ASPECTS OF PLASMA TRIGLYCERIDE METABOLISM IN CHILDREN

Aan mijn moeder.
Aan mijn vrouw.

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PROEFSCHRIFT

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Abbreviations

FA	Fatty acid
FFA	Free fatty acid
GSD	Glycogen storage disease
IVFTT	Intravenous fat tolerance test
LPL	Lipoprotein lipase
PHLA	Postheparin lipolytic activity
PHLPL	Postheparin lipoprotein lipase
PN	Parenteral nutrition
SEM	Standard error of the mean
TG	Triglyceride

General aspects of plasma triglyceride metabolism

Plasma lipids consist of triglycerides, phospholipids, cholesterol, cholesterol esters and free fatty acids (FFA). Because of their water insolubility plasma lipids do not circulate as such but combine with proteins to form hydrophilic lipoprotein complexes. Plasma lipoproteins can be separated in different classes by ultracentrifugation or by electrophoresis. Four groups of lipoproteins are important in clinical diagnosis. They are: high density or α-lipoproteins, low density or β-lipoproteins, very low density or pre-β-lipoproteins and chylomicrons. The latter two groups have a higher fat and triglyceride content than the former two. In clinical situations, associated with an increased plasma triglyceride content, hyperchylomicronemia, hyper pre-β-lipoproteinemia or a combination of these will be found.

The source of plasma chylomicrons is dietary fat. Dietary triglycerides are hydrolysed into monoglycerides and fatty acids which are absorbed and reesterified into triglycerides in the small intestinal mucosa. The intestinal cells secrete most of these triglycerides as chylomicrons and a small fraction as very low density lipoproteins into lymph channels collecting into the thoracic duct and thence into the venous circulation. Plasma chylomicrons consist of particles having a diameter of 750 to 12.000 Å. Clearance of chylomicrons from the plasma occurs mainly in the extrahepatic tissues and is mediated by the enzyme lipoprotein lipase. Hydrolysis of triglyceride molecules by lipoprotein lipase occurs concurrently with their uptake by the tissues. This process probably takes mainly place in the capillary endothelium of muscle and adipose tissue (Blanchette-Mackie, and Scow 1971).

The main source of pre-β-lipoprotein is the liver which synthesizes pre-β-lipoprotein particles and secretes these into the blood stream. Fatty acids of pre-β-lipoprotein triglycerides derive from two main sources:

1 Synthesis *de novo* from acetyl-CoA which is generated by glycolysis from carbohydrates. This pathway plays quantitatively an important role in the fed state.
2 Adipose tissue triglycerides lipolysis. This is regulated by a hormone sensitive triglyceride lipase located in the fat cells. In situations characterized by an increased lipolysis (fasting, diabetes mellitus) plasma FFA represents the main source of plasma pre-β-lipoprotein triglyceride fatty acids. Clearance of plasma pre-β-lipoproteins triglycerides in the extrahepatic tissues does not seem to differ qualitatively from that of chylomicron triglycerides (Havel, 1965).

Triglyceride bloodlevels represent the sum of triglyceride molecules incorporated in the

different lipoproteins, chylomicrons and pre-β-lipoproteins having the highest triglyceride content. When no fat is consumed for a period of about 12 hours prior to blood-sampling, chylomicrons have disappeared from the plasma. Consequently, in the fasting state plasma triglycerides are mainly derived from pre-β-lipoproteins.
Generally speaking the concentration of triglycerides in the plasma depends on two processes:

1 The rate of entry into plasma (production rate).
2 The rate of efflux from plasma (clearance rate).

In order to understand the pathogenesis of hypertriglyceridemia one should investigate whether an increased plasma triglyceride level is due to an increased triglyceride production rate, a decreased triglyceride clearance rate or both. In a steady state these two rates are equal and represent triglyceride turnover. Methods for the measurement of plasma triglyceride turnover involve different techniques such as measurement of disappearance rate of intravenously injected labelled lipoproteins, the determination of the rate of entry into plasma, or clearance from plasma of endogenous lipoproteins labelled from various precursors in vivo (Nikkilä et al. 1971, Adams et al. 1974). Among other techniques used in the investigation of plasma triglyceride metabolism, the intravenous fat tolerance test (introduced in clinical studies by Boberg et al. in 1969) has been used by many workers in the field. Studies of plasma triglyceride metabolism by these different techniques (labelled precursors on the one hand, the IVFTT on the other) have yielded concordant results in all situations where comparative studies have been performed (Nikkilä, and Kekki 1972, Kissebah et al. 1974, Kissebah et al. 1973) with the exception of idiopathic hypertriglyceridemia (Adams et al., 1974). There is, at present, no clearcut explanation on this last point. We did not make use of labelled precursors in vivo in our studies of pediatric patients on the following grounds:

1 Ethical problems associated with the use of radioactive products in children.
2 The radioactive decay methods require a long fasting period (unacceptable for many of our patients, who were prone to hypoglycemia).

