. Gordon Jr. Tissue distribution of ^{14}C after the intravenous injection of labeled chylomicrons and unes terified fatty acids in the rat. J. Clin. Invest. 37: 574-579, 1958.

9. Chen, Y-D.I., T.R. Risser, M. Cully and G.M. Reaven. Is the hypertriglyceridemia associated with insulin deficiency caused by decreased lipoprotein lipase activity ? Diabetes 28: 893-898, 1979.

10. Dole, V.P. and J.T. Hamlin. Particulate fat in lymph and blood. Phys. Rev. 42: 674-701, 1962.

11. Fielding, C.J. Lipoprotein lipase. Evidence for high- and low affinity enzyme sites. Biochemistry 15: 879-884, 1976.

12. Fielding, C.J. and R.J. Havel. Lipoprotein lipase. Arch. Pathol. Lab. Med. 101: 225-229, 1977.

13. Frederickson, D.S. and R.S. Gordon.Jr. Transport of fatty acids. Phys. Rev. 38: 585-630, 1958.

14. Garfinkel, A.S. and M.C. Schotz. Separation of molecular species of lipoprotein lipase from adipose tissue. J. Lipid Res. 13: 63-68, 1972.

15. Ho, R.J. and H.C. Meng. A technique for the cannulation and perfusion of isolated rat epididymal fat pad. J. Lipid Res. 5: 203-209, 1964.

16. Jeanrenaud, B. Dynamic aspects of adipose tissue metabolism. Metab. Clin. Exp. 10: 535-581, 1961.

17. Kompiang, I.P., A. Bensadoun and Ming-Whui Wang Yang. Effect of an anti-lipoprotein lipase serum on plasma triglyceride removal. J. Lipid Res. 17: 498-505: 1976.

18. Laurell, S. and G. Tibbling. Colorimetric micro-determination of free fatty acids in plasma. Clin. Chim. Acta 16: 57-62, 1967.

19. Morley, N. and A. Kuksis. Lack of fatty acid specificity in the lipolysis of oligo- and polyunsaturated triacylglycerols by milk lipoprotein lipase. Biochim. Biophys. Acta 487: 332-342, 1977.

20. Nilsson-Ehle, P. and M.C. Schotz. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J. Lipid Res. 17: 536-541, 1976.

21. Robinson, D.S. The function of the plasma Triglycerides in Fatty acid transport. In: Comprehensive Biochemistry Amsterdam, Elsevier, edited by. Florkin and E.H. Stot 1970: 18, pp. 51-116.

22. Scow, R.O. Perfusion of isolated adipose tissue:

FFA release and blood flow in rat parametrial fat body. In: Handbook of Physiology. section 5: Adipose Tissue. Baltimore USA, Waverly Press Inc. 1965: pp. 437-455.

23. Rodbell, M. and R.O. Scow. Chylomicron metabolism: uptake and metabolism by perfused adipose tissue. In: Handbook of Physiology. section 5: Adipose Tissue. Baltimore USA, Waverly Press Inc. 1965: pp. 491-498.

24. Scow R.O., M. Hamosh, E.J. Blanchette-Mackie and A.J. Evans. Uptake of blood triglyceride by various tissues. Lipids 7: 497-505, 1972.

25. Taskinen, M.R., E.A. Nikkilä, J.K. Huttunen and H. Hilden. A micromethod for assay of lipoprotein lipase activity in needle biopsy samples of human adipose tissue and skeletal muscle. Clin. Chim. Acta. 104; 107-117, 1980.

26. Vaughan, M. The metabolism of adipose tissue in vitro. J. Lipid Res. 2: 293-316, 1961.

CHAPTER III

THE EFFECT OF SYNACTHEN ADMINISTRATION ON LIPOPROTEIN LIPASE ACTIVITY IN THE EPIDIDYMAL FAT PAD OF THE RAT.

M.G.A.Baggen, R.Lammers, H.Jansen and J.C.Birkenhäger.

Metabolism 1985, 34: 1053-1056.

SUMMARY

Conflicting data have been reported on the influence of (excess) glucocorticoids on lipoprotein lipase (LPL) activity in adipose tissue. To solve this problem hypercorticism was induced in rats by treatment for varying periods with Synacthen, a synthetic corticotrophin-1-24 preparation, and LPL was measured in the epididymal fat pads using different methods. In extracts of defatted tissue preparations from overnight fasted rats treated for 3 days with Synacthen we observed an increase in LPL activity (acetone-ether powder LPL) to values similar to those found in normally fed controls. In contrast, the heparin-elutable part of LPL activity in the tissue was not influenced by the Synacthen treatment. This activity remained significantly lower in overnight fasted animals, whether with Synacthen treated or not, than in normally fed rats. Adrenalectomy lowered the acetone-ether powder LPL activity of the epididymal adipose tissue in fasted as well as in fed rats. In fasted rats it prevented the stimulation of the LPL activity by Synacthen.

INTRODUCTION

Hypercortisolism leads to profound changes in lipid metabolism (1,2). Besides an increase in plasma lipid levels (3,4) the fat distribution in the body changes. Subcutaneous fat depots are depleted and the size of the omental fat stores increases. While an increased lipogenesis may contribute to the elevation of triglyceride-rich plasma lipoproteins, the mechanisms leading to the fat redistribution are not clear. In the rat experimentally induced hypercorticism leads to a situation comparable to that found in man. The omental fat tissue reflected in the epididymal fat pad, maintains its weight while the size of the subcutaneous fat depots and body weight are lowered (5). Lamberts et al. (6) demonstrated a potentiating effect of glucocorticoids on the mobilisation of triglyceride in the epididymal fat pad of the rat. Since the fat pad weight does not change, an increased input of fatty acids into the triglyceride pool of the fat pad has to be assumed. These fatty acids can be derived from de novo synthesis or from plasma triglycerides taken up after

prior hydrolysis under influence of lipoprotein lipase (LPL). The activity of this enzyme is generally considered to be the rate-limiting factor in the uptake of serum triglycerides. Some investigators found a decrease in LPL activity in the adipose tissue of rats during hypercorticism (7-10). Others however found an opposite effect (11). In humans no difference between adipose tissue LPL activity of patients with Cushing's syndrome and controls was found (12). These conflicting results may have been caused by several factors: different methods for the in vitro LPL assay have been used, studies have been conducted in fed and fasted animals treated for different periods with different hormone preparations. Moreover the genesis of the Cushing's syndrome varies (pituitary adenoma with raised corticotrophin levels or cortisol secreting adrenal tumors with suppression of corticotrophin secretion). To study to what extent these various factors can contribute to the differential effects on LPL observed we made rats hypercorticistic using a corticotrophin analogue for varying periods of time and measured the LPL activity of the epididymal fat pad with different methods in intact and adrenalectomized rats.

MATERIALS AND METHODS

Male rats of the Wistar strain with a weight of 200-250 g were used. The rats had free access to standard lab chow and tap water unless noted otherwise. To assure survival for a short period adrenalectomized rats received 0.9% NaCl (w/v) in the drinking water. Lights were on from 8.00 a.m. till 6.00 p.m. Hypercorticism was induced by daily subcutaneous injections in the suprascapular region with 50 ug (= 5 I.U.) of a synthetic 1-24 corticotrophin (Synacthen depot, Ciba Geigy NV, Belgium). Treatment was for 3 or 20 days. The drug was administered late in the afternoon. This regimen leads in intact rats to hypercorticism with plasma corticosterone levels of 610 ± 137 ng/ml vs. 258 ± 120 ng/ml in the controls (13). For each experimental condition 6 animals were used. The control groups were injected with 0.9% NaCl (w/v). The fasted groups were deprived of all caloric intake 16 hours before use. The rats were killed by

decapitation between 9.00 and 10.00 a.m. The blood was collected in ice-cooled tubes containing 10 mg Na_2 EDTA. Half of the right epididymal fat pad was defatted with acetone and diethyl-ether as described by Nilsson-Ehle et al. (15). (acetone-ether powder). LPL activity was measured in Tris-ethylene glycol buffer extracts (1-3 mg powder/ml) on the same day (14,15). This activity is referred to as acetone-ether powder LPL activity. The other half of the fat pad was cut into pieces of about 20 mg, and placed into 10 ml medium consisting of Krebs-Ringer buffer (pH: 8.3), supplemented with albumin (5% w/v, bovine serum albumin, Fraction V, Sigma St.Louis, USA), heparin (50 IU/ml, Tromboliquine, Organon, Oss, The Netherlands) and glucose (5mM). After incubation for 40 minutes at 30°C in a shaking water bath, the tissue was removed and 100 µl of the medium was incubated with an equal amount of a substrate mixture to measure LPL (Heparin-elutable LPL (16)). The LPL substrate consisted of a glycerol stabilized glycerol-tri 9,10(n)-^{3}H-oleate, phospholipid emulsion as described in detail by Nilsson-Ehle et al. (15). The activity is expressed in milli Units (nmoles free fatty acid released per min). Serum of overnight fasted male rats served as LPL activator. Insulin (IRI) was measured with a I^{125} radioimmunoassay (Insulin RIA, Immuno Nuclear Cooperation, Stillwater, Minn, USA). The statistical significance of differences between groups was calculated comparing each experimental group with its control group using the unpaired Student's t-test.

RESULTS

"Physiological" effects of Synacthen treatment.

Synacthen administration affected body- and adrenal weight, but not the weight of the epididymal fat pads. Water intake in the several experimental groups was similar. Food intake was lower in the adrenalectomized rats than in the non-adrenalectomized controls and Synacthen treated rats. (Adrenenalectomized rats: 13 g per day, controls: 20 g per day and Synacthen treated rats: 19 g per day per rat). There was no difference in food intake by the rats treated with Synacthen for 3 or 20 days. The adrenal

Table I. Effect of Synacthen on Body, Epididymal Fat Pad, and Adrenal Weight in Rats*

Feeding Condition	Intact/ adx†	Treatment Saline/Synacthen	Duration of treatment (d)	Body Weight (g)	Epididymal Fat Pad (mg)	Adrenals (mg)	Insulin μU/mL
Fasted☆	Intact	Saline	3	214 ± 3	1,417 ± 160	30 ± 3	7.8 ± 4.7
	intact	Synacthen	3	199 ± 10	1,556 ± 188	64 ± 5	20.6 ± 8.4
	intact	Saline	20	275 ± 12	1,614 ± 288	27 ± 3	10.1 ± 2.0
	intact	Synacthen	20	183 ± 16	1,728 ± 315	104 ± 18	22.7 ± 5.6
	adx	Saline	3	192 ± 9	763 ± 164	—	ND
	adx	Synacthen	3	190 ± 18	988 ± 372	—	ND
Fed	intact	Saline	3	233 ± 14	1,034 ± 202	27 ± 2	25.0 ± 3.7
	intact	Synacthen	3	209 ± 11	813 ± 113	64 ± 10	45.3 ± 7.8
	intact	Saline	20	282 ± 13	1,651 ± 303	25 ± 3	22.7 ± 2.9
	intact	Synacthen	20	198 ± 8	1,580 ± 401	121 ± 12	40.7 ± 4.4
	adx	Saline	3	176 ± 5	470 ± 46	—	ND
	adx	Synacthen	3	186 ± 11	533 ± 156	—	ND

* rats were adrenalectomized ten days before starting Synacthen injections, which they received for three days.
† adx; bilateral adrenalectomized.
☆ rats were fasted for 16 hours before being killed.

weight showed an increase up to 4-5 times the basal value. Insulin levels were elevated after three days of Synacthen treatment and did not change during further Synacthen treated fasted rats and in the normally fed controls insulin levels were similar. Synacthen treatment induced a further increase (Table I) of insulin levels in the fed state.

Lipoprotein lipase activity in control, Synacthen-treated and adrenalectomized rats.

LPL activity in epididymal fat pads was measured in rats treated with Synacthen with or without prior adrenalectomy. Adrenalectomy led to a lowered acetone-ether powder LPL activity in normally fed and overnight fasted rats (Table II). Upon Synacthen administration the LPL in overnight fasted intact animals was increased nearly to the values measured in normally fed controls. This effect was completely abolished by adrenalectomy. In normally fed rats no further (significant) increase in the LPL activity during Synacthen induced hypercorticism was observed. In normally fed rats acetone-ether powder LPL activity was after adrenalectomy comparable to that found in overnight fasted intact rats.

Table II. Effect of Synacthen-Administration for 3 Days and/or Adrenalectomy on Acetone-Ether Powder Lipoprotein Lipase Activity in Normally Fed and Overnight Fasted Rats

Treatment	Lipoprotein Lipase (mU/g wet weight)	
	Fasted Rats	Fed Rats
Saline	84 ± 15†	150 ± 47*
Saline and adrenalectomy§	48 ± 13	81 ± 16
Synacthen	140 ± 25☆	180 ± 11
Synacthen and adrenalectomy	65 ± 35	94 ± 33

* significantly different from the fasted untreated group ($p < 0.02$) and adrenalectomized groups.
† significantly different from the fasted adrenalectomized group ($p < 0.05$).
☆ significantly different from the untreated fasted group ($p < 0.001$).
§ rats were adrenalectomized ten days before starting Synacthen treatment.

LPL activity in control and Synacthen treated rats determined with different methods.

When measured with the heparin-elution method the LPL activity in normally fed rats was much higher than with the acetone-ether powder method. In fasted animals the opposite was found (compare Tables III and IV). This resulted in a fed/fasted ratio of LPL activity of about two with the acetone-ether method and of more than ten with the heparin-elution method.

Table III. Effect of Synacthen Treatment on Acetone-Ether Powder LPL Activity of the Epididymal Fat Pad in Normally Fed and Overnight Fasted Rats*

Treatment Saline/Synacthen	Duration of Treatment (d)	Lipoprotein Lipase (mU/g wet weight) Fasted	Fed
Saline	3	84 ± 15	150 ± 47☆
Synacthen	3	140 ± 25†	180 ± 11
Saline	20	105 ± 26	133 ± 34
Synacthen	20	133 ± 61	143 ± 81

* The mean LPL activities ± 1 SD are given for different periods of Synacthen treatment. Each experimental group consisted of six animals in the fasted and four animals in the fed state.
† significantly different from the untreated fasted group ($p < 0.001$).
☆ significantly different from the untreated fasted group ($p < 0.02$).

After Synacthen treatment for 3 days the acetone-ether powder LPL activity was 66% higher than in non-treated controls if the rats were fasted overnight prior to use. The LPL activity was then comparable to that in normally fed controls. After 20 days the difference in LPL between Synacthen-treated and non-treated fasted rats was much less (27%) and statistically not significant. The increase in LPL after three days of Synacthen

treatment was not found if the rats were not fasted overnight (Table III). With the heparin-elution method no change in LPL activity was found after treatment with Synacthen of fasted or fed rats (Table IV).

Table IV. Effect of Synacthen Treatment on Heparin Elutable LPL Activity of the Epididymal Fat Pad in Normally Fed and Overnight Fasted Rats*

Treatment Saline/Synacthen	Duration of Treatment (d)	Lipoprotein Lipase (mU/g wet weight) Fasted	Fed
Saline	3	29 ± 7	444 ± 102†
Synacthen	3	24 ± 12	464 ± 164†
Saline	20	28 ± 7	382 ± 75
Synacthen	20	19 ± 9	317 ± 160

* The mean LPL activities are given ± SD for different states and periods of Synacthen treatment. Each experimental group consisted of six animals in fasted and four animals in fed state.
† significantly different from the fasted group ($p < 0.001$).

DISCUSSION

The role of LPL in the uptake of fatty acids derived from triglycerides in the blood (TG-FA) by adipose tissue has received considerable interest during the past twenty years (17-20). Chylomicrons and very low-density lipoproteins (VLDL) are hydrolyzed by LPL at or close to the surface of the endothelial cells of the blood capillaries so that free fatty acids (FFA) are generated. The FFA can enter the fat cells to be reesterified and stored as TG. The mobilisation of FFA from endogenous TG pools in isolated fat cells derived from the epididymal fat pad of rats pretreated with glucocorticoids (6,21,22) is enhanced. The net effect of this treatment on the fat pad weight is dependent on the de novo synthesis of FA in the adipocytes and/or by the flux of FFA from plasma TG into the fat cells. Lau and Roncari (23) found a decreased fat pad weight and a lowered activity of lipid synthesizing enzymes in adipocytes after treatment of rats with dexamethasone, while others have described a decreased activity of LPL in the epididymal fat pad after administration of glucocorticoids. In some studies (5,14) pretreatment of rats with glucocorticoids had no effect on the epididymal fat pad weight. If the intracellular lipolysis is increased under these conditions then an increased de novo

synthesis and/or increased FFA uptake should occur to compensate for the loss of FFA from the tissue. We studied the effect of excess glucocorticoids on LPL activity by treatment of rats with Synacthen, a synthetic corticotrophin analogue. We found no significant changes of fat pad weight in the fasted nor in the fed state, even during long-term hypercorticism. Adrenalectomy led to a pronounced decrease in body- and fat pad weight, which was not prevented by Synacthen treatment. In fasted Synacthen-treated rats we consistently found a LPL activity elevated to the level in normally fed controls. This effect was already seen after 3 days of treatment. Adrenalectomy resulted in a pronounced (about 50%) reduction in LPL activity.

LPL is probably synthesized in the fat cell. Its synthesis is stimulated by insulin and glucocorticoids (24). After synthesis, the enzyme is transported to the luminal side of the endothelial cells, where it is assumed to exert its function, and is attached to heparan sulphate lining the vessel wall (20). Heparin with a structural analogy to heparan sulphate can release LPL from its binding site. Following elution of the enzyme with heparin one can measure LPL activity in the cell-free eluate. It is generally assumed that this heparin-elutable LPL represents the physiologically active form of the enzyme (25). Using another method, the acetone-ether powder method, it is suggested that the intracellular LPL pool is measured in addition to the elutable enzyme. We found no effect of Synacthen treatment on heparin-elutable LPL activity, in the fasted nor the fed state. However, as discussed above, the LPL activity in the acetone-ether powders of the tissue turned out to be higher at least in animals in the fasted state. This increase is probably caused by an enhanced synthesis of the enzyme and is apparently confined to a pool, which is not elutable by heparin in vitro. Insulin may stimulate the synthesis of LPL in the adipocyte. During Synacthen treatment a pronounced hyperinsulinemia develops in the fasted groups comparable to those of the normally fed controls. In spite of a further increase of the insulin level in the fed Synacthen-treated animals no further increase in LPL activity was observed. It is possible, therefore, that the

capacity to synthesize the enzyme is utilized maximally in the fed controls and fasted treated rats. The discrepancy in behaviour of acetone-ether powder and heparin-elutable LPL activity to glucocorticoid excess suggests that the synthesis of the enzyme in the adipocyte is stimulated during pretreatment with Synacthen, while the transfer to the endothelial cell is impaired. Our results demonstrate once more the necessity to evaluate these two frequently applied methods for determining LPL activity in respect to their physiological significance.

REFERENCES

1. Adlersberg, D.: Hormonal influences on the serum lipids. Am. J. Med. 23: 769-789, 1957.
2. Rudman, D., di Girolamo, M.: Effect of adrenal cortical steroids on lipid metabolism. In human Adrenal Cortex., edited by Christy, N.P. New York. Harper Row, 1971, pp. 241-255.
3. Hill, R.B., Droke, W.E., Hays, A.P.: Hepatic lipid metabolism in the cortisone-treated rat. Exp. Mol. Path. 4: 320-327, 1965.
4. Klausner, H., Heimberg, M.: Effect of adrenal cortical hormones on release of triglycerides and glucose by liver. Am. J. Physiol. 212: 1236-1246, 1967.
5. Lamberts, S.W.J., Birkenhäger, J.C.: Exogenous hypercortisolism and epididymal fat cell count in young rats. Hormone Res. 7: 158-163, 1976.
6. Lamberts, S.W.J., Timmermans, H.A.T., Kramer-Blankestijn, M. et al.: The mechanism of the potentiating effect of glucocorticoids on catecholamine-induced lipolysis. Metabolism 24: 681-689, 1975.
7. de Gasquet, P., Pequignot-Planche, E., Tonnu, N.T. et al. Effect of glucocorticoids on lipoprotein lipase activity in rat heart and adipose tissue. Horm. Metab. Res. 7: 152-157, 1975.
8. Krotkiewski, M., Björntorp, P., Smith, U.: The effect of long-term dexamethasone treatment on lipoprotein lipase activity in rat fat cells. Horm. Metab. Res. 8: 245-246, 1976.
9. Krausz, Y., Bar-On, H., Shafrir, E.: Origin and pattern of glucocorticoid-induced hyperlipidemia in rats. Biochim. Biophys. Acta. 663: 69-82, 1981.
10. Bagdade, J.D., Yee, E., Albers, J. et al.: Glucocorticoids and triglyceride transport: Effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat. Metabolism 25: 533-542, 1976.

11. de Gasquet, P., Pequignot, E.: Changes in adipose tissue and heart lipoprotein lipase activities and in serum glucose, insulin and corticosterone concentrations in rats adapted to a daily meal.
Horm. Metab. Res. 5: 440-443, 1973.

12. Taskinen, M.R., Nikkilä, E.A., Pelkonen, R. et al.: Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome.
J. Clin. Endocrinol. Metab. 57: 619-626, 1983.

13. Jansen, H., Schoonderwoerd, G., Baggen, M.G.A., et al.: The effect of corticotrophin on liver type lipase activity in adrenals, liver and HDL-subfractions in the rat.
Biochim. Biophys. Acta 753: 205-212, 1983.

14. Garfinkel, A.S., Schotz, M.C.: Separation of molecular species of lipoprotein lipase from adipose tissue.
J. Lipid Res. 13: 63-68, 1972.

15. Nilsson-Ehle, P., Schotz, M.C.: A stable, radioactive substrate emulsion for assay of lipoprotein lipase.
J. Lipid Res. 17: 536-541, 1976.

16. Taskinen, M.R., Nikkilä, E.A., Huttunen, J.K. et al.: A micro method for assay of lipoprotein lipase activity in needle biopsy samples of human adipose tissue and skeletal muscle.
Clin. Chim. Acta 104: 107-117, 1980.

17. Robinson, D.S.: The clearing factor lipase activity of adipose tissue. Handbook of Physiology, section 5: Adipose Tissue. Baltimore USA, Waverly Press Inc., 1965, pp. 295-299.

18. Scow, R.O.: Perfusion of adipose tissue: FFA release and blood flow in rat parametrial fat body. Handbook of Physiology, section 5: Adipose Tissue. Baltimore USA, Waverly Press Inc., 1965, pp:437-455.

19. Borensztajn, J., Robinson, D.S.: The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase

(lipoprotein lipase) releasable by heparin in the perfused rat heart.
J. Lipid Res. 11: 111-117, 1970.

20. Cryer, A.: Tissue lipoprotein lipase activity and its action in lipoprotein metabolism.
Int. J. Biochem. 13: 525-541, 1981.

21. Jeanrenaud, B., Renold, A.E.: Studies on rat adipose tissue in vitro. VII Effects of adrenal cortical hormones.
J. Biol. Chem 235: 2217-2223, 1960.

22. Jeanrenaud, B.: Effect of glucocorticoid hormones on fatty acid mobilization and re-esterification in rat adipose tissue.
Biochem. J. 103: 627-633, 1967.

23. Lau, D.C.W., Roncari, D.A.K.: Effects of glucocorticoid hormones on lipid-synthetic enzymes from different adipose tissue regions and from liver.
Can. J. Biochem. Cell. Biol. 61: 1245-1250, 1983.

24. Ashby, P., Robinson, D.S.: Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose tissue lipoprotein lipase.
Biochem. J. 188: 185-192, 1980.

25. Borensztajn, J., Rone, MS., Sandros, T.: Effects of colchicine and cycloheximide on the functional and non-functional lipoprotein lipase fractions of rat heart.
Biochim. Biophys. Acta 398: 394-400, 1975.

CHAPTER IV

EFFECTS OF SYNACTHEN ON LIPID METABOLISM IN THE PERFUSED EPIDIDYMAL FAT PAD OF THE RAT.

M.G.A. Baggen, R. Lammers, H. Jansen, L. Verschoor and J.C. Birkenhäger.

Metabolism 1987; 36: 544-547.

SUMMARY

Rats were treated with Synacthen, a synthetic corticotrophin analogue, to induce hypercorticism. The epididymal fat pad was selectively cannulated and perfused.

In fasted rats acetone ether powder lipoprotein lipase (LPL) activity rose during treatment to levels found in fed controls. In fed animals no further rise in LPL activity was observed during Synacthen treatment. However, the heparin-elutable LPL activity did not change in fasted nor fed animals. Pharmacologic levels of insulin in the perfusion medium, caused an increase in heparin releasable LPL activity as a percentage of total fat pad LPL activity (15 % vs 48 %).

Hydrolysis of chylomicrons was higher in fasted three days treated animals than in controls (10 ± 4% vs 2 ± 2%). In this group a higher uptake of liberated free fatty acids was found (2.6 ± 1.5% vs 1.0 ± 0.5% in controls). The increase in hydrolysis rate and uptake of fatty acids in the treated fasted animals could not be explained by an increase in releasable LPL activity.

Fatty acid release from the fat pad was lower in treated animals than in controls (fasted and fed), basally as well as after adrenalin stimulation. The observation that the epididymal fat pad retains its weight during hypercorticism may therefore be ascribed to an increased influx of fatty acids from increased hydrolysis of TG-rich particles and to an inhibited efflux of fatty acids from the adipocyte. The discrepancy between the LPL activity extractable from an acetone ether powder and the heparin-releasable LPL activity suggests impairment of the transport of LPL from the adipocyte to the heparin releasable pool at the endothelium.

INTRODUCTION

Hypercorticism leads to profound changes in lipid metabolism, and is associated with a characteristic centripetal redistribution of fat in humans. Rats do not have this typical redistribution of adipose tissue, but it is suggested that glucocorticoids have differential effects on the weight of different adipose

depots (1). In hypercorticistic rats total body weight is decreased while the weight of the epididymal fat pad is maintained (2,3).

The influence of glucocorticoids on adipose tissue has been studied in isolated fat cells (2,4-6). Glucocorticoids are either necessary for or markedly increase the effect of stimuli of hormone sensitive lipase in adipose tissue (2,4-10). Therefore the effect of glucocorticoids, if unopposed, is to increase lipolysis, resulting in free fatty acid release.

In the intact organism the lipolytic action of glucocorticoids is counteracted by insulin. The typical distribution of fat in humans, and the preservation of the epididymal fat pad in the rat, associated with excess levels of glucocorticoids may be due to the fact that the insulin response predominates in the regions of increased fat mass, and that the direct steroid response predominates in the areas of fat loss (6). Trying to elucidate part of this problem we made rats hypercorticistic by treating them with Synacthen, a synthetic corticotrophin analogue, and found an increase in tissue LPL activity in the treated animals (3). LPL is the rate limiting enzyme in the hydrolysis of triglycerides (TG) from chylomicrons. To study the effect of glucocorticoids on the handling of exogenous lipid by the fat pad, we perfused epididymal fat pads in vitro (11) and studied the hydrolysis and uptake of chylomicron-triglycerides under different conditions. In addition the effect of glucocorticoids on adrenalin stimulated lipolysis was determined.

MATERIALS AND METHODS

Male rats of the Wistar strain with a weight of 200-250 g were used. The rats had free access to standard lab chow and tap water. Lights were on from 09.30 a.m. to 9.30 p.m. Hypercorticism was induced by daily subcutaneous injections of 50 µg (= 5 I.U.) of synthetic 1-24 corticotrophin (Synacthen depot, Ciba Geigy, Belgium)in the suprascapular region. Treatment was for 3 or 10 days. The drug was administered late in the afternoon. In intact rats this regimen leads to hypercorticism with plasma corticosterone levels of 610 ± 137 ng/ml vs. 258 ± 120 ng/ml in

control rats (12). For each experimental condition four animals were used, unless noted otherwise.

The control groups were injected with 0.9% NaCl (w/v). The fasted groups were deprived of all caloric intake 16 hours before use. Rats were used for perfusion between 09.30 and 10.00 a.m.

Perfusion of the epididymal fat pad was carried out for 60 min according to Ho and Meng (13) extensively modified by us (11; chapter II). Where noted insulin (Actrapid NOVO, Copenhagen, Denmark), adrenalin or heparin were added to the perfusion medium.

Chylomicrons labeled with ^{14}C-palmitate were prepared as described earlier (11; chapter II,14). Extraction of fatty acids and triglycerides from the perfusate occurred according to Bligh and Dyer (15). Subsequently thin-layer chromatography was performed. The specific activity of TG-FA ranged from 0.95 to 1.09 x 10^6 dpm/μmol TG.

Hydrolysis of chylomicron-triglycerides was expressed as the quotient of the increament in radioactive free fatty acids and the total radioactivity in the TG fraction (100 x delta dpm FA/ dpm TG-FA = % hydrolysis).

The uptake of fatty acids in the perfused fat pad was calculated as the ratio of radioactivity accumulated in the fat pad and the total radioactivity, that passed through the pad during perfusion. LPL activity was determined in defatted preparations (acetone-ether powder) of the fat pad (16,17) and in the medium after perfusion. The LPL substrate consisted of a glycerol stabilized glycerol-tri 9,10 (n)-^{3}H-oleate, phospholipid emulsion as described in detail by Nilsson-Ehle et al. (16).

RESULTS

LPL activity and plasma TG during Synacthen treatment.

As previously observed during Synacthen treatment (3) LPL activity increased significantly in acetone-ether extracts of the epididymal fat pad of fasted rats (Table I; $p < 0.02$) and was not significantly different from the activity in the fed groups, either treated with Synacthen or not. Plasma TG concentration was not changed by the treatment in the fasted animals. The

Table I. Lipoprotein Lipase Activity in the Epididymal Fat Pad and Plasma Triglyceride Concentrations in Controls and Synacthen-Treated Rats

Feeding Conditions	Group	n	Treatment (Saline/Synacthen)	Duration of Treatment (d)	TG (mmol/L)	LPL● (mU/gww)
Fasted	I	7	Saline	10	0.65 ± 0.11	97 ± 39†
	II	7	Synacthen	3	0.63 ± 0.09	390 ± 190☆
	III	7	Synacthen	10	0.62 ± 0.14	240 ± 57§
Fed	IV	7	Saline	10	1.28 ± 0.30	440 ± 158
	V	7	Synacthen	3	2.40 ± 0.47	378 ± 81
	VI	7	Synacthen	10	1.92 ± 0.92	436 ± 140

● Measured in the nonperfused epididymal fat pad.
† Significantly different from group IV ($p < .01$).
☆ Significantly different from group I ($p < .02$).
§ Significantly different from group I ($p < .05$).
Group II and III *v* IV; IV *v* V and VI are not significantly different.

Synacthen treated fed rats were hypertriglyceridemic.

Effect of insulin on heparin-releasable LPL activity.

To study the possible underlying cause for the observed discrepancy between the increase in total and heparin-releasable LPL we determined the effect of a relatively low (100 μU/ml) and high (10 mU/ml) insulin concentration on the distribution of LPL over heparin-releasable and non-releasable pools. In fasted rats treated for 3 days with Synacthen the low insulin concentration did not affect the heparin-releasable activity but with the high insulin concentration a significantly ($p < 0.01$) higher lipase activity was found (Table II). This suggests that insulin in high doses in Synacthen treated rats promotes the transport of LPL to the heparin-releasable pool.

Effect of Synacthen treatment on the hydrolysis of chylomicron--TG and uptake of chylomicron-TG-FA in perfused epididymal fat pad.

The impact of changes in LPL activity during Synacthen treatment on the hydrolysis and uptake of chylomicron-TG-FA were studied. Hydrolysis of chylomicron-TG in fasted untreated animals is low: 5-7 times lower than in the fed controls (Table III). In the three and ten days treated and fasted rats hydrolysis of

chylomicrons rose significantly ($p < 0.001$ and < 0.05, respectively).

Table II. Heparin-Releasable LPL Activity in Controls and Synacthen-Treated Fasted Rats With Two Different Insulin Concentrations in the Perfusion Medium

Insulin	n	Controls	n	Synacthen
0.1 mU/mL	4	8 ± 4	4	15 ± 11
10 mU/mL	4	15 ± 11*	4	48 ± 14†

LPL activity is given as percentage of the acetone-ether extractable LPL of the non-perfused fat pad.
* NS *v* the low insulin concentration group.
† Significantly different from all other groups $p < .01$.

Table III. Effect of Synacthen Treatment on the Hydrolysis of Chylomicron-TG in the Perfused Epididymal Fat Pad of the Rat

	n	Fasted	n	Fed
0	7	2 ± 2	7	14 ± 4*
3	7	10 ± 4†	7	15 ± 3
10	7	7 ± 2☆	7	12 ± 5

Hydrolysis is expressed as the percentage of labeled fatty acids freed from the labeled TG-FA during the whole of the perfusion (60 minutes).
* Significantly different from the untreated fasted group ($p < .001$)
† Significantly different from the untreated fasted group ($p < .001$)
☆ Significantly different from the untreated fasted group ($p < .05$)

In the untreated groups heparin-releasable LPL activity and hydrolysis rate were significantly correlated ($r = 0.9$, $n = 7$). In contrast in none of the Synacthen treated groups a correlation was observed.

The effect of Synacthen treatment on the uptake in the perfused tissue was similar to that on hydrolysis of chylomicron-TG (Table IV). A significant increase in uptake in the fasted animals treated with Synacthen for three and ten days was found ($p < 0.05$), resulting in a percentage of uptake identical to that found in the fed controls.

Table IV. Effect of Synacthen Treatment on the Uptake of Chylomicron-Fatty Acids in the Perfused Epididymal Fat Pad

		Fasted		Fed
0	7	1.0 ± 0.5	7	2.7 ± 0.9†
3	7	2.6 ± 1.5*	7	3.2 ± 0.8
10	7	3.3 ± 1.6*	7	3.2 ± 0.9

Uptake is expressed as percentage of TG-FA that has been extracted from the perfusate.
* Significantly different from the untreated fasted group ($p < .05$)
† Significantly different from the untreated fasted group ($p < .05$).

Effect of adrenalin on FA release from the fat pad from Synacthen treated rats.

Besides uptake of FA, release of FA may determine the size of the fat body. Therefore the extend of adrenalin stimulated lipolysis was measured in Synacthen treated animals. In the absence of adrenalin FA release from the fat pad in fasted controls was significantly higher than in fed ones ($p < 0.02$). During prolonged perfusions without adrenalin in the medium FA-release did not change in both situations. After adding adrenalin to the medium (1 µg/ml) FA release was largely stimulated in both fasted and fed animals (Fig. 1). Fed pads of Synacthen treated rats released significantly less fatty acids in both conditions than those of the controls (Fig. 1).