We used the following two tests in our investigation. Firstly, we performed the intravenous fat tolerance test (IVFTT) with Intralipid fat emulsion. With this method the fractional Intralipid turnover rate can be calculated. Secondly, by measuring the plasma postheparin lipoprotein lipase activity we tried to get more insight into the physiological significance of lipoprotein lipase which plays a predominant role in triglyceride elimination from the blood. As it has been suggested by Boberg (1972) that blood samples taken 5 and 40 minutes after the intravenous injection of heparin could reflect lipolytic activities from different pools, not equally accessible for injected heparin, both 5 and 40 minutes postheparin lipoprotein lipase (PHLPL) activities were measured in our investigation. Recent litterature data concerning these two tests are presented in the following chapter.

Chapter I

The intravenous fat tolerance test and the plasma postheparin lipoprotein lipase activity

1. The intravenous fat tolerance test

1.1. Introduction

Hypertriglyceridemia in man has initially been investigated by oral fat tolerance tests (Angerwall, 1964). The serum triglyceride curves obtained after such tests depend on many factors such as intestinal absorption, lymph flow and triglyceride clearance. Because of these many operating factors oral fat tolerance tests are difficult to interpret and do not allow a precise estimation of triglyceride clearance. A better estimation of the latter can be obtained by performing an intravenous fat tolerance test bypassing in this way the gastrointestinal tract.

1.2 Validation of Intralipid emulsion for the measurement of plasma triglyceride metabolism

For a fat emulsion to be a valid reflector of chylomicrons metabolism it should have properties similar to chylomicrons. It has been shown that, when incubated in vitro with postheparin plasma, chylomicrons and Intralipid presented a similar enzymic kinetic behaviour whereas this was not the case with other fat emulsions (Boberg, and Carlson, 1964). Furthermore, Intralipid elimination from the blood is known to follow identical kinetic rules as chylomicron elimination in the dog (Carlson, and Hallberg, 1963) and in man (Hallberg, 1965 b). These reported similarities in behaviour of chylomicrons and Intralipid probably validate the use of Intralipid fat emulsion for the measurement of chylomicron metabolism. The second question is whether Intralipid, being a good reflector of exogenous triglyceride elimination, is also a good reflector of endogenous triglyceride elimination. This is probably the case, as it has been shown that a good correlation exists between the Intralipid fractional turnover rate as measured by the IVFTT and the fractional turnover rate of endogenous triglycerides measured by several techniques (Rössner, 1974). It should be noted that contrary to the findings of Boberg and Carlson (1964), Huttunen and Nikkilä (1973) recently reported differences in enzymic kinetic behaviour between Intralipid and chylomicrons. These authors found affinity differences for lipoprotein lipase between Intralipid on the one hand and chylomicrons on the other. These divergent results seem to be due to differences in methodology. Huttunen and Nikkilä found that the addition of 1 M NaCl in their test system

resulted in an increased lipolytic activity, while Boberg and Carlson (1964) reported a 90% decrease of lipolytic activity under these conditions. As it is known that lipoprotein lipase is inhibited by NaCl the results of Huttunen and Nikkilä (1973) are difficult to interpret. Although we realize that the discrepant results of different authors cannot be fully explained at present, we used Intralipid emulsion to perform the intravenous fat tolerance test in order to investigate plasma triglyceride removal.

1.3 Kinetics of the elimination of exogenous lipids from the blood

The kinetics of the elimination of exogenous lipids from the blood after intravenous injection has been studied by Hallberg (1965a). Elimination is regulated by two rate constants according to this author. A zero order reaction takes place at high plasma triglyceride concentrations, a first order reaction at low plasma triglyceride concentrations. The zero order part of the elimination has been interpreted to be a maximal elimination capacity operating above a critical Intralipid concentration. This elimination constant has been designated as K_1. Below the critical concentration a first order reaction takes place, its elimination constant has been designated as K_2. If a low Intralipid dose (0.1 gr/kg body weight) is used the IVFTT only measures K_2. If higher Intralipid doses are used one can measure both K_1 and K_2. Boberg et al. (1969) used the IVFTT with high fat doses. It appeared that K_1 was the same in young and old controls and normal in patients with primary hypertriglyceridemia. On the other hand K_2 was found to be subnormal in old when compared to young controls, the former having higher triglyceride bloodlevels in the fasting state. K_2 was also found to be subnormal in patients with primary hypertriglyceridemia (see 1.5.1). It thus appeared that K_1 and K_2 values were not correlated, and that K_2 yielded important clinical information on the pathogenesis of hypertriglyceridemia, whereas K_1 did not. In our clinical studies we measured K_2 only when performing the IVFTT (see chapters 2 and 3).