DISCUSSION

Hypercorticism in rats induced by daily injections of Synacthen leads to an increase in adipose tissue LPL activity in acetone ether powder extracts of the fat pad. However, the in vitro by heparin-incubation releasable lipase activity remains unaltered (3). It is generally assumed that the last mentioned activity represents the physiologically active enzyme pool. In hypercorticistic rats and in controls the fat pad weight is similar (2,3) while it has been reported that in the adipocyte glucocorticoids have a potentiating effect on the intracellular adrenalin stimulated lipolysis (6), an increased input of FA into the TG pool of the fat pad has to be assumed. However, as discussed above the physiologically active LPL pool seems not to be enhanced. To study this apparent discrepancy we induced hypercorticism in rats by Synacthen treatment and carried out a

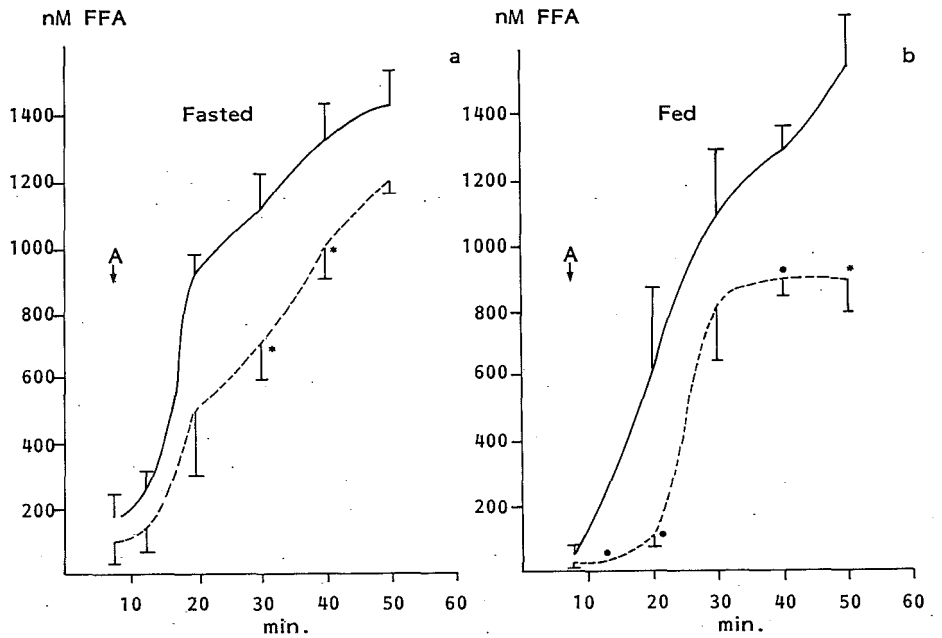


Fig 1. Effect of adrenaline on fatty acid release from the fat pad in the perfusate in controls and Synacthen-treated animals. Data are given as mean ± SEM. Perfusion during 60 minutes was carried out in fasted (A) and fed (B) animals; controls (_) and Synacthen-treated (- - -) animals. Synacthen treatment with 5 IU sc for three days. After ten minutes perfusion, adrenaline (1 μg/mL) was added to the medium (↓). * Significantly different from the untreated group $P < .05$. ° Significantly different from the untreated group $P < .02$.

selective perfusion of the epididymal fat pad.

However in the hypercorticistic rats, the heparin-releasable LPL activity in the perfusate rose from 3 ± 1 to 12 ± 8 mU in fasted animals. In fed animals the heparin releasable pool of LPL increased to 36 ± 20 mU in controls, while Synacthen treatment did not further increase this pool (30 ± 13 mU). This is in contrast to the lack of influence of Synacthen treatment on the heparin-elutable LPL activity in in vitro experiments (3). This increase may explain the observed rise in TG-hydrolysis in situ and the increase of the uptake of TG-FA. In controls hydrolysis of chylomicron-TG correlated strongly with the heparin releasable LPL activity in the perfusate. Such a correlation was not found in the hypercorticistic animals, although it seems that a correlation is present between acetone-ether LPL and TG-hydrolysis. Hydrolysis rate in the short-term Synacthen treated and fasted

animals, was significantly higher than in the fasted controls and approached the level found in fed controls. We cannot easily explain why the increased hydrolysis did not correlate with the heparin-releasable LPL activity. It seems that not under all conditions LPL is the only or major determinant of the hydrolysis of chylomicron-TG. In line with this observation Verschoor et al. (18) reported that diet induced changes in adipose tissue LPL activity (acetone-ether powder and heparin-elutable) did not furnish any useful information about VLDL-TG kinetics. It is possible that Synacthen treatment induces changes in the enzyme configuration lowering the K_m of the enzyme for chylomicrons leading to a higher rate of hydrolysis with a given in vitro LPL activity (capacity). The increase in hydrolysis of chylomicrons in short-term treated and fasted rats leads to an increase in uptake of FA by the epididymal pad to levels found in the fed ones. Therefore, apparently hypercorticism leads in situ to an increase of the FA uptake by the adipose tissue. The discrepancy in chylomicron hydrolysis and FA-uptake indicates a dissociation between the capacity of the epididymal fat pad to hydrolyze and esterify FA under the influence of Synacthen. In other words the hydrolysis is stimulated by Synacthen to a greater extent than the uptake.

The increased FA influx into the adipocyte from the vascular compartment alone cannot explain the retaining of the pad weight during hypercorticism for prolonged periods. The data represented in Fig. 1 show that the FA release from the adipocytes into the vascular compartment was reduced in the Synacthen treated rats, both in the fasted and fed state. This lowering effect was more pronounced during stimulation of the intracellular lipolytic process with adrenalin. These findings are in contrast with experiments in isolated epididymal adipocytes from fasted rats in which pretreatment of the animal with cortisol lead to a further increase of the adrenalin induced lipolysis (6). This remarkable discrepancy may be explained by the fact that in vivo (or in situ) the secondary hyperinsulinism in hypercorticistic rats counteracts the increased intracellular lipolysis. This results in an increase in total LPL content of the adipose

tissue (acetone-ether powder LPL activity is presumed to reflect total tissue enzyme) an increase of the FA uptake and a decrease of the intracellular lipolysis leading to a balanced state in which the fat pad can retain its weight.

Spirovski et al. (19) who studied lipolysis in the perfused parametrial fat of the fed rat, found an increased reesterification in intact and adrenalectomized Synacthen treated animals. Although the results of the present study do not contradict those of Spirowski et al. (19), the conditions in our experiments are different to such a degree that it makes comparison difficult.

Synacthen treatment in fasted rats leads to a marked increase in LPL activity in acetone-ether powders of the adipose tissue, a (slight) increase in heparin-releasable LPL (Table III) but not to changes in the heparin-elutable LPL activities in vitro (3). These data may indicate a situation in which the enzyme is confined to a pool not elutable by heparin in vitro or in situ.

In hypercorticistic rats a pronounced hyperinsulinemia develops in the fasting state. The insulin levels become comparable to that of the normally fed controls. Many studies have shown that insulin administration leads to a rise in LPL in adipose tissue (18,20,21). In the tissue culture insulin stimulates the release of LPL from adipocytes (22-24). There is evidence that the insulin effect on enzyme synthesis is enhanced by glucocorticoids in vitro (25). Possibly the synthesis of LPL in adipose tissue of hypercorticistic rats is maximal, but the transfer of LPL to the endothelial cell is impaired. To study whether a relative shortage of insulin (insulin resistance) may have contributed to an impaired transfer of LPL to the heparin releasable pool we added pharmacologic insulin concentrations (10 mU/ml) to the perfusion fluid in Synacthen treated and control fasted rats to overcome possible blockade of the transport of LPL from the adipocyte to the vascular compartment. Under these conditions the heparin releasable LPL rose from 15% to 48% of total LPL activity measured in the fat pad of the Synacthen treated rats. In these experiments with insulin added to the perfusion medium, insulin appeared to cause a shift of LPL from the intracellular

to the heparin-releasable pool, while the total enzyme activity (the sum of the heparin-released LPL and the remaining activity in the perfused pad) did not change.

The results of these in situ experiments confirm the hypothesis that the transport of LPL from intra- to extracellular loci in adipose tissue is insulin dependent (24,25). More detailed studies of this transport system and of the effects of insulin, glucocorticoids and other hormones thereon are in progress.

REFERENCES

1. Lau, D.C.W., Roncari, D.A.K.: Effects of glucocorticoid hormones on lipid-synthetic enzymes from different adipose tissue regions and from liver. Can. J. Biochem. Cell. Biol. 61: 1245-1250, 1983.

2. Lamberts. S.W.J., Birkenhäger, J.C.: Exogenous hypercortisolism and epididymal fat cell count in young rats. Hormone Res. 7: 158-163,1976.

3. Baggen, M.G.A., Lammers, R., Jansen, H. et al.: The effect of Synacthen administration on lipoprotein lipase activity in the epididymal fat pad of the rat. Metabolism 34: 1053-1056, 1985.

4. Robinson, C.A., Butcher, R.W., Sutterland, E.W.: Cyclic AMP. New York Academic Press 1971.

5. Fain, J.: Effect of dibutyryl-3,5-AMP, theophylline and norepinephrine on the lipolytic action of growth hormone and glucocorticoid in white fat cells. Endocrinology 82: 125-163, 1968.

6. Lamberts, S.W.J., Timmermans, H.A.T., Kramer-Blankestijn M et al.: The mechanism of the potentiating effect of glucocorticoids on catecholamine-induced lipolysis. Metabolism 24: 681-689, 1975.

7. Rudman, D., Di Girolamo, M. : Effect of adrenal cortical steroids on lipid metabolism. In Human Adrenal Cortex. edited by Christy, N.P. New York, Harper Row, 1971, pp. 241-255.

8. Smith, O.K., Long, C.H.M.: Effect of cortisol on plasma amino nitrogen of eviscerated adrenalectomized diabetic rats. Endocrinology 80: 561-565, 1967.

9. Havel, R.J., Goldfein, A.: Adrenal and free fatty acid mobilisation. J. Lipid Res. 1: 102-110, 1959.

10. Shapiro, E., Steinberg, D.: The essential role of the adrenal cortex in the response of plasma free fatty acids, cholesterol and phospholipids to epinephrine injection. J. Clin. Invest. 39: 310-316, 1960.

11. Baggen, M.G.A., Jansen, H,, Lammers, R. et al.:

Lipoprotein lipase activity and triglyceride metabolism in the perfused epididymal fat pad. (Submitted.)

12. Jansen, H., Schoonderwoerd, G., Baggen, M.G.A. et al.: The effect of corticotrophin on liver type lipase activity in adrenals, liver and HDL-subfractions in the rat. Biochim. Biophys. Acta 753: 205-212, 1983.
13. Ho, R.J., Meng, H.C.: A technique for the cannulation and perfusion of isolated rat epididymal fat pad. J. Lipid Res. 5: 203-209, 1964.
14. Bolman, J.L., Cain, J.C., Grindlay, J.H.: Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. J. Lab. Clin. Med. 33: 1349-1356, 1948.
15. Bligh, E.G., Dyer, C. : A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917, 1959.
16. Nilsson-Ehle, P., Schotz, M.C.: A stable radioactive substrate emulsion for assay of lipoprotein lipase. J. Lipid Res. 17: 536-541, 1976.
17. Garfinkel, A.S., Schotz, M.C.: Separation of molecular species of lipoprotein lipase from adipose tissue. J. Lipid Res. 13: 63-68, 1972.
18. Verschoor, L., Chen, Y-D.I., Reaven, G.M.: In search of a relationship between physiologically induced variations in adipose tissue lipoprotein lipase activity and very low density lipoprotein kinetics in normal rats. Metabolism 31: 499-503, 1982.
19. Spirovski, M.Z., Kovacev, V.P., Spasovska, M. et al.: Effect of ACTH on lipolysis in adipose tissue of normal and adrenalectomized rats in vivo. Am. J. Physiol. 228: 382-385, 1975.
20. Hollenberg, C.H.: The effect of incubation on characteristics of the lipolytic activity of rat adipose tissue. Can. J. Biochem. Physiol. 40: 703-707, 1962.

21. Robinson, D.S.: The function of the plasma Triglycerides in fatty acid transport. In Comprehensive Biochemistry. vol. 18. edited by M.Florkin and E.H. Stotz. Elsevier Amsterdam, pp 51-116, 1970.

22. Salamon, M.R., Robinson, D.S.: Clearing-factor lipase in adipose tissue. A medium in which the enzyme activity of tissue from starved rats increases in vitro. Biochem. J. 99: 640-647, 1966.

23. Spooner, P.M., Chernick, S.S., Garrison, M.M. et al.: Development of lipoprotein lipase activity accumulation of triacylglycerol in differentiating 3T3-L1 adipocytes. J. Biol. Chem. 254: 1305-1311, 1979.

24. Spooner, P.M., Chernick, S.S., Garrison, M.M. et al.: Insulin regulation of lipoprotein lipase activity and release in 3T3-L1 adipocytes. J. Biol. Chem. 254: 10021-10029, 1979.

25. Ashby, P., Robinson, D.S.: Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose tissue lipoprotein lipase. Biochem. J. 188: 185-192, 1980.

CHAPTER V

DISPARATE EFFECTS OF ACTH (1-24) AND CORTICOSTERONE ON LIPOPROTEIN LIPASE IN RAT ADIPOSE TISSUE.

M.G.A. Baggen, H. Jansen, R. Lammers, L. Verschoor and J.C. Birkenhäger.

J. Endocr. 1987; 114:369-372

SUMMARY

The effects of corticosterone and ACTH (1-24) on lipoprotein lipase (LPL) activity of rat epididymal fat tissue were studied. Hypercorticism induced by s.c. administration of 10 mg corticosterone acetate for 3 days led to a decrease in LPL activity. This decrease could be prevented by treatment of the rats simultaneously with synthetic ACTH (1-24). Adrenalectomy also reduced LPL activity. Corticosterone and ACTH (1-24) treatment had a similar effect on LPL activity in adrenalectomized and intact rats.

These results indicate that ACTH (1-24) may affect adipose tissue LPL in the rat by a mechanism in which corticosterone is not involved.

INTRODUCTION.

Lipoprotein lipase (EC 3.1.1.34) is the enzyme that catalyses the hydrolysis of triacylglycerols in chylomicrons and very low-density lipoproteins. In adipose tissue the enzyme is synthesized in the adipocytes from which it is secreted and transported to the luminal surface of the capillary endothelial cells were it exerts its effect (1,2). Environmental changes lead to large variations in the activity of the enzyme, and many of these variations are under hormonal control (3). There is good evidence, for example, that insulin promotes the synthesis of the enzyme in the adipocyte. The role of corticosteroids in the regulation of lipoprotein lipase activity (LPL) is less clear. Some investigators have reported an increase in LPL activity after the administration of corticosteroids to rats (3,4), while others have found an opposite effect (5-8). We have previously described an increase in total LPL activity in the epididymal fatpad of the rat during short- and long-term hypercorticism induced by Synacthen, a synthetic corticotrophin (1-24) (ACTH 1-24) preparation (9). However, LPL activity which could be released from the tissue by heparin and which probably represents the functional pool of the enzyme was not influenced by this treatment. In this paper we report further studies on

the effects of ACTH (1-24) and corticosterone on LPL activity in the epididymal fat pad of the rat.

MATERIALS & METHODS.

Animals.

Male rats of the Wistar strain weighing of 200 to 250 g were used. They had free access to standard lab chow and drinking water unless noted otherwise. To ensure survival for a short period, adrenalectomized rats received 0.9% NaCl (w/v) in their drinking water. Lights were on from 9.30 till 21.30. Some rats were adrenalectomized ten days before starting treatment.

Treatments.

Corticotrophin (1-24) (Synacthen depot, Ciba Geigy, Brussels, Belgium) was administrated for three days by daily subcutaneous injections in the suprascapular region (50 µg; 5 I.U.per animal per day). In addition, or alternatively, various doses of corticosterone 21-acetate (Sigma, St. Louis, MO, USA;1, 5 or 10 mg per animal) were given. All treatments were administered late in the afternoon. The control groups were injected with 0.9% (w/v) NaCl. The fasted groups were deprived of all food for 16 h before use. Rats were killed by decapitation between 9.30 and 10.30 h.

Biochemical methods.

Blood was collected in ice-cooled tubes containing 10 mg Na_2-EDTA. The right epididymal fat pad was defatted with acetone and diethyl-ether as described by Nilsson-Ehle & Schotz (10). activity was measured in Tris-ethylene glycol buffer extracts of the defatted tissue (1-3 mg powder/ml) on the same day (10,11). This activity is referred to as acetone-ether powder LPL activity. The left pad was cut into pieces of about 20 mg and placed in 10 ml medium consisting of Krebs-Ringer buffer (pH 8.3), supplemented with bovine serum albumin (5% w/v, fraction V, Sigma, St. Louis, USA), heparin (50 I.U./ml; Tromboliquine, Organon, Oss, The Netherlands), glucose (5 mmol/l). After incubation for 40 min at 30°C in a shaking water bath, the tissue was removed and 100 µl medium were incubated with an equal amount of a substrate mixture to measure LPL activity

Table I. Effect of corticosterone treatment on lipoprotein lipase (LPL) activity in adipose tissue and on plasma hormone concentrations in normally fed rats and in rats fasted overnight. Values are means ± S.E.M. for four rats per group.

	Acethone-ether powder LPL** (mu./g wet wt)	Heparin-elutable LPL*** (mu./g wet wt)	Insulin (pmol/l)	Corticosterone (ng/ml)
Treatment				
Fasted rats				
Saline	75 ± 9	53 ± 5	60.9 ± 4.9	192 ± 70
Corticosterone (1 mg)	56 ± 9	36 ± 3	63.3 ± 7.7	190 ± 85
Corticosterone (5 mg)	51 ± 5	29 ± 3	73.5 ± 4.9	281 ± 75
Corticosterone (10 mg)	43 ± 6*	19 ± 5*	105 ± 9.1*	506 ± 35*
Fed rats				
Saline	322 ± 38	288 ± 39	126.0 ± 14.0	150 ± 75
Corticosterone (1 mg)	224 ± 19	352 ± 57	119.0 ± 2.1	110 ± 80
Corticosterone (5 mg)	224 ± 19	336 ± 47	182.0 ± 26.6	288 ± 80
Corticosterone (10 mg)	181 ± 22*	198 ± 25	217.0 ± 26.6*	415 ± 79*

* $P < 0.025$ compared with control (saline) group (unpaired Student's t-test); ** Lipoprotein lipase activity measured in an extract from the defatted epididymal fat pad; *** lipoprotein lipase activity measured in a heparin-containing medium after a 40-min incubation of pieces of the epididymal fat pad.

(heparin-elutable LPL) (12). The substrate mixture for the LPL measurement consisted of a glycerol-stabilized glycerol--tri[9,10-^{3}H] oleate-phospholipid emulsion. (10). The activity is expressed in milli Units (mU; nmol fatty acid released per min) per gram tissue wet weight. Serum from male rats, which had been fasted overnight, served as the LPL activator. Insulin (IRI) was measured with a ^{125}I radioimmunoassay (Insulin RIA; Immuno Nuclear Cooperation, Stillwater, MN. USA). Corticosterone was measured with a ^{125}I radioimmunoassay.

Statistical analysis.

The statistical significance of differences between groups was calculated by comparing each experimental group with its control group using the unpaired Student's t-test.

RESULTS.

Effects of corticosterone administration.

Treatment of rats with increasing amounts of corticosterone led to a progressive reduction of the acetone-ether powder LPL activity in animals fasted overnight (Table I). The decrease in activity (-43 %) was statistically significant at the highest dose of corticosterone administered (10 mg/rat per day). The decrease in heparin-elutable LPL activity followed a similar pattern. In rats not fasted overnight, a decrease in the

heparin-elutable activity was apparent only at the highest dose of corticosterone (Table I). In both fasted and fed rats, corticosterone administration led to a dose dependent increase in plasma concentrations of insulin and corticosterone .

Table II. Effect of ACTH (1-24) and/or corticosterone on adipose tissue lipoprotein lipase activity (LPL) in rats fasted overnight. Values are means ± S.E.M.

	n	Fat pad weight (mg)	Acetone ether powder LPL (mu./g wet wt)
Treatment			
Intact			
Saline	(12)	877 ± 12	91 ± 6
Corticosterone	(11)	634 ± 10	41 ± 3*
Corticosterone + ACTH (1-24)	(12)	590 ± 11	106 ± 6☆
Adrenalectomized			
Saline	(8)	463 ± 12	72 ± 5
Corticosterone	(14)	665 ± 9	45 ± 4
Corticosterone + ACTH (1-24)	(8)	609 ± 9	100 ± 5★#

* $P < 0.01$ compared with intact control (saline) group; ☆ $P < 0.001$ compared with corticosterone-treated intact group; ★ $P < 0.01$ compared with adrenalectomized corticosterone-treated group; # $P < 0.05$ compared with adrenalectomized control (saline) group (unpaired Student's t-test).
Rats were adrenalectomized 10 days before starting treatment with ACTH (1-24) (5 i.u.) and/or corticosterone (10 mg).
Acetone-ether powder LPL activity was measured in extracts from defatted epididymal fat pads.

Effects of administration of corticosterone plus ACTH (1-24).

We have shown previously that hypercorticism induced by administration of ACTH (1-24) (Synacthen) leads to an increase in acetone-ether powder LPL activity of fasted rats (9). We therefore wondered whether, during hypercorticism, LPL activity is differentially affected in the presence or absence of ACTH (1-24). Administration of ACTH (1-24) plus corticosterone to intact rats, was found to abolish completely the lowering effect on LPL of corticosterone alone (Table II). In adrenalectomized rats acetone-ether powder LPL activity was lower than in intact rats. Corticosterone administration lowered further LPL activity per gram tissue. The total activity in the fat pads was not affected because of a corticosterone-induced rise in the fat pad weight. Concomitant administration of ACTH (1-24) and corticosterone led to increase in LPL activity above basal values.

DISCUSSION.

Several hormones influence the activity of LPL with insulin and glucocorticoids seeming to be especially important. The exact role of glucocorticoids however is not clear. Opposite effects of corticosteroids on LPL activity have been reported. We have described the effects of hypercorticism, induced by the administration of ACTH (1-24), on LPL activity (9). During this type of hypercorticism LPL activity, extractable from a defatted tissue preparation, which presumably represents the total LPL activity in the tissue, was enhanced if the rats were fasted overnight. In normally fed rats however, the activity was not further increased. The functional part of the enzyme, releasable from the tissue by heparin was not affected. This indicated that situations can exist in which only part of the total LPL activity in the tissue is hormonally affected. The present study shows that induction of hypercorticism by the administration of corticosterone leads to a decrease in the total, as well as in the heparin-releasable LPL activity in the epididymal adipose tissue, an effect which was found in normally fed and in those fasted overnight. These effects are opposite to those found after induction of hypercorticism by ACTH (1-24) administration. This apparent discrepancy was not due to a different level of corticosterone or insulin in the plasma, as both hormones reached comparable levels in both types of hypercorticism (cf.9). We therefore studied whether a combination of a higher concentration of corticosterone plus ACTH (1-24) or ACTH (1-24) alone, stimulated LPL activity differentially. The latter possibility appears to be unlikely as, after adrenalectomy, when endogenous ACTH levels are high, LPL activity is low. Moreover we have shown previously that treatment of adrenalectomized rats with ACTH (1-24) does not affect LPL activity (9). Simultaneous administration of ACTH (1-24) and corticosterone completely abolished the lowering effect of LPL activity by corticosterone in intact rats. In adrenalectomized rats LPL activity after administration of ACTH (1-24) plus corticosterone reached values even higher than those in adrenalectomized rats not treated with any hormone. The experiments with the adrenalectomized rats also

indicate that no other hormone is involved in the stimulatory effect of ACTH (1-24) on LPL.

Based on these results, we postulate a direct stimulating effect of ACTH on the activity of LPL in the adipocyte of the rat. The inhibition of LPL during corticosterone administration may be due to suppression of endogenous ACTH secretion. Stimulation of LPL by ACTH (1-24) was seen only in the presence of corticosterone (endogenous or exogenous). Therefore, both corticosterone and ACTH appear to be necessary for the regulation of adipose tissue LPL.

In conclusion, in addition to insulin and glucocorticoids, ACTH seems to regulate LPL activity in adipose tissue of the rat.

REFERENCES.

1. Robinson, D.S. The function of the plasma triglycerides in fatty acid transport. Comprehensive Biochemistry, vol. 18, pp. 51-116, 1970, Elsevier, Amsterdam. Edited by M. Florkin and E.H. Stotz.

2. Scow, R.O., Blanchette-Mackie, E.J. & Smith, L.C. Role of capillary endothelium in the clearance of chylomicrons. Circulation Research 39: 149-162, 1976.

3. Robinson, D.S. & Wing, D.R. Studies on tissue clearing factor lipase related to its role in the removal of lipoprotein triglyceride from the plasma. Biochemical Society Symposium 1971, no: 33, Plasma Lipoproteins, pp. 123-135. Academic Press London. Edited by R.M.S. Smellie.

4. de Gasquet, P., Pequignot-Planche, E., Tonnu, N.T. & Diaby, F.A. Effect of glucocorticoids on lipoprotein lipase activity in rat heart and adipose tissue. Hormone and Metabolic Research 7: 152-157, 1975.

5. Krotkiewski, M.,Björntorp, P. & Smith, U. The effect of long-term dexamethasone treatment on lipoprotein lipase activity in rat fat cells. Hormone and Metabolic Research 8: 245-246, 1976.

6. Krausz, Y., Bar-On, H. & Shafrir, E. Origin and pattern of glucocorticoid induced hyperlipedemia in rats. Biochimica et Biophysica Acta 663: 69-82, 1981.

7. de Gasquet, P. & Pequignot-Planche, E. Changes in adipose tissue and heart lipoprotein lipase activities and in serum glucose, insulin and corticosterone concentrations in rats adapted to a daily meal. Hormone and Metabolic Research 5: 440-443, 1973.

8. Bagdade, J.D., Yee, E., Albers, J. & Pykälistö, O.J. Glucocorticoids and triglyceride transport: effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in

the rat. Metabolism 25: 533-542, 1976.

9. Baggen, M.G.A., Lammers, R., Jansen, H. & Birkenhäger, J.C. The effect of Synacthen administration on lipoprotein lipase activity in the epididymal fat pad of the rat. Metabolism 34: 1053-1056, 1985.

10. Nilsson-Ehle, P. & Schotz, M.C.. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. Journal of Lipid Research 17: 536-541, 1976.

11. Garfinkel, A.S. & Schotz, M.C. Separation of molecular species of lipoprotein lipase from adipose tissue. Journal of Lipid Research 13: 63-68, 1972.

12. Taskinen, M.R., Nikkilä, E.A., Huttunen, J.K. & Hilden, H. A micromethod for assay of lipoprotein lipase activity in needle biopsy samples of human adipose tissue and skeletal muscle. Clinica Chimica Acta 104: 107-117, 1980.

CHAPTER VI

EFFECTS OF DOXAZOSIN AND PROPRANOLOL ADMINISTRATION ON LIPOPROTEIN LIPASES IN CHOLESTEROL-FED RATS

H. Jansen and M.G.A. Baggen.

J. Cardiovasc. Pharmacol. 10: Suppl. 16-19, 1987.

SUMMARY

The effects of α-1-adrenergic receptor inhibition with doxazosin, and ß-blockade with propranolol on tissue lipoprotein lipases and plasma lipids were studied in rats.In rats fed a normal lab chow, doxazosin increased heart lipoprotein lipase (LPL) activity (+ 14%), while propranolol had the opposite effect (-20%). These effects were not statistically significant when compared with nontreated controls,although the difference between the doxazosin and propranolol groups was significant ($p < 0.05$). There were no significant effects on adipose tissue LPL activity or hepatic lipase (HL) activity.In rats fed a cholesterol-enriched diet there were similar but smaller effects on heartLPL activity (+5% and -12% respectively). In these rats α-1-inhibition also tended to increase adipose tissue LPL (+14%) and HL (+13%), while ß-blockade had an opposite effect (-20% and -9% respectively). The lipase activities were significantly different between the treatment groups in liver and heart but not in the adipose tissue. Doxazosin and propranolol did not affect plasma triglyceride or total cholesterol, but high-density lipoprotein cholesterol was increased during α-1-blockade (+24%).

INTRODUCTION

Anti-hypertensive treatment with α- or ß-blockers produces disparate effects on plasma lipid and lipoprotein levels (1). Although the results from different studies are variable, the emerging general picture is that α-1-adrenergic receptor inhibition (α-1-inhibition) leads a more favorable lipid profile than ß-blockade. Plasma triglycerides are decreased, while high density lipoprotein (HDL) cholesterol is increased. The mechanisms that lead to these effects are at present unknown. Two lipases located in the vascular endothelium, LPL in extrahepatic tissues, and HL in the liver, play a key role in the metabolism of plasma triglycerides and HDL (2-4). Therefore changes in the activity of these enzymes could lead to the variations in plasma lipid concentration that occur during α- and ß-blockade. Little is known about the adrenergic control of lipase activity although the effects of adrenergic stimuli have

been described for rat heart and adipose tissue LPL (5,6) and for rat HL (7).

In a number of recent human studies, LPL activity in postheparin plasma was higher after α-1-inhibition than after ß-blockade (8-10). In humans, the mechanisms of drug-induced changes in metabolism are difficult to assess. Moreover, beneficial or adverse effects of changes in plasma lipoproteins may become overt only after many years. Therefore we established the effects of a long-acting α-1-inhibitor, doxazosin, and of ß-blockade with propranolol on lipoprotein lipases and plasma lipids in rats under different feeding conditions.

MATERIALS AND METHODS

Animals

Rats (males from the Wistar strain,Hannover,F.R.G., 250-300 g) were housed under controlled conditions, (temp. 20°-23°C ,lights on between 08.00 and 19.00 h) and had free access to food and water, unless noted otherwise.

Treatment procedures

The food consisted of normal lab chow on its own or supplemented with 2% cholesterol (Hope's Farms, Woerden, The Netherlands). Drugs were added to the drinking water. Doxazosin (donated by Pfizer Central Research, Sandwich, U.K.) was dissolved in 0.4 M lactic acid (100 mg/ml) before being added to tap water (final concentration 100 mg/l) (7). Propranolol was dissolved in tap water at a concentration of 37.5 mg/l and lactic acid was added later. The concentration of lactic acid in the drinking water of treatment groups and controls was in the same concentration (10^{-4}M). The average water consumption was 40 ml/rat/day, therefore the rats received average daily doses of 4 mg of doxazosin or 1.5 mg of propranolol. This dose of propranolol has been shown to produce effective ß-blockade in rats during intravenous administration (11). The dose of prazosin used has been reported to affect lipid parameters in rats (7). Drinking water was renewed every other day. Cholesterol was given for 3 weeks, and drugs and lactic acid were added to the drinking water over the last 7 days.

Biochemical methods

The rats were killed by decapitation. Blood was collected in ice-cooled tubes, the liver was removed and a portion quickly frozen in liquid nitrogen. The heart was placed in ice-cold 0.9% NaCl pH 7.2 and homogenized. Homogenates were diluted with a buffer containing heparin and sodium dodecyl sulphate (12), and LPL activity was determined within 1 h. Epididymal fat pads were immediately delipidated with acetone and diethylether (13). The defatted tissues were stored at -20°C and assayed for LPL activity within 1 day. LPL activity was measured using a glycerol-stabilized tri-[^{3}H]oleoyl glycerol emulsion as described by Nilson-Ehle and Schotz (13). HL activity was measured in liver homogenates within 1 day using a gum acacia stabilized tri-[^{3}H]oleoyl glycerol emulsion in the presence of 1 M NaCl (14). Triglyceride and cholesterol concentrations were determined enzymatically (Testkit combinations, Boehringer Mannheim, F.R.G.). HDL in pooled sera from five rats was separated from other lipoproteins by ultracentrifugation (15).

Statistics: The statistical significance of differences between groups was determined using the unpaired Student's t-test.

RESULTS

Effect of doxazosin and propranolol on lipoprotein lipase in rat heart and adipose tissue

Lipoprotein lipase activity in rat heart and adipose tissue, and hepatic lipase activity in the liver was determined in rats treated with doxazosin or propranolol (Fig.1) and in age- and weight-matched controls that were not treated with either drug. In doxazosin-treated animals, heart LPL activity was higher than in the controls; in the propranolol-treated animals this activity was lower, but the effects were not statistically significant. However, heart LPL activity was significantly higher with doxazosin than with propranolol. Adipose tissue LPL and HL activity were unaffected by either drug. When the results were expressed on a tissue wet weight basis, heart LPL activity was higher with doxazosin than with propranolol (719 ± 121 mU/g vs 527 ± 115 mU/g).

FIG. 1.

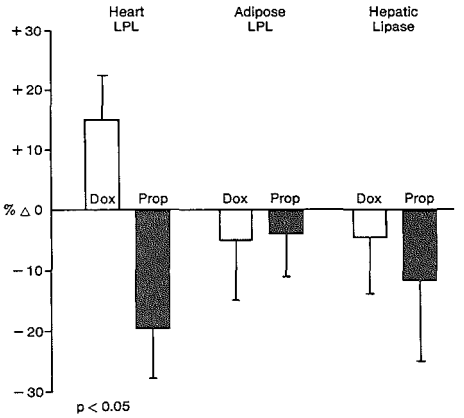


FIG. 2.

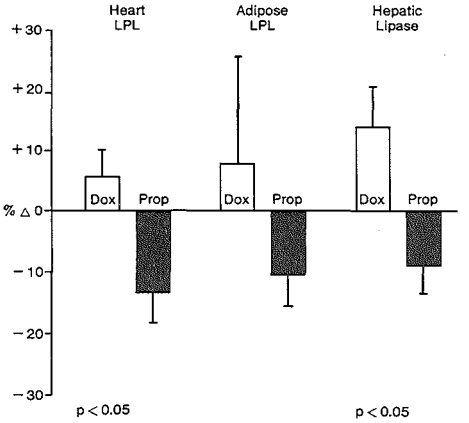


FIG. 1. Percentage change in heart and adipose tissue lipoprotein lipase and hepatic lipase acitivity during treatment with doxazosin (Dox) and propranolol (Prop) in rats fed a normal lab chow. Mean ± SEM lipase activities per heart, left fat pad or liver are expressed as a percentage of a mean control value (heart lipoprotein lipase 478 mU, n = 5; adipose tissue lipoprotein lipase 215 mU, n = 11; hepatic lipase 4115 mU, n = 15). The percentage differences from controls (100%) are given. The significance of the between-groups differences are given (Student's t-test).

FIG. 2. Percentage change in heart and adipose tissue lipoprotein lipase and hepatic lipase activity in cholesterol-fed rats during treatment with doxazosin (Dox) and propranolol (Prop). Results are expressed as in Fig. 1. Control values were: heart lipoprotein lipase 899 mU, n = 5; adipose tissue lipoprotein lipase 180 mU, n = 5; hepatic lipase 3120 mU, n = 15.

Effect of adrenergic blockade on lipoprotein lipase and hepatic lipase in cholesterol-fed rats.

In the hearts of rats fed a cholesterol-enriched diet the LPL activity was higher than in the hearts of rats fed a normal lab diet (683 ± 112 mU/heart vs 899 ±95 mU/heart, means ± SD, n = 10). HL activity was lower in the cholesterol-fed rats than in the control-fed rats (4115 ± 53, n =10 vs. 3119 ± 444, n = 15 mU/liver). LPL activity in adipose tissue was similar in rats on both diets.

In cholesterol-fed rats, the effects of α-1- and β-blockade on heart LPL were similar to those in control fed rats, with a relative increase during doxazosin treatment when compared with propranolol treatment (Fig.2). In adipose tissue the same trends were observed although the changes were statistically not significant.