1.4 Methodology of the intravenous fat tolerance test

A methodological study of the IVFTT with Intralipid fat emulsion has been performed by Carlson and Rössner (1972). After a single intravenous injection of 0.1 g Intralipid per kg body weight the turbidity of bloodsamples taken during the following 40 minutes was determined nephelometrically. The following points were clarified. Firstly, identical results were obtained with venous plasma and capillary whole bloodsamples. This makes the test easy to perform in children. Secondly the test has been shown to be highly reproducible in the same individual from one day to another.

1.5 Situations characterized by a decreased value of the IVFTT. Correlation with triglyceride bloodlevels

1.5.1 Primary hypertriglyceridemia

A decrease of the IVFTT value in patients with primary hypertriglyceridemia has been found by many investigators. Boberg (1969) published the first study on this subject. He found K_2 to be significantly decreased in hyperlipemic patients. When K_2 values and plasma triglyceride concentrations were plotted against each other, K_2 was found to decrease with increasing concentration of plasma triglycerides in a hyperbolic fashion. The same inverse relationship between K_2 and serum triglyceride level in a group of normal, hyperlipemic and diabetic adult patients was found by Lewis et al. (1972). Adams et al. (1974) performed and extensive study of triglyceride transport kinetics in patients with type IV and type V hyperlipoproteinemia. Triglyceride removal as measured by the IVFTT was found to be decreased in both hyperlipoproteinemias.

1.5.2 Dietary influences

Nestel and Barter (1973) studied triglyceride clearance during diets rich in carbohydrates or fats. Clearance rates of Intralipid fat emulsion were measured from steady state triglyceride increments during constant infusion of the fat emulsion. Diets rich in polyunsaturated fat lead to a faster Intralipid clearance than high carbohydrate diets or diets rich in saturated fat.

Mancini et al. (1973) studied the mechanisms of carbohydrate induced hyperlipemia in normal man. High carbohydrate diets lead to higher triglyceride bloodlevels and to a decrease of Intralipid clearance as measured by the IVFTT.

Cahlin et al. (1973) studied, among other things, the effect of sucrose feeding on removal of intravenous fat in man. They showed that sucrose feeding leads to a decrease of the IVFTT value.

1.5.3 Obesity

Rössner et al. (1974b) studied the IVFTT in subjects with massive obesity. The patients were studied before and after a jejuno-jejunal bypass operation. Before operation high triglyceride bloodlevels were found concomitantly with a decrease of the IVFTT value. There was a negative correlation between triglyceride bloodlevels and values of the IVFTT. With postoperative weight reduction, plasma triglyceride levels decreased significantly while the IVFTT values rose. The changes varied considerably in different patients, however, some patients losing much weight without showing changes of the IVFTT value while others with a very moderate weight loss showed a marked rise of the IVFTT value.

1.5.4 Maturity onset diabetes mellitus

In 1972 Guisard et al. showed that in adults with maturity onset diabetes Intralipid clearance was decreased. Kissebah et al. (1974) studied plasma FFA and triglyceride transport kinetics in maturity onset diabetes. In non obese diabetics the IVFTT value was found to be decreased. In obese diabetics a slight decrease of the IVFTT value was found. Basal triglyceride levels, though increased in both groups of patients, were highest in the non obese group who showed the most important reduction of the IVFTT values.

1.5.5 Hypothyroidism

Nikkilä and Kekki (1972) studied plasma triglyceride metabolism in thyroid disease. In

hypothyroid patients triglyceride bloodlevels were elevated. Triglyceride removal as measured by the IVFTT was found to be decreased. There was no significant correlation between triglyceride bloodlevels and the IVFTT values.

1.5.6 Alcoholic hyperlipemia

Chait A. et al. (1972) performed a metabolic study of alcoholic hyperlipemia. The value of the IVFTT was found to be decreased before as well as after alcohol withdrawal. Triglyceride bloodlevels on the other hand decreased very significantly after alcohol withdrawal. Thus no correlation was found between triglyceride bloodlevels on the one hand and the IVFTT value on the other.

1.6 Situations characterized by an increased value of the IVFTT. Correlation with triglyceride bloodlevels

1.6.1 Dietary influences

Nestel and Barter (1973) studied the effect of different diets on Intralipid elimination. Diets rich in unsaturated fat resulted in the highest Intralipid fractional removal rates and the lowest fasting plasma triglyceride levels (see also 1.5.2).

1.6.2 Thyrotoxicosis

Nikkilä and Kekki (1972) studied plasma triglyceride metabolism in thyroid disease. In thyrotoxicosis the IVFTT values were higher than normal. Triglyceride bloodlevels were slightly increased (see also 1.5.5).

1.6.3 Pharmacologic agents

Many pharmacologic agents have been shown to lower plasma triglyceride levels and increase the IVFTT concommitantly. These agents are for example: nicotinic acid and clofibrate derivatives (Boberg et al., 1970), oxandrolone (Olsson et al., 1974), progesterone or megestrol (Kissebah et al., 1973).

1.7 Discussion

The first clinical studies using the IVFTT in the investigation of primary hypertriglyceridemia showed that there exists an inverse relationship between triglyceride bloodlevels and the IVFTT values (Boberg et al., 1969). This finding can be interpreted in two ways. A decreased triglyceride elimination rate could induce hypertriglyceridemia or the reverse sequence could be the case. In the former case the IVFTT would be a valuable tool in the investigation of hypertriglyceridemia. In the latter case the lowering of the IVFTT value could simply be explained by a competitive effect of endogenous triglyceride with Intralipid for common removal sites (Adams et al. 1974). The IVFTT would merely be a reflection of endogenous triglyceride pool size and not of triglyceride removal efficiency. If the IVFTT value merely reflects the triglyceride bloodlevels one should expect the original inverse relationship found between the IVFTT values and

triglyceride bloodlevels in primary hypertriglyceridemia to be a constant finding in all clinical situations presenting with hypertriglyceridemia. The following studies do not support his hypothesis.

A study on alcoholic hyperlipemia (Chait et al., 1972) has demonstrated that in that situation triglyceride bloodlevels and the IVFTT values were unrelated and furthermore that the IVFTT could remain unaffected by major variations of triglyceride bloodlevels. A study on the mechanism of hypertriglyceridemia associated with the use of contraceptive steroids (Rössner et al., 1971) has further demonstrated that the IVFTT could remain unaffected by changes in triglyceride bloodlevels. The study performed by Nestel and Barter (1973) on the effects of different diets on Intralipid clearance revealed that changes in Intralipid clearance rates cannot be fully explained by changes in triglyceride pool size.
In a study of patients with thyroid diseases Nikkilä and Kekki (1972) showed that elevated triglyceride bloodlevels can be found concomitantly with elevated IVFTT values (thyrotoxicosis). Consequently, the IVFTT seems to be relatively independent of triglyceride pool size and probably measures triglyceride removal efficiency. Investigation of hypertriglyceridemia with the IVFTT can lead to one of the following possible results:

1 High triglyceride bloodlevels are negatively correlated with decreased IVFTT values. In this situation an inefficient triglyceride removal is probably the most important pathogenetic factor.

2 High triglyceride bloodlevels are unrelated to normal or even increased IVFTT values. In this situation triglyceride hyperproduction is probably the most important pathogenetic factor.

3 High triglyceride bloodlevels are not correlated with decreased IVFTT values. In this situation the hypertriglyceridemia is probably due to both an increased triglyceride production and an inefficient triglyceride removal.

2 Plasma postheparin lipoprotein lipase

2.1 Introduction

The discovery of the 'clearing factor' dates back to 1943. Lipoprotein lipase or 'clearing factor' was discovered during research aimed at explaining the mechanism by which heparin injection reduced lactescence in hyperlipemic plasma (Hahn, 1943). Since then much work has been performed and a few review articles on the subject have been published (Robinson 1963, Persson 1971). Lipoprotein lipase is one of several triglyceride lipases with the following properties (Korn, 1955). It specifically requires lipoprotein and heparin as 'co-factor' and it is inhibited by a high NaCl concentration and protamine. The function of LPL is to hydrolyse triglyceride molecules of plasma lipoproteins. This

hydrolysis takes place at the capillary endothelium of LPL containing tissues. The most important of these are adipose tissue, muscle, the mammary gland, heart and aorta.