Table I. Effects of doxazosin and propranolol on plasma cholesterol and triglycerides

Drug	Plasma concentration (mM) TG	TC	HDL cholesterol
None (11)	0.46 ± 0.16	1.83 ± 0.59	0.49 ± 0.07[a]
Doxazosin (11)	0.68 ± 0.25	1.85 ± 0.60	0.61 ± 0.03[b]
Propranolol (11)	0.47 ± 0.20	1.74 ± 0.46	0.48 ± 0.03[a]

Mean plasma values ± SD after the treatment period, number of rats given in brackets.
TG. = plasma triglycerides, TC = total cholesterol.
[a] versus [b], $p < 0.05$.

HL increased in the doxazosin group and increased in the propranolol group, although these changes were not significant. However, the difference between both treatment groups was statistically significant ($p < 0.05$).

Plasma lipid values in cholesterol-fed rats during adrenergic blockade

Doxazosin and propranolol did not affect total plasma cholesterol or triglyceride content in cholesterol-fed rats (Table I). HDL-cholesterol increased significantly in the doxazosin group when compared with controls and propranolol treated rats. HDL-cholesterol was unaffected in the propranolol group.

Table II. Insulin and thyroid hormones in cholesterol-fed rats during α- and β-blockade

Treatment	Insulin (mU/ml)	T_3 (nM)	T_4 (nM)
None (10)	11.5 ± 5.3	1.10 ± 0.20	44 ± 10
Doxazosin (10)	15.2 ± 8.6[a]	1.21 ± 0.17[c]	43 ± 8
Propranolol (10)	8.5 ± 2.7[b]	0.96 ± 0.22[d]	45 ± 9

Mean plasma values ± SD after the treatment period a versus b and c versus d, $p < 0.05$.

Insulin and thyroid hormone levels

As the secretion of several hormones may be affected by adrenergic stimuli, plasma levels of insulin and thyroid hormones were determined (Table II). Doxazosin and propranolol did not significantly affect these hormone levels when rats treated with these drugs were compared with controls. However, insulin and T_3 in the doxazosin group were significantly higher than in the

propranolol group.

DISCUSSION

This study demonstrates that short-term treatment of rats with the long-acting α-1-adrenergic inhibitor doxazosin leads to a significantly higher heart muscle LPL activity than short-term treatment with propranolol. In cholesterol-fed rats HL activity and HDL-cholesterol were relatively enhanced during the α-1-inhibition. Adipose tissue LPL was not significantly affected. In human studies a relative increase in post-heparin LPL activity has been described during α-1-blockade (8-10). As the post-heparin lipase is derived from several tissues, the origin of an enhanced activity in postheparin LPL activity is not clear, but in view of our present studies it is tempting to suggest that in humans also, LPL, mainly (heart) muscle, is affected by adrenergic blockers. In patients on hemodialysis, ß-blockade was found to lower HL activity (8), while in another study no effects were observed (10). In rats, a differential effect of α- and ß-blockade on HL was found only when they were fed a cholesterol-rich diet. The reason for these discrepancies is unclear but may relate to changing adrenergic activity under differing conditions. However, it is generally true that the effects of α- and ß-blockade on lipoprotein lipases in rats and humans are similar. In the present study prazosin was applied at a dose comparable to that used in humans. However, the propranolol dose was less than that used in humans; whether a higher dose would have led to exaggerated lipid effects was not studied.

Dall'Agglio et al. (16), described an increase in HDL-cholesterol and a decrease in plasma triglycerides during α-blockade in rats fed normally. They suggested that the decrease in triglycerides may result from an impaired VLDL secretion. In view of the present findings it seems likely that an increased catabolism of VLDL under the influence of elevated LPL activity plays a role. This mechanism would also explain the increase in HDLcholesterol. Of special interest are the effects of α- and ß-blockade in the cholesterol-fed rats. It was previously found that norepinephrine treatment of cholesterol-fed (but not of

normal lab chow-fed) rats leads to hypercholesterolemia and to a decrease in HDL-cholesterol and HL activity (H. Jansen, unpublished results).

The question arises, does α- or ß-blockade would reverse these effects? As can be seen from Fig.2, the decrease of hepatic lipase activity during cholesterol feeding is partly abolished by doxazosin treatment, while in propranolol-treated rats the lipase activity was further lowered. The difference in HL activity between both groups was significant ($p < 0.05$). As HL may play a role in uptake of HDL-cholesterol by the liver (4,17,18), this may be facilitated during α-1-inhibition in contrast to during ß-blockade.

In the cholesterol-fed rats this did not reduce HDL-cholesterol concentration; instead HDL-cholesterol increased during α-1-inhibition, which indicates that other mechanisms are operative. One such mechanism may consist, at least partly, of an enhanced rate of triglyceride catabolism under the influence of an elevated LPL activity.

These data taken together suggest that in normally fed and cholesterol-fed rats, α-1-inhibition with doxazosin produces changes in plasma lipids and lipoprotein lipases that can be interpreted as beneficial in the sense that they may contribute to a more efficient cholesterol transport to the liver (18), which is in contrast to the effects of the ß-blocker propranolol.

This view requires experimental confirmation. It is not known whether the effects obtained are exerted directly via adrenergic receptors or via other hormones. In the cholesterol-fed rats plasma insulin and T_3 levels were slightly affected. As these hormones may also affect lipase activities, the influence of the adrenergic blockers may be indirect. It is therefore important to determine the levels of these hormones during intervention with adrenergic blockers.

REFERENCES

1. Weinberger, M.H.: Antihypertensive therapy and lipids. Am. J. Med. 80 (suppl 2A): 64-70, 1986.

2. Robinson, D.S.: The function of plasma triglycerides in fatty acid transport. Comp. Biochem. 18: 51-105, 1970.

3. Nikkilä, E.A., Kuusi, T., Tikkanen, M., Taskinen, M.R. Lipoprotein lipase and hepatic endothelial lipase are key enzymes in the metabolism of plasma high density lipoproteins, particularly HDL_2. In: Atherosclerosis V. Gott, A.M., Smith, L.C., Allen, B. eds. New York: Springer-Verlag, 387-392, 1980.

4. Jansen, H., Hülsmann, W.C.: Enzymology and physiology role of hepatic lipase. Biochem. Soc. Trans. 13: 24-26, 1985.

5. Friedman, G., Chajek- Shaul, T., Stein, O., Noe, L., Etienne, J., Stein, Y.: ß-adrenergic stimulation enhances translocation, processing and synthesis of lipoprotein lipase in rat heart cells. Biochim. Biophys. Acta 877: 112-120, 1986.

6. Ball, K.L., Speake, B.K., Robinson, D.S.: Effects of adrenaline on the turnover of lipoprotein lipase in rat adipose tissue. Biochim. Biophys. Acta 877: 399-405, 1986.

7. Schoonderwoerd, G.C., Hülsmann, W.C., Jansen, H.: Regulation of liver lipase II. Involvement of the alpha-1-receptor. Biochim. Biophys. Acta 795: 481-486, 1984.

8. Harter, H.R., Meltzer, V.N., Tindira, C.A., Naumorich, A.D., Goldberg, A.P.: Comparison of the effects of prazosin versus propranolol on plasma lipoprotein lipids in patients receiving hemodialysis. Am. J. Med. 80 (suppl 2A): 82-89, 1986.

9. Ferrara, L.A., Marotta, T., Rubba, P., et al.: Effects of α-adrenergic receptor blockade on lipid metabolism. Am. J. Med. 80 (supp 12A): 104-108, 1986.

10. Jansen, H., Laird- Meeter, K., Baggen, M.G.A. et al.: The effects of prazosin and propranolol treatment on plasma lipoproteins and postheparin plasma lipase activities. Vth Washington Spring Symposium 1986: abstract 299.

11. Smits, J.F.M., Coleman, T.G., Smith, T.L., Kasbergen, C.M., van Essen, H., Struyker-Boudier, H.A.J.: Antihypertensive effect of propranolol in concious spontaneously hypertensive rats: central hemoplasmavolume, adrenal function during ß-blockade with propranolol. J. Cardiovasc. Pharmacol. 4: 903-914, 1982.

12. Hülsmann, W.C., Dubelaar, M.L.: Lipoprotein lipases and stress hormones: studies with glucocorticoids and choleratoxin.
Biochim. Biophys. Acta 875: 69-75, 1986.

13. Nilsson-Ehle, P., Schotz, M.C.: A stable radioactive substrate emulsion for assay of lipoprotein lipase.
J. Lipid Res. 17: 536-541, 1976.

14. Jansen, H., Birkenhäger, J.C.: Liver lipase-like activity in human and hamster adrenocortical tissue.
Metabolism 30: 428-430, 1981.

15. Jansen, H., Schoonderwoerd, G.C., Dallinga-Thie, G.M.: Separation of rat plasma HDL subfractions by density gradient centrifugation and the effect of incubation on these fractions.
Biochim. Biophys. Acta 754: 271-278, 1983.

16. Dall'Agglio, E., Chang, H., Reaven, G.M.: Disparate effects of prazosin and propranolol on lipid metabolism in a rat model. Am. J. Med. 76: 85-88, 1984.

17. Kuusi, T., Kinnunen, P.K.J., Nikkiläm E.A.: Hepatic endothelial lipase antiserum influences rat plasma low and high density lipoproteins in vivo.
FEBS Lett 104: 384-388, 1979.

18. Jansen, H., Hülsmann, W.C.: Heparin-releasable (liver) lipase(s) may play a role in the uptake of cholesterol by steroid-secreting tissues.
Trends Biochem. Soc. 5: 265-268, 1980.

CHAPTER VII

A COMPARATIVE STUDY ON THE EFFECTS OF PRAZOSIN AND PROPRANOLOL TREATMENT ON PLASMA LIPOPROTEINS AND POST-HEPARIN PLASMA LIPASE ACTIVITIES IN MALE PATIENTS AFTER CORONARY BYPASS GRAFTING.

H. Jansen, K. Laird-Meeter, M.G.A. Baggen, J.D. Barth, G.A. van Es, J. Lubsen and J.C. Birkenhäger.

submitted for publication.

SUMMARY.

Male patients (52) with mild hypertension who had undergone coronary bypass grafting (CABG) were randomly allocated to propranolol or prazosin treatment. Plasma lipid parameters were measured on 3 occasions: at the start of the treatment 6 weeks postoperatively (visit 1), 3 months (visit 2) and 6-9 months (visit 3) later. The effects of both drugs on lipid parameters were compared using analysis of covariance. During the first period (visit 1 to visit 2) prazosin treatment, in comparison to propranolol, led to a lower plasma triglyceride (mean treatment difference (m.t.d.) -25%) and a higher HDL-cholesterol/total cholesterol ratio (m.t.d. +14%). During prolonged treatment (6-9 months) prazosin resulted in, beside a consistant lower triglyceride level (m.t.d. -20%) and a higher HDL-cholesterol/total cholesterol ratio (m.t.d. +14%), a significantly higher HDL-cholesterol level (m.t.d. +12%) and lipoprotein lipase activity (m.t.d. +20%) than propranolol. Propranolol and prazosin did not differentially affect hepatic lipase activity. These results indicate that effects of adrenoceptor blockers on lipoprotein lipase activity may be part of the mechanisms leading to differential influences of alpha- and beta-blockers on plasma lipid levels.

INTRODUCTION.

Hypertension has been recognized as a major risk factor for the development of premature atherosclerosis leading to coronary heart disease. However antihypertensive treatment with beta-blockers fails to substantially prevent the occurrence of coronary heart disease. The explanation may be that this treatment also affects lipid metabolism. The changes in plasma lipid levels are such that the possible beneficial effect of the lowering of the blood pressure is counteracted by adverse effects on plasma lipid levels, especially on cholesterol and triglycerides. In a number of studies it has been shown that beta-blockade can lead to an increase in plasma low-density lipoprotein (LDL) cholesterol and triglycerides and a decrease in high-density-lipoprotein (HDL) cholesterol (1-4).

Alpha-adrenoceptor blockade has been reported to have an opposite effect (1-4). Little is known about the mechanisms which lead to the changes in lipoprotein levels during adrenoceptor blockade. Two endothelially located lipases, lipoprotein lipase (LPL) in extrahepatic tissues and hepatic lipase (HL) in the liver play an important role in lipoprotein metabolism and seem to be major determinants of plasma triglyceride and HDL levels (5). To study the possible involvement of these enzymes in the lipoprotein changes during treatment of hypertension with alpha- or beta-blockers we determined the activity of the enzymes in patients who had undergone coronary bypass grafting and were treated with either the beta-blocker, propranolol, or the alpha-$_1$-specific blocker, prazosin.

PATIENTS AND METHODS.

Fifty two male patients entered the study. They had undergone a first isolated coronary artery bypass grafting (CABG) and had moderate hypertension (systolic 150-190 mmHg, diastolic 90-120 mmHg) and were not in need of beta-blocking agents. Exclusion criteria were severe heart failure, valvular heart disease, recent (< 3 months) myocardial infarction, slow heart rate (< 50 b.p.m.), pulmonary disease for which medication had to be taken, anaemia (Hb < 6.5 mM/l), diabetes mellitus, hypothyroidism and secondary hyperlipidemia.

Study design.

After informed consent was obtained, randomization was carried out before CABG. The assignment to a treatment group was determined by drawing an envelope containing a note on the drug to be used. Six weeks after CABG the patients were seen at the outpatient service of the Thoraxcenter of Rotterdam (visit 1). During this visit blood samples were taken to obtain base-line information on lipid profile, biochemistry and enzymes and the hypertension treatment started. Visit 2 was 3 months after visit 1 and visit 3, the end of the study period, 6-9 months after visit 1.

Drug administration.

Prazosin was given twice daily, starting with 2 mg per day.

Propranolol was taken 3 times a day, starting with 60 mg per day. The dosage of the drugs was adjusted dependent on the effect on the bloodpressure until a level equal to or below 90 mmHg diastolic and 150 mmHg systolic was reached. Prazosin was prescribed with a mean dose of 2.8 mg per day, range 1.5-15 mg, Propranolol at 110 mg per day, range 20-160 mg.

Chemical methods.

Plasma triglycerides (6), and total cholesterol (7) were measured with standard enzymatic laboratory methods. HDL cholesterol was determined after precipitation of other lipoproteins with heparin and manganese^{2+} and with phosphotungstic acid plus magnesium^{2+} (8). HDL subfractions were separated as described by Gidez et al. (9). The fraction that is precipitated with dextran-sulphate is indicated as HDL_2 and the fraction that remains in solution as HDL_3. LDL cholesterol was calculated with the Friedewald formula (10).

LPL and HL activities in postheparin blood plasma were measured with the immunochemical method described by Huttunen et al. (11). The blood samples were withdrawn 20 min after the intravenous administration of 50 IU heparin/kg bodyweight. Intra-assay variation in LPL was 2.6% and inter-assay variation 4.4%. Intra-assay variation in HL 3.5% and inter-assay variation 6.8%.

Statistical Methods.

All results are expressed as means ± 1 s.d. Lipid parameters at different time points were compared with the values at visit 1 using the paired t-test (12). To compare the influence of prazosin and propranolol treatment on the lipid profile, analysis of covariance (13) was performed to correct for a possible incomparability of the base-line data. Data concerning the lipid profile of 24 patients treated with prazosin and 26 patients treated with propranolol at visit 1 and visit 2 were compared. The same comparison was made for visit 1 and visit 3 with 22 patients on prazosin and 24 on propranolol. The mean treatment differences were adjusted for baseline incomparability. The analysis showed no evidence for inequality of slopes between the two treatment groups.

Table I

Plasma lipid and Lipoprotein Concentrations during Propranolol Treatment.

Variable (mM)	Visit 1 (n = 26)	Visit 2 (n = 26)	Visit 3 (n = 24)
Triglyceride	2.24 ± 1.10	2.04 ± 0.87	2.18 ± 0.82
Total cholesterol	6.20 ± 1.33	6.92 ± 1.29*	6.90 ± 1.32*
LDL-cholesterol	4.24 ± 0.99	4.98 ± 0.94*	4.87 ± 1.03*
HDL-cholesterol	0.90 ± 0.23	1.00 ± 0.25*	1.03 ± 0.25*
HDL_2-cholesterol	0.16 ± 0.09	0.16 ± 0.08	0.14 ± 0.08
HDL_3-cholesterol	0.70 ± 0.14	0.80 ± 0.23*	0.83 ± 0.15***
Ratio HDL-C/TC	0.14 ± 0.03	0.14 ± 0.03	0.15 ± 0.04

* denotes a statistically significant difference from visit 1 with $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. LDL-cholesterol = low density lipoprotein cholesterol, HDL-cholesterol, HDL-C = high density lipoprotein cholesterol, TC = total plasma cholesterol.

Table II

Plasma Lipid and Lipoprotein Concentrations during Prazosin Treatment.

Variable (mM)	Visit 1 (n = 25)	Visit 2 (n = 24)	Visit 3 (n = 22)
Triglyceride	2.25 ± 1.47	1.47 ± 0.68**	1.74 ± 1.04*
Total cholesterol	5.85 ± 1.27	6.37 ± 1.00*	6.18 ± 1.03
LDL-cholesterol	3.90 ± 1.12	4.52 ± 0.88*	4.27 ± 0.74
HDL-cholesterol	0.92 ± 0.21	1.09 ± 0.21**	1.13 ± 0.24**
HDL_2-cholesterol	0.17 ± 0.09	0.18 ± 0.13	0.20 ± 0.15
HDL_3-cholesterol	0.74 ± 0.16	0.88 ± 0.14***	0.89 ± 0.14***
Ratio HDL-C/TC	0.16 ± 0.04	0.17 ± 0.04	0.18 ± 0.04++

* denotes a statistical significant difference from visit 1 with $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. LDL-cholesterol = low density lipoprotein cholesterol, HDL-cholesterol, HDL-C = high density lipoprotein cholesterol, TC = total plasma cholesterol.

RESULTS.

Patient population follow-up.

One of the patients admitted to the randomization procedure was excluded from the analysis because of protocol violations before the start of the study therapy. From the remaining 51 patients 25 were placed in the prazosin and 26 in the propranolol group. The median age of the men receiving prazosin was 57 years, range 41-69, and propranolol: 57 years, range 38-68. Three patients on prazosin discontinued trial medication prematurely. The reasons for discontinuation were: severe angina pectoris (2), dizziness

and palpitations (1). One patient's data on propranolol were incomplete due to an administrative error and one patient, participating in the propranolol group, deceased. Hence 22 patients of the prazosin group and 24 patients of the propranolol group completed the study.

Plasma lipid values.

In both treatment groups considerable changes in plasma lipid values in comparison to visit 1 had occurred at visit 2 and visit 3 (Tables I,II). In the propranolol group total cholesterol, LDL cholesterol and HDL cholesterol levels were increased on both occasions (Table I). The elevation in HDL cholesterol was mainly in the HDL_3 fraction. In the Prazosin group the total cholesterol and the LDL cholesterol concentrations were increased at visit 2, returning to basal values at visit 3 in comparison to visit 1. HDL cholesterol showed an increase at visit 2 and 3, also mainly due to a higher level of HDL_3 cholesterol. The ratio of HDL- cholesterol/total cholesterol was in the prazosin group significantly enhanced at visit 3. Another major effect in the prazosin group was a substantial decrease in the plasma triglyceride concentration at visits 2 and 3.

While part of the changes may due to recovery from the post-surgical situation probably present at visit 1 covariance analysis of the data was carried out to compare the specific effects of the treatment with either drug. Prazosin treatment resulted in a significantly lower plasma triglyceride concentration than propranolol treatment at visit 2 and at visit 3. The m.t.d. are shown in Fig 1. There was no statistically significant difference between the treatment effects on HDL cholesterol levels at visit 2. At visit 3 the prazosin treatment was found to result in a higher HDL cholesterol concentration than the propranolol treatment (mean treatment difference = 0.11 mM ; 12% of the initial value, $p < 0.05$). If this fraction was determined after precipitation of the other lipoproteins with phosphotungstic acid and magnesium the trend was the same, but the treatment difference was not statistically significant (0.088 mM, $p = 0.14$). Prazosin treated men had an increased HDL

cholesterol/total cholesterol ratio at visit 2 and visit 3 compared to the patients taking propranolol (m.t.d. on both occasions = 0.021; 14% of initial value, with $p < 0.05$).

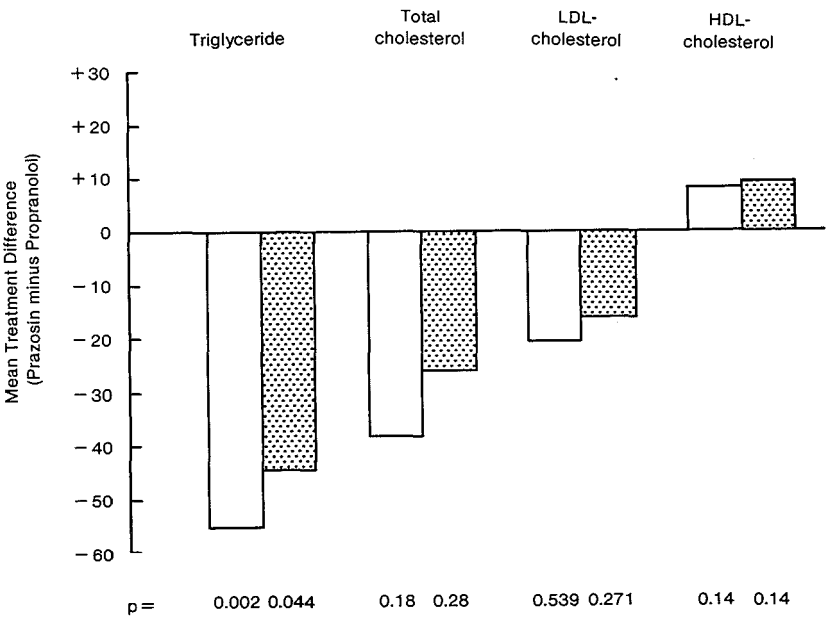


Fig. 1. Mean treatment differences in plasma lipid during Prazosin or Propranolol treatment.
The mean treatment difference in the values during Prazosin treatment minus the values during Propranolol treatment are given. At the bottom of the fig. the actual P values are shown. The open bars relate to the data at visit 2, the black bars relate to the data at visit 3.

Lipase activities.

At visit 2 the LPL activity in the prazosin group was higher than at visit 1 (Table III). At visit 3 the activity was less enhanced and the difference with visit 1 was no longer statistically significant. LPL activity did not change during the study period in the propranolol treated patients.

Comparing the effect of treatment with prazosin with the treatment with propranolol using covariance analysis the prazosin treatment was found to lead to a higher LPL activity than propranolol treatment. The mean treatment difference at visit 2 and visit 3 about 20 % of the initial value ($p = 0.05$, Table III). HL activity tended to increase at visit 2 in all patients but this effect was only statistically significant

Table III

Postheparin Plasma Lipase Activities during Treatment with Prazosin or Propranolol.

Enzyme (mUnits/ml)	Visit	Prazosin	Propranolol
Lipoprotein Lipase	1	67 ± 24	69 ± 28
	2	89 ± 33*	75 ± 27
	3	78 ± 27	64 ± 20
Hepatic Lipase	1	281 ± 106	251 ± 91
	2	295 ± 98	301 ± 110*
	3	263 ± 65	268 ± 105

* denotes a statistically significant difference from visit 1 with $p < 0.05$.

in the propranolol group. At visit 3 neither in the propranolol nor in the prazosin group a substantial difference from visit 1 in HL activity was found. Covariance analysis showed no differential effect between treatment with either drug on HL activity. The mean treatment differences were less than 7% of the initial values.

DISCUSSION.

In this study the possible involvement of LPL and/or HL in the changes in plasma lipid levels provoked by adrenoceptor blockade was studied. Therefore the effects of prazosin and propranolol treatment on plasma lipids, lipoproteins and postheparin lipase activities were compared in mildly hypertensive patients who had undergone coronary artery bypass grafting. Two main conclusions could be drawn: prazosin treatment leads in comparison to propranolol to 1. a more favorable lipid profile (a lower plasma triglyceride, a higher HDL-cholesterol and a higher HDL-cholesterol/total cholesterol ratio) and 2. a relatively higher LPL activity. Opposite changes in the concentration of HDL and plasma triglycerides may causally be related. During the degradation of triglycerides in VLDLs or chylomicrons by LPL surface material is released from these lipoproteins and becomes associated with the HDL fraction (5,14). A rapid VLDL catabolism may therefore lead to a low plasma triglyceride and a high HDL

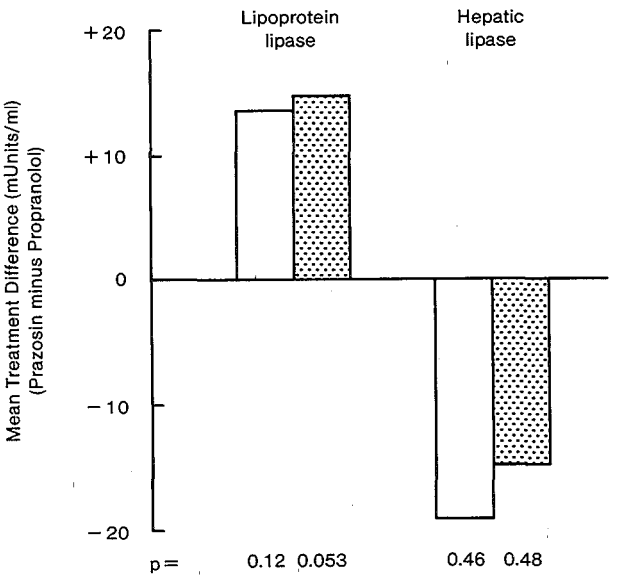


Fig. 2. Mean treatment differences in postheparin plasma lipase activities. The data are presented in the same way as in Fig. 1.

concentration. Plasma triglycerides were at the start of the study period (visit 1) higher (means around 2.25 mM) and HDL cholesterol lower (around 0.90 mM) than normally found. LPL was in the low-normal range (mean LPL patients around 68 mU/ml, normal 65-120 mU/ml. This picture, probably a postsurgical phenomenon, suggests a situation of impaired triglyceride catabolism at least partly due to the relatively low LPL activity. LPL activity was inversely correlated to the plasma triglyceride concentration ($r = -0.30$), which illustrates the importance of this lipase activity as a de-terminant of the triglyceride level. The simultaneous decrease in plasma triglyceride (-35%) and increase in HDL cholesterol (+ 16%) that occurred between visit 1 and 2 in the prazosin treated patients probably results from an enhanced degradation rate of VLDL triglyceride resulting from an increased LPL activity (+33%). During propranolol administration the changes in this period were much smaller (triglyceride -10%, HDL cholesterol +10%, LPL +9%) and only significant in HDL cholesterol. Since in the period between visit 1 and visit 2 presumably the effect of a recovery of the postsurgical to a normal situation also

influences the plasma lipid concentrations, the effects observed cannot completely be ascribed to the drug treatment. Therefore to compare the effects of both drugs analysis of covariance was carried out. From this analysis appeared that prazosin treatment leads to a statistically lower plasma triglyceride and a higher HDL cholesterol/total cholesterol value in comparison to propranolol treatment. On the long-term (visit 3) the differences in these variables remained, while differences in other lipoprotein variables between prazosin treatment and propranolol treatment that were already apparent at visit 2 became statistically significant, notably: a higher HDL cholesterol level and a higher LPL activity during prazosin than during propranolol treatment. Why the effects on HDL cholesterol concentrations were more clear when the other lipoproteins were precipitated with heparin-manganese instead of phosphotungstic acid and magnesium is not clear. It seems to indicate that both methods are not completely comparable. As discussed above the opposite effects of alpha- and betablockade on plasma lipids may partly result from inverse effects of the blockers on LPL activity. It is clear however that not all changes in plasma lipoproteins can be ascribed to alterations in the activity of this enzyme. For example, LPL activity in the prazosin group was not significantly different from visit 1 and still triglycerides were lowered and HDL cholesterol enhanced. HL seems not to be involved as this enzyme showed similar activity in both groups. An effect on plasma triglyceride clearing during adrenergic blockade has been suggested before. Ferrara et al. (15) found an increase in postheparin lipolytic activity during short-term treatment with prazosin. LPL and HL were however not separately measured in this study. Harter et al. (16) found, very small, inverse changes in LPL (+2.5% and -2.5 %, resp.) during alpha- and beta blockade in patients on hemodialysis, while Day et al. (17) demonstrated a decrease in the catabolic rate of plasma triglyceride following beta-blockade. In this latter study plasma triglycerides increased during beta-blockade and the authors suggested a lowering in LPL activity. Lithell and coworkers (18) found no effect of prazosin on LPL in biopsies of

muscle and adipose tissue. However, in their study women who had initially a low plasma triglyceride and a relatively high HDL level were studied. This suggests that in contrast to the initial situation in our study VLDL catabolism was already rapid and LPL activity high. In this case LPL may not be further stimulated by alpha-blockade, but, as in the study by Day et al. (17), may indeed be lowered by beta-blockade. While the differential effect of alpha- and beta blockade on LPL at least partly explains the inverse alterations in HDL and plasma triglycerides other mechanisms may be operative too. In conclusion: antihypertensive treatment with the $alpha_1$-specific blocker, prazosin leads to a more favorable lipid profile than the beta-blocking agent propranolol; an important factor in the generation of this favorable lipid profile may be a higher LPL activity in the prazosin treated patients.

REFERENCES

1. Leren, P., Hegeland, A., Holme, I., Foss, P.O., Hjermann, I., Lund-Larsen, P.G. Effect of propranolol and prazosin on blood lipids. The Oslo study. Lancet, ii: 4-6, 1980.
2. Lowenstein, J., Neusy, A.J. Effects of Prazosin and Propranolol on serum lipids in patients with essential hypertension. Am. J. Med., 76: 79-84, 1984.
3. Grimm, R.H., Hunninghale, D.B. Lipids and hypertension. Am. J. Med., 80 (suppl. 2A) : 56-63, 1986.
4. Weinberger, M.H. Antihypertensive therapy and lipids. Am. J. Med., 80 (suppl. 2A): 64-70, 1986.
5. Nikkillä, E.A., Kuusi, T., Tikkanen, M., Taskinen, M.R. Lipoprotein lipase and hepatic endothelial lipase are key enzymes in the metabolism of plasma HDL, particularly HDL_2. In: Atherosclerosis V (Gotto, A.M., Smith L.C., Allen B. eds), New York, Springer Verlag, 387-397, 1980.
6. Nägele, U., Lehman, P., Wiedeman, E. and Wahlefeld, A.W. An enzymatic colour test for the determination of triglycerides using an alpha-glycerophosphate oxidase/-PAP method. Clin. Chim. 29: 1229, 1983.
7. Kattermann, R., Jaworek, D. and Möller, G. Multicentre study of a new enzymatic method of cholesterol determination. J. Clin. Chim. Clin. Biochem. 22: 245-251, 1984.
8. Warnick, G.R., Cheung, M.C., Albers, J.J. Comparison of current methods for HDL cholesterol quantitation. Clin. Med., 25: 596-604, 1979.
9. Gidez, L.I., Miller, G.J., Burstein, M., Slagle, S., Eder, H.A. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J. Lipid Res., 23: 1206-1223, 1982.
10. Friedewald, J.T., Levy, R.I. and Frederickson, D.S. Estimation of the concentration of low-density

lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge.
Clin. Chem., 18: 1463-1467, 1972.

11. Huttunen, J.K., Ehnholm, C, Kinnunen, P.K. and Nikkilä, E.A. An immunochemical method for selective measurement of two triglyceride lipases in human postheparin plasma. Clin. Chim. Acta, 63: 335-347, 1975.
12. Duncan, R.C., Knapp, R.G. and Clinton Miller III, M. In: Introductory biostatistics for the health sciences, John Wiley & Sons, New York/ London/ Sydney/ Toronto, 79, 1979.
13. BMDP statistical software, University of California Press, Berkely, California 1983.
14. Eisenberg, S. High density lipoprotein metabolism, J. Lipid Res., 25: 1017-1058, 1984.
15. Ferrara, L.A., Marotta, T., Rubba, P., de Simone, B., Leccia, L.A., Soro, S., Mancini, M. Effects of alpha-adrenergic and beta-adrenergic receptor blockade on lipid metabolism. Am. J. Med., 80 : 104-108, 1986.
16. Harter, H.R., Meltzer, V.N., Tindira, C.A., Naumovich, A.D., Goldberg, A.P. Comparison of the effects of Prazosin versus Propranolol on plasma lipids in patients receiving hemodialysis, Am. J. Med., 80: 82-89, 1986.
17. Day, J.L., Melcalfe, J., Simpson, N., Lowenthal, L. Adrenergic mechanisms in the control of plasma lipids in man. Am. J. Med., 80: 82-89, 1986.
18. Lithell, H., Waern, N., Vessby, B. Effect of Prazosin on lipoprotein metabolism in premenopausal hypertensive women. J. Cardiovasc. Pharm., 4: 5242-5244, 1982.

CHAPTER VIII

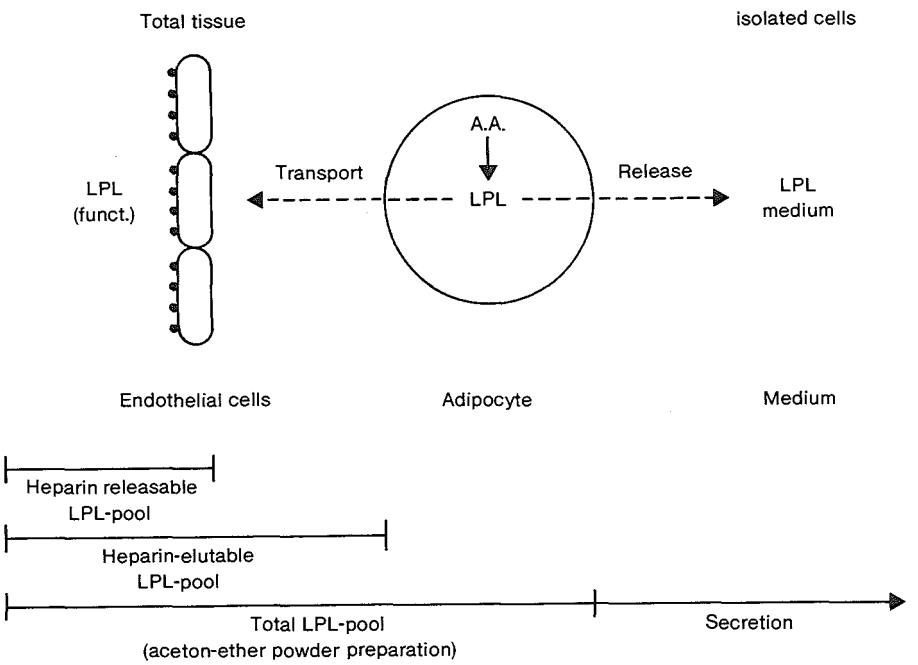
GENERAL DISCUSSION

GENERAL DISCUSSION.

Lipoprotein lipase (LPL; EC 3.1.1.34) activity in adipose tissue was studied in vitro, in the selectively perfused epididymal fatpad of the rat, and in post-heparin plasma of humans.

The synthesis of LPL in the adipocyte is under control of several hormones, of which insulin is probably the most important. The enzyme is transported via an unknown mechanism, but probably is a glycosylation step necessary, while the intracellular microtubular system is involved. The enzyme is functional at the endothelial site of the capillary wall, bound to heparan-sulphate, from which binding sites it can be released by heparin.

Fig. 1: A schematical representation of the measarable LPL pools in adipose tissue.



Some groups have found an inhibitory effect of several weeks administration of glucocorticoids on LPL activity, whileothers reported a stimulating effect of one dose. We made rats hypercorticistic by giving them a daily subcutaneous injection of Synacthen, a synthetic corticotrophin analogue for 3 days or

longer (Chapter III). This regimen leads to an important elevation of the corticosterone production and plasma corticosterone levels in fasted and fed animals (610 ± 137 ng/ml, controls 258 ± 120 ng/ml). While total body weight is decreasing the weight of the epididymal fat pad of the hypercorticistic animal is preserved. This hypercorticism leads also to an increase of plasma insulin levels. From 7.8 ± 4.7 μU/ml in fasted controls to 20.6 ± 8.4 μU/ml in fasted hypercorticistic animals. These last levels are the same as the insulin levels in fed controls (25.0 ± 3.7 μU/ml). Longterm treatment (20 days) does not raise insulin levels further in fasted groups, but do so in the fed treated groups (45.0 ± 7.8 μU/ml).

LPL activity, measured in an extract of an acetone-ether powder of the epididymal fat pad is stimulated by feeding. Adrenalectomy lowered the LPL-activity in overnight fasted as well as in fed animals. Synacthen treatment induced an increase of the LPL-activity in intact fasted animals (from 84 ± 15 to 140 ± 25 mU/gww), that is to levels found in the untreated fed group. In fed animals no increase in LPL-activity by Synacthen is observed.

This is in contrast to the data of Lawson et al. (1) who found a decrease in adipose tissue LPL activity. However, he used a much lower dose of ACTH and studied the enzyme only after feeding. Moreover he did not find the effect throughout the year, but only in the winter. We did not observe any in vitro effect of ACTH on LPL after feeding and the stimulating effect of ACTH after overnight fasting was observed the whole year through. The fact that Chernick et al. (2) did not find an effect of ACTH on LPL activity in 3T3-L1 adipocytes, is in line which our observation that ACTH needs corticosterone to express the effect on adipose tissue LPL activity: Synacthen treatment did not raise the LPL-activity in fasted nor fed adrenalectomized animals. The heparin-elutable LPL-activity, which is to be believed to represent the physiologically active LPL pool of the epididymal fat pad <u>in vitro</u> is an order of magnitude higher after feeding the rat, (29 ± 7 and 444 ± 102 mU/gww, respectively). The differences in milli-units enzyme activity found with

the acetone-ether powder and heparin-elutable method may be explained by the fact that heparin stabilizes LPL. It is also possible that a fraction of the LPL-activity is inactivated by the procedure in making an acetone-ether powder. Synacthen does not have any effect on the heparin-elutable LPL-activity in fasted, nor fed animals in vitro.

Table I. Scheme of Intracellular Regulation of Lipoprotein Lipase Synthesis.

Parenchyma	Stimulation		Inhibition	Inactivation
		Amino Acids Energy		
	Insulin ACTH Glucocorticoids	↓ (synthesis) PROENZYME	← Cycloheximide Glucocorticoids	
	Glucose	Glycosylation	← Tunicamycin	
		↓ (activation)		
		ACTIVE ENZYME	←- - - - - - - - - - - -	Catecholamines Theophylline
		↓		
		Microtubular transport	← Colchicine	
		(secretion)		
Interstitium		? (transport)		
Endothelium	α_1-adrenergic blockade	? (transport)		
Capillary		Active Site		

The induction of hypercorticism by the administration of corticosterone instead of ACTH leads to a decrease in the LPL activity from an extract of an acetone-ether powder as well as in the heparin-elutable epididymal adipose tissue LPL activity in vitro (Chapter V).

This effect was found in normally fed and in overnight fasted animals. This is in line with data reported by de Gasquet et al. (3), Krotkiewski et al. (4), Bagdade et al. (5) and Cigiloni &

Smith (6). Although in another study De Gasquet et al. (7) found an increase in adipose tissue LPL activity several hours after an intraperitoneally injection of dexamethasone or corticosterone in fed and fasted animals. Ashby et al. (8) found in vitro a stimulation of LPL activity after administration of glucocorticoids. This is not necessarily in contrast to our findings, since he used a much higher concentration of insulin in the incubation medium. Moreover he found the strongest effects with synthetic glucocorticoids and not with corticosterone. The effects of corticosterone that we observed are opposite to those found after induction of hypercorticism by corticotrophin administration. This apparent discrepancy was not due to a different level of corticosterone or insulin in the plasma as both hormones reached comparable levels in both types of hypercorticism (Chapter III). Therefore we investigated the possibility whether a combination of a high corticosterone level plus corticotrophin or corticotrophin alone may stimulate LPL. The last possibility appears to be unlikely as after adrenalectomy, when endogenous corticotrophin levels are high, LPL activity is low. Moreover we showed also that Synacthen treatment of adrenalectomized rats does not affect LPL activity (Chapter III). When corticotrophin and corticosterone were simultaneously administered to intact rats the lowering of LPL activity by corticosterone was completely abolished. In adrenalectomized rats treated with corticotrophin plus corticosterone the LPL activity reached values significantly higher than in the adrenalectomized rats not treated with either hormone. The experiments with the adrenalectomized rats also indicate that in the stimulatory effect of corticotrophin on LPL probably no other adrenal hormone is involved than corticosterone. Based on these results it is clear that corticotrophin has a direct stimulating effect on the activity of LPL in the adipocyte of the rat. The inhibition of LPL during corticosterone administration may be due to suppression of the endogenous corticotrophin secretion. Stimulation of LPL by corticotrophin was only seen in the presence of (endogenous or exogenous) corticosterone. Therefore corticosterone

and corticotrophin appear to be both necessary for the regulation of adipose tissue LPL.

To study the physiological meaning for the adipose tissue of the effects of Synacthen on LPL activity and on the balance of in- and efflux of fatty acids, the epididymal fat pad was selectively perfused (Chapter II). We applied some modifications to the perfusion technique, of the epididymal fat pad as described by Ho and Meng (9). We aimed at preventing swelling of the tissue and at maintaining it in a good condition during the procedure. Judged by the ATP content and energy charge of the adipose tissue before and after perfusion, the tissue could be kept in a metabolically healthy state for at least 150 minutes. This was accomplished using a perfusion rate of maximally 100 µl medium per min (in vivo the flow rate is 70 µl/g/min) and a medium containing albumin, glucose, insulin, amino acids and red blood cells as oxygen carrier. During all experiments we observed a spontaneous LPL-release into the perfusion medium. This has also been reported by Scow et al. (10), but not by Fielding et al. (11) . The activity released into the medium by adipose tissue from fasted animals was about 1 o/oo per minute of the total LPL activity in the tissue. In the fed state the absolute spontaneous release of LPL activity was 4 - 5 times higher, but as a percentage of the LPL activity present in the fat pad not different from that in fasted animals. Upon addition of heparin to the perfusion medium the release of the enzyme rose to 20 - 25 times over the basal value.

As described above the enzyme activity released by heparin elution in vitro was from the adipose tissue of fed rats 10 or more times higher than from the fasted animals, while a factor 2 to 4 was found in acetone-ether powder extracts of the epididymal fat pads of the fed and fasted animals (Chapter III). In the perfused epididymal fat pad LPL activity was measured in the post-perfusion medium (during perfusion heparin was added to the medium) and we found that the heparin-releasable LPL was 3-5 times higher in the fed than in the fasted animals. Therefore, with the in vitro heparin elution method, to determine LPL activity, in the adipose tissue from the fed animals not only the

enzyme in the vascular compartment appears to be measured. However, LPL is a labile enzyme and its activity rapidly decreases at 37°C. Although the sum of the LPL activity released from the endothelium and the remaining LPL activity in the fat pad after the perfusion procedure (acetone-ether method) was equal to the activity extracted from the non-perfused fat pad (acetone-ether method), this does not exclude the possibility of an underestimation of LPL activity during the heparin perfusion.

The heparin-releasable pool of LPL activity measured by perfusion of the epididymal fat pad is 10%-20% of the total pool, in the fasted as well as in the fed state, which means that not only the synthesis, but also the transport of LPL to the vascular wall is enhanced. A strong correlation was found between the rate of chylomicron-TG hydrolysis and the total amount of heparin-releasable LPL in the perfusion medium ($r = 0.94$ in fasted and $r = 0.96$ in fed animals). This once more establishes the conclusion that the LPL activity in the vascular compartment is the physiologically active fraction.

We found that the LPL activity measured in vitro is influenced by the FA-concentration: the enzyme activity is low if fatty acid-free albumin was used and increases to a maximum when the FA-load was raised to a FA/albumin ratio of 5 : 1. Albumin saturated with FA in the medium inhibited the LPL activity. In the perfusion model of the fat pad when fatty acid free albumin was used in the medium also no hydrolysis of chylomicrons could be detected. Using albumin with varying amounts of FA complexed to it, hydrolysis of chylomicron-TG and uptake of the FA released occurred. The rate of these processes did not change with different FA/albumin ratios. So in situ the relationship between FA-albumin and LPL activity appears less important than it seems to be in vitro. One of the possible explanations for this difference could be that under the conditions of the perfusion the adipose tissue by itself is a FA acceptor too. As to the influence of flow rate Scow et al. (10) described a relationship between the FFA released from the fat pad with that variable, but he did not report on an influence of flow rate on chylomicron hydrolysis. We found a percentual increase of the

chylomicron hydrolysis if the flow rate was lowered, but the uptake of liberated fatty acids did not increase.
In fact not the hydrolysis of TG by LPL, but the uptake of FA by the adipose tissue appears to be the limiting factor.
The hydrolysis of chylomicrons and VLDL is low in the fasted state. In fed animals the hydrolysis of chylomicron-TG is higher, but VLDL-TG hydrolysis remains unchanged. These differences were also found for the uptake of the liberated TG-FA. This is in line with the general idea that chylomicron-TG is preferentially taken up by adipose tissue and VLDL-TG by muscle tissue. However it should be kept in mind that in contrast to the chylomicrons used VLDL isolated from plasma has already been in contact with LPL. Therefore, the particles isolated may be regarded as remnant particles from VLDL and a less good substrate for LPL. It is difficult to compare our results with data derived from in vivo studies on the uptake of liberated FA from chylomicron-TG by adipose tissue, since the liver is removing most of it after an intravenous injection. However, the uptake of chylomicron-TG-FA in perfused adipose tissue in our hands is comparable to that calculated by Scow et al. (10) from their experiments with perfused parametrial fat.
The uptake of liberated FA is correlated with the chylomicron-TG concentration in the medium at least up to 4 mM TG; higher concentrations were not used.
In the hypercorticistic rats (by Synacthen treatment), the heparin-releasable LPL activity in the effluent of the perfused epididymal fat pad rose from 3 ± 1 mU to 12 ± 8 mU (in a sixty minutes lasting perfusion; mean ± SD) in fasted animals. However, in fed animals the heparin-releasable LPL-pool has a much higher activity than in the fasted treated animals (36 ± 20 respectively 12 ± 8 mU), and Synacthen treatment did not further increase this pool in the fed animals (30 ± 13 mU). This is in contrast to the lack of influence of Synacthen treatment on the heparin-elutable LPL activity in in vitro experiments with the epididymal fatpad (Chapter III). The increase in the heparin-releasable LPL pool <u>in situ</u> explains the observed rise in TG-hydrolysis and the increase of the uptake of TG-FA. In controls

hydrolysis of chylomicron-TG correlated strongly with the heparin-releasable LPL activity in the perfusate. Such a correlation was not found in the hypercorticistic animals. Because there was not a significant correlation between hydrolysis of chylomicron-TG and the heparin-releasable LPL-activity in the Synacthen-treated animals, it seems that not under all conditions LPL is the only or major determinant of the hydrolysis of chylomicron-TG. It is possible that Synacthen treatment induces changes in the enzyme configuration lowering the K_m of the enzyme for chylomicrons leading to a higher rate of hydrolysis with a given in vitro LPL activity (capacity). The increase in hydrolysis rate of chylomicrons in short-term treated and fasted rats leads to an increase in uptake of FA by the epididymal pad to levels found in the fed animals. Apparently hypercorticism leads in vivo to an increase of the FA uptake by the adipose tissue (Chapter IV). The discrepancy in the effects on chylomicron hydrolysis and FA-uptake (the hydrolysis is stimulated by Synacthen to a greater extent than the uptake) indicates a dissociation between the capacity of the epididymal fat pad to hydrolyze and esterify FA under the influence of Synacthen. The percentage of the FA liberated that subsequently is taken up in the tissue of the fasted rats falls after Synacthen treatment to about the same value as in the tissue of the fed rats.

The increased FA influx into the adipocyte from the vascular compartment cannot alone explain the retaining of the pad weight during hypercorticism for prolonged periods, when during hypercorticism the intracellular hormone sensitive lipase activity is enhanced. The FA release from the adipose tissue into the vascular compartment mediated by the hormone-sensitive lipase was reduced in the Synacthen treated rats, both in the fasted and fed state. This lowering effect was more pronounced during stimulation of the intracellular lipolytic process with adrenalin. These findings are in contrast with the results of experiments in isolated epididymal adipocytes from fasted rats in which pretreatment of the animal with cortisol or pre-incubation of the tissue with dexamethasone led to an enhancement of the adrenalin and glucagon induced lipolysis. This remarkable

discrepancy may be explained by the fact that in vivo or (in situ) the secondary hyperinsulinism in hypercorticistic rats counteracts the increased intracellular lipolysis.
Furthermore corticosteroids stimulate the reësterification rate of fatty acids in the adipocyte. This results in a netto influx (decreased efflux) of fatty acids in (from) the epididymal fat pad leading to a balanced state in which the fat pad can retain its weight during hypercorticism.
Synacthen treatment in fed rats leads to a marked increase in LPL activity in acetone-ether powders of the adipose tissue, a slight increase in heparin-releasable LPL in situ, but not to changes in the heparin-elutable LPL activities in vitro. These data may indicate a situation in which the enzyme is confined to a pool not elutable by heparin in vitro and limited releasable in situ.
In hypercorticistic rats a pronounced hyperinsulinemia develops in the fasting state. The insulin levels become comparable to that of the normally fed controls. Many studies have shown that insulin administration leads to a rise in LPL activity in adipose tissue, and that the insulin effect on enzyme synthesis is enhanced by glucocorticoids in vitro. Possibly the synthesis rate of LPL in adipose tissue of hypercorticistic rats is maximal, while the transfer of LPL to the endothelial cell is impaired. A relative deficiency of insulin (insulin resistance) appeared to cause an impaired transfer of LPL to the heparin-releasable pool as discussed above. By addition of pharmacologic levels of insulin (10 mU/ml) to the perfusion medium the heparin-releasable LPL activity rose from 15% to 48% of total LPL activity measured in the fat pad of the Synacthen treated fasted rats (Chapter IV). In these experiments with excess insulin added to the perfusion medium, insulin appeared to cause a shift of LPL from the intracellular to the heparin-releasable pool, while the total enzyme activity (the sum of the heparin-released LPL and the activity remaining in the perfused pad) did not change. Under these circumstances the stimulating effect of Synacthen treatment on the release of LPL was even more out-

spoken than in the experiments with less (0.1 mU/ml) insulin in the medium.
The results of these in situ experiments confirm the hypothesis that the transport of LPL from intra- to extracellular loci in adipose tissue is insulin-dependent.

The anti-hypertensive treatment with beta-adrenergic receptor blocking agents fails to prevent the occurrence of coronary heart disease. The explanation may be that this treatment also affects lipid metabolism. The changes in plasma lipid levels may be such that the possible beneficial effects of the lowering of the blood pressure are counteracted by adverse effects on cholesterol and TG. For example, in a number of studies it has been shown that beta-blockade can lead to an increase of plasma low-density lipoprotein cholesterol and of plasma TG and a decrease of high-density lipoprotein cholesterol. Alpha-adrenergic receptor blockade has an opposite effect. The effects of alpha- and beta-blockade have been summarized by several authors (12-15). Lipoprotein lipase may play an important role in the mechanism of action of both types of adrenergic blockers, leading to the changes in lipoprotein concentrations in plasma. In a clinical trial, that we have conducted, male patients with hypertension and coronary heart disease were randomly assigned to propranolol or prazosin treatment to begin 6 weeks after the coronary artery bypass grafting (Chapter VII). Prazosin use (mean 2,8 mg/day) for three months led to a decrease in plasma TG (-35 %). Total cholesterol (+9%), HDL-cholesterol (+16%), especially the HDL-3 fraction, and LPL activity in fasting post-heparin plasma increased. Propranolol treatment (mean 110 mg/day) had no effect on the TG concentration, but plasma cholesterol (+12%) and HDL-cholesterol (+10%), again the HDL-3 fraction, increased, as did the hepatic lipase activity. In the prazosin group long-term treatment (6-9 months) had essentially the same effect on TG and HDL-cholesterol, (HDL-3), while total cholesterol returned to basal levels. Analysis of covariance showed that prazosin treatment induced a lower plasma triglyceride and a higher high-density lipoprotein cholesterol concentration, a higher high-den-

sity lipoprotein/total cholesterol ratio and a higher lipoprotein lipase activity than propranolol treatment. Opposite effects of alpha- and beta-blockade on plasma lipids can be explained as resulting from inverse effects of the blockers on LPL activity; for prazosin possibly at the endothelial cell, because the adipocyte does not have alpha-1 adrenergic receptors. A role for lipoprotein lipases in plasma lipid changes during adrenergic blockade has been suggested by Ferrara et al. (16).

Post-heparin plasma LPL-activity originates from several tissues, especially from adipose tissue and skeletal muscles. We found that in the rat short-term alpha-adrenergic blockade leads to an increase in heart LPL activity (+14%), without an effect on adipose tissue LPL (Chapter VI). Beta-blockade has the opposite effect (-20%). In rats fed a cholesterol-enriched diet a similar, but smaller effect on heart LPL was found (+5%, -12% respectively). In these rats a slight increase in adipose tissue LPL was observed (+14%), as well as for hepatic lipase (+13%), while treatment with propranolol had an opposite effect (-20% and -9% respectively). It is well known that alpha- and/or beta-adrenergic stimuli have effects on the plasma levels of insulin and thyroid hormone. Insulin is an important hormone in the regulation of adipose tissue LPL and thyroid hormone in the regulation of heart-muscle LPL. In our study (Chapter VI) alpha-blockade tended to raise and propranolol to lower insulin levels. The same opposite effects were observed on tri-iodothyronine levels. Inverse changes in adipose tissue and heart LPL are generally observed, for example during feeding and fasting. Under these conditions insulin seems to be the major determinant of the adipose tissue LPL activity.

The experiments were all carried out in overnight fasted animals when insulin secretion is already depressed. It is possible that the effects of either drug are more pronounced in the fed animal.

This thesis shows disparate effects of ACTH and corticosterone on adipose tissue LPL-activity in the rat, while ACTH needs

corticosterone to achieve its positive effect on LPL activity. In hypercorticistic rats, a block in the transport of LPL from the adipocyte to the endothelial site of the vascular wall exists which can be overcome by an excess of insulin.

The effects of alpha- and beta-receptor blocking agents on lipoprotein levels in plasma could be explained by the changes in LPL-activity. The effects of the adrenenergic-receptor blocking drugs on LPL-activity are probably indirect and may be explained by the changes in insulin and thyroid hormone levels.

REFERENCES

1. Lawson, N., Pollard, A.D., Jennings, R.J., Gurr, M.I. and Brindley, D.N. The activities of lipoprotein lipase and of enzymes involved in triacylglycerol synthesis in rat adipose tissue.
Biochem. J. 200: 285-294, 1981.

2. Chernick, S.S., Spooner, P.M., Garrison, M.M. and Scow, R.O. Effect of epinephrine and other lipolytic agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 adipocytes.
J. Lipid Res. 27: 286-294, 1986.

3. De Gasquet, P. and Pequignot, E. Changes in adipose tissue and heart lipoprotein lipase activities and in serum glucose, insulin and corticosterone concentrations in rats adapted to a daily meal.
Horm. Metab. Res. 5: 440-443, 1973.

4. Krotkiewski, M., Björntorp, P. and Smith, U. The effect of long-term dexamethasone treatment on lipoprotein lipase activity in rat fat cells.
Horm. Metab. Res. 8: 245-246, 1976.

5. Bagdade, J.D., Yee, E., Albers, J. and Pykälistö, O.J. Glucocorticoids and triglyceride transport:effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat.
Metabolism 25: 533-542, 1976.

6. Cigolini, M., Smith, U. Human adipose tissue in culture. VIII. Studies on the insulin-antagonistic effect of glucocorticoids. Metabolism 28: 502-510, 1979.

7. De Gasquet, P., Pequignot-Planche, E., Tonnu, N.T. and Diaby, F.A. Effect of glucocorticoids on lipoprotein lipase activity in rat heart and adipose tissue.
Horm. Metab. Res. 7: 152-157, 1975.

8. Ashby, P. and Robinson, D.S. Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipase.
Biochem. J. 188: 185-192, 1980.

9. Ho, R.J. and H.C. Meng. A technique for the cannulation and perfusion of isolated rat epididymal fat pad. J. Lipid Res. 5: 203-209, 1964.

10. Scow, R.O. Perfusion of isolated adipose tissue: FFA release and blood flow in rat parametrial fat body. In: Handbook of Physiology. section 5: Adipose Tissue. Baltimore USA, Waverly Press Inc. 1965: pp. 437-455.

11. Fielding, C.J. Lipoprotein lipase. Evidence for high- and low affinity enzyme sites. Biochemistry 15: 879-884, 1976.

12. Leren, P., Hegeland, A., Holme, I., Foss, P.O., Hjermann, I., Lund-Larsen, P.G. Effect of propranolol and prazosin on blood lipids. The Oslo study, Lancet, ii : 4-6, 1980.

13. Lowenstein, J., Neusy, A.J. Effects of Prazosin and Propranolol on serum lipids in patients with essential hypertension. Am. J. Med., 76: 79-84, 1984.

14. Grimm, R.H., Hunninghale, D.B. Lipids and hypertension. Am. J. Med., 80 (suppl. 2A): 56-63, 1986.

15. Weinberger, M.H. Antihypertensive therapy and lipids. Am. J. Med., 80 (suppl. 2A): 64-70, 1986.

16. Ferrara, L.A., Marotta, T., Rubba, P., de Simone, B., Leccia, L.A., Soro, S., Mancini, M. Effects of alpha-adrenergic and beta-adrenergic receptor blockade on lipid metabolism. Am. J. Med., 80 : 104-108, 1986.

SUMMARY

The enzyme lipoprotein lipase (LPL) plays an important role in the lipoprotein metabolism. It is the rate-limiting enzyme in the degradation of triglycerides (TG) from chylomicrons and VLDL. Therefore knowledge of the regulation of the enzyme is of great importance.

In this thesis different aspects of the regulation of LPL are described. The regulation was investigated in vitro as well as in situ and in man in vivo.

First an in situ model was established. The epididymal fat pad of the rat was selectively perfused. After we had made the conditions optimal, the metabolism of chylomicrons and VLDL were investigated in this perfused pad (Chapter II).

We found a strong relation between the hydrolysis of chylomicrons and the heparin-elutable LPL pool. Furthermore, chylomicron-TG was a better substrate for LPL than VLDL-TG, at least in adipose tissue.

The role of corticosteroids in the regulation of LPL was investigated by treating the animals with Synacthen (ACTH 1-24) and /or corticosteron. Synacthen treatment in the doses used led to hypercorticism, and induced an increase of the LPL activity in adipose tissue in fasted animals to levels found in fed controls (Chapter III). The increased LPL activity was found when the activity was measured in an extract of an acetone-ether powder of the epididymal fat pad. No change in LPL activity was found in the heparin-elutable fraction of pieces of the pad in vitro. In the Synacthen treated animals, the heparin-releasable LPL activity in the selectively perfused fat pad was higher than in fasted controls, but remained lower than that found in fed controls. We found an increase of the hydrolysis of chylomicron-TG and of the uptake of the liberated fatty acids by the perfused fat pad, to levels found in fed controls (Chapter IV).

By using farmacologic concentrations of insulin in the perfusion medium, the heparin-releasable fraction LPL increased from 15% to 48% of the total LPL pool in the pad. These results are an indication that insulin plays an important role in the transport of LPL from the adipocyte to the endothelial cell.

The fact that the Synacthen-induced increase in releasable LPL activity was also seen in the presence of an insulin excess indicates that the effect of Synacthen cannot be due to hyperinsulinism only.

The efflux of fatty acids from the adipose tissue, measured in the medium after perfusion, was less than in controls, basally as well as after stimulation of the intracellular lipolysis with adrenalin.

The earlier observation, that the rat epididymal fat pad retained its weight during hypercorticism, while the total body weight is decreasing could therefore be explained by several factors. First: an increased influx of fatty acids, liberated by LPL from chylomicrons. Second: an inhibition of intracellular lipolysis leading to a decreased efflux of fatty acids from the adipocyte.

In Chapter V the results are described of experiments in which we treated the animals with corticosterone and/or Synacthen. We consistently found a decreased LPL activity in adipose tissue in animals treated with corticosterone alone (in intact as well as in adrenalectomized rats). This negative effect of corticosterone on LPL could be overcome by treating the animals simultaneously with Synacthen. These results indicate that ACTH (1-24) has a direct effect on LPL activity in the adipose tissue of the rat.

The effects of alpha-1- and beta-adrenergic blockade on lipolytic enzymes and plasma lipids in rats as well as in humans are described in the Chapters VI and VII.

Treatment with doxazosin, a selective blocker of the alpha-1 adrenergic receptor, leads to an increment of the LPL activity in the heart, while propranolol, a non-selective blocker of the beta-adrenergic receptor, had an opposite effect (Chapter VI). Using animals, who were feeded with a cholesterol-enriched diet, the same, albeit smaller effect was found. Liver lipase and adipose tissue LPL activity increased, non-significantly, in the doxazosin treated animals, and decreased in the propranolol treated group. Both forms of treatment had no effect on plasma TG and total cholesterol. However the cholesterol content of the HDL fraction rose in cholesterol-rich fed rats treated with

doxazosin.

The effects of doxazosin and propranolol on lipolytic enzymes and plasma lipids can, at least partially, be ascribed to an increase and a decrease, respectively, of the plasma insulin concentration.

In Chapter VII the results of alpha-1- and beta-blockade (prazosin and propranolol respectively) on plasma lipids and lipolytic enzymes in humans are presented.

Fifty-two men with mild hypertension, after coronary bypass grafting, were divided in two groups at random and treated for nine months with one of these medicaments.

In comparison with the propranolol-treated patients plasma TG decreased in the prazosin treated group, while plasma total cholesterol and LDL cholesterol levels increased initially, but were back at basal levels at the end of the study. In the propranolol treated group plasma TG did not change, while total and LDL-cholesterol increased. In both treatment groups the HDL-cholesterol concentration rose essentially by changes in the HDL-3 fraction. LPL activity in post-heparin serum was higher in the prazosin than in the propranolol treated group.

In conclusion: antihypertensive treatment with an alpha-1 selective adrenergic blocker gives a more favorable plasme lipid pattern than treatment with a non-selective beta-blocker. An important factor leading to this pattern is the increment in LPL activity.

SAMENVATTING

Het enzym lipoproteine lipase (LPL) speelt een belangrijke rol in de vetstofwisseling. Het is het snelheidsbepalende enzym bij de afbraak van triglyceriden uit chylomicronen en VLDL. Inzicht in de regulatie van het enzym is dan ook van groot belang.
In dit proefschrift worden verschillende aspecten van de regulatie van LPL beschreven. De regulatie werd zowel in vitro als in situ (en bij de mens in vivo) onderzocht.
Eerst werd een in situ model opgesteld. Het epididymale vetkwabje van de rat werd selectief geperfundeerd. Nadat de condities optimaal waren gemaakt werd het metabolisme van chylomicronen en VLDL onderzocht in dit geperfundeerde vetkwabje (hoofdstuk II).
Hierbij bleek dat de hydrolyse van triglyceriden in chylomicronen sterk gecorreleerd is met de door heparine te elueren pool LPL. Tevens kwam naar voren dat chylomicron-TG een beter substraat is voor vetweefsel LPL dan VLDL-TG.
De rol van corticosteroiden in de regulatie van LPL werd nagegaan door proefdieren te behandelen met Synacthen (ACTH 1-24) en/of corticosteron. Het bleek dat hypercorticisme op basis van Synacthen de LPL activiteit verhoogt in vetweefsel van gevaste dieren tot een niveau gevonden in gevoede controle dieren (hoofdstuk III). De verhoging van LPL activiteit werd gevonden als we de activiteit maten in een aceton-ether poeder van het totale vetkwabje en niet in de met heparine te elueren LPL-fractie uit stukjes vetweefsel in vitro. Hoewel bij selectieve perfusie van het epididymale vetkwabje, van het met Synacthen voorbehandelde dier, bleek dat de in situ met heparine te elueren LPL pool wel steeg, maar niet tot het niveau van het gevoede dier. Er werd wel een stijging waargenomen van de hydrolyse van TG en opname van de hierbij vrijgekomen vetzuren door het geperfundeerde kwabje in gevaste, behandelde dieren, tot het niveau gevonden in gevoede ratten (hoofdstuk IV).
Door gebruik te maken van farmacologische concentraties insuline in het perfusie medium, steeg de met heparine in situ te elueren fractie LPL van 15% tot 48% van de totale LPL activiteit in het

vetkwabje. Deze bevinding duidt erop dat insuline een (belangrijke) rol speelt bij het transport van LPL vanuit de adipocyt naar de endotheelcel. Tevens dat het positieve effect van Synacthen op de heparine-elueerbare LPL pool in situ niet alleen verklaard kan worden door het hyperinsulinisme op zich. Het vrijkomen van vetzuren uit het vetweefsel, gemeten in het perfusie medium na perfusie, was minder bij met Synacthen voorbehandelde dieren dan bij onbehandelde, zowel basaal als na stimulatie van de intracellulaire lipolyse met adrenaline.
De al eerder gedane waarneming, dat het epididymale vetkwabje van de rat zijn gewicht behoudt gedurende hypercorticisme,terwijl het totale lichaamsgewicht daalt, zou dus als volgt verklaard kunnen worden: a. verhoogde influx van vetzuren vrijgemaakt uit TG-rijke partikels ten gevolge van de gestegen LPL activiteit. b. verminderde efflux van vetzuren vanuit de adipocyt ten gevolge van geremde lipolyse.
In hoofdstuk V worden de effecten beschreven van corticosteron en/of Synacthen in intacte en bijnierloze proefdieren op LPL activiteit in vetweefsel. Een consistente bevinding was dat hypercorticisme op basis van corticosteron leidt tot een verlaging van de LPL activiteit bij intacte zowel als bij geadrenalectomeerde ratten. Deze verlaging kan teniet gedaan worden door het dier tegelijkertijd te behandelen met Synacthen. Deze resultaten wijzen erop dat ACTH (1-24) een direct effect heeft op de LPL activiteit in vetweefsel van de rat. In bovengenoemde experimenten viel tevens op dat de hypercorticistische dieren met vergelijkbare corticosteron concentraties in het bloed (na Synacthen dan wel corticosteron behandeling), ook vergelijkbare insuline concentraties hadden. Gelet op de tegengestelde effecten van beide behandelingen op de LPL activiteit, is ook dit een aanwijzing dat het effect van ACTH (1-24) op LPL niet alleen verklaard kan worden door het optredende hyperinsulinisme.
De hoofdstukken VI en VII beschrijven de effecten van alpha- en beta-adrenerge receptor blokkade op lipolytische enzymen en plasma lipiden bij de rat en bij de mens.
In de rat leidt doxazosin, een medicament dat de alpha-1 adrenerge receptoren blokkeert, tot een stijging van de LPL activi-

teit in het hart, terwijl propranolol, dat niet-selectief de beta-adrenerge receptor blokkeert, een tegengesteld effect heeft (hoofdstuk VI). Werden de dieren gevoed met een met cholesterol-verrijkt dieet dan werd een zelfde, doch kleiner effect gevonden op de LPL activiteit in het hart. De activiteiten van leverlipase en vetweefsel LPL stegen niet significant in de met doxazosin behandelde dieren en daalden in de met propranolol behandelde groep.

Beide behandelingen hadden geen effect op plasma TG of het totaal cholesterol. Het cholesterolgehalte in de HDL-fractie steeg in de met doxazosin behandelde dieren. De gevonden effecten van doxazosin en propranolol op vetweefsel LPL en plasma lipiden kunnen, tenminste deels, toegeschreven worden aan verhoging respectivelijk verlaging van de insuline concentratie in het plasma.

Hoofdstuk VII geeft de resultaten van alpha-1- en beta-blokkade (respectievelijk prazosin en propranolol) op plasma lipiden en lipolytische enzymen bij de mens. Tweeënvijftig mannen met milde hypertensie, werden na een kransslagaderoperatie, "at random" behandeld met één van beide medicamenten gedurende negen maanden.

In vergelijking met propranolol behandelde patiënten daalt de plasma TG-concentratie in de met prazosin behandelde groep, terwijl het plasma totaal cholesterol en LDL-cholesterol aanvankelijk stegen. De laatste twee grootheden waren echter aan het eind van het onderzoek terug op het uitgangsniveau. In de met propranolol behandelde mensen bleef het plasma-TG constant, en stegen zowel het totale als het LDL-cholesterol blijvend. In beide behandelingsgroepen steeg het HDL-cholesterol, voornamelijk ten gevolge van toename van de HDL-3 fractie.

De LPL activiteit in post-heparine serum was in de met prazosin behandelde groep hoger dan in de met propranolol behandelde.

Concluderend leidt anti-hypertensieve therapie met een selectieve alpha-1 adrenerge blokker als prazosin tot een gunstiger plasma lipiden patroon dan tijdens behandeling met propranolol. Een belangrijke factor bij de tot standkoming van dit patroon is de verhoogde LPL activiteit.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 13 oktober 1953 geboren te Leur. Na het behalen van het HBS-B diploma aan het Thomas More College te Oudenbosch werd in 1971 begonnen met de studie geneeskunde aan de Rijksuniversiteit te Utrecht, alwaar in 1978 het artsexamen werd afgelegd. Tot juni 1979 was hij werkzaam als arts-assistent inwendige geneeskunde in het St.Elisabeth ziekenhuis te Tilburg (hoofd: Dr. V.A.M. Terwindt), waarna hij in opleiding tot internist kwam op de afdeling inwendige geneeskunde III van het academisch ziekenhuis Dijkzigt te Rotterdam (hoofd: Prof.Dr. J.C. Birkenhäger). In deze periode werd een begin gemaakt met het onderzoek beschreven in dit proefschrift. Op 1 januari 1984 werd hij ingeschreven in het specialistenregister, en is sedert die tijd verbonden als staflid aan de afdeling inwendige geneeskunde III.