Because LPL plays such an important role in the removal of plasma triglycerides its activity should be studied in situations associated with abnormal triglyceride bloodlevels. In clinical studies there are two possible ways of investigation. LPL can be measured in adipose tissue biopsies or in plasma. The last approach has been most often chosen. In preheparin plasma LPL activity is very low. After heparin injection LPL activity in plasma rises rapidly. The precise mechanism involved is unknown.

In the last few years terminology has changed from PHLPL to postheparin lipolytic activity (PHLA) or postheparin triglyceride lipase activity. The reason for this is that evidence for the presence of at least two triglyceride lipases in postheparin plasma has been provided (LaRosa et al., 1970). One of these triglyceride lipases has been shown to have properties specific of lipoprotein lipase, the other not. The latter seems to be of hepatic origin. Consequently, when measuring postheparin lipolytic activity one measures a higher total activity than that due solely to LPL.

When referring to literature data we shall keep to the terminology used by the original author. Comparison between different authors' findings is difficult as different heparin doses have been used and bloodsamples have not been collected at the same times after heparin injection. It has been shown that the use of different heparin doses or blood-sampling times result in the measurement of different PHLA in rat (Jansen and Hülsmann, 1974) and in man (Krauss et al., 1974). Furthermore, the methodology for the measurement of PHLPL activity is not standardized. All these factors can lead to the finding of very different PHLPL activities in similar clinical situations. Finally, it should be pointed out that PHLPL represents the overall result of enzyme activity changes in different tissues. Total PHLPL activity can consequently remain unaffected when enzyme activity changes occur in opposite directions in different tissues. This is probably one of the reasons why the functional significance of PHLPL is at present poorly understood.

2.2 *Situations characterized by a decreased plasma PHLPL activity. Correlation with triglyceride bloodlevels*

2.2.1 Idiopathic hypertriglyceridemia (primary hyperlipoproteinemia with elevated plasma triglyceride concentration)

Boberg (1972) measured PHLPL activities in patients with hypertriglyceridemia. Activities were measured in bloodsamples collected 5 and 40 minutes after i.v. injection of 100 units heparin/kg body weight. Lower PHLPL activities were found in men and women with idiopathic hypertriglyceridemia. A significant negative correlation between 40 minutes PHLPL activities and the logarithm of plasma triglyceride concentrations was found in men and women. 5 minutes PHLPL activities correlated negatively with plasma triglyceride concentrations in women only. Adams et al. (1974) measured plasma postprandial lipolytic activity in the same clinical situation. Heparin dose was 10 U/kg

body weight, blood was collected 10 minutes after heparin injection. Significantly decreased PHLA were found in patients with type IV and type V hyperlipoproteinemia. Other authors, however, reported postheparin lipolytic activities to be normal or elevated in primary hypertriglyceridemia (Havel 1969, Ahrens et al. 1961, Krauss et al 1974 b)

2.2.2 Dietary influences

Mancini et al. (1973) showed that isocaloric replacement of fat by carbohydrate in the diet led to a reduction of PHLA while triglyceride bloodlevels increased.
The heparin dose was 10 U/kg body weight, bloodsamples were collected 10 min. after heparin injection.

2.2.3 Diabetes mellitus

Kissebah et al. (1974) measured PHLA in maturity onset diabetes. The heparin dose was 10U/kg body weight, bloodsamples were collected 10 min. after heparin injection.
PHLA was significantly decreased while triglyceride bloodlevels were elevated. Patients with the highest triglyceride bloodlevels (non obese diabetics) had the lowest PHLA.
Decreased PHLA in diabetes has also been found by Guisard et al. (1972).

2.2.4 Hypothyroidism

Nikkilä and Kekki (1972) studied PHLA in thyroid disease. The heparin dose was 20 U/Kg body weight; blood was collected 10 min after heparin injection.
In the hypothyroid state PHLA was decreased, while triglyceride blood levels were elevated. No correlation between PHLA and triglyceride bloodlevels was found however.

2.2.5 Oral contraceptives

Kissebah et al. (1973) measured PHLA in women taking contraceptive steroids. The heparin dose was 10 U/kg body weight. Blood was sampled 10 min. after heparin injection. Estrogen administration was associated with a significant reduction of PHLA while triglyceride bloodlevels increased.
In a similar investigation Rössner et al. (1971) studied PHLPL activity in bloodsamples taken 5 and 40 minutes after intravenous injection of 100 U heparin/kg body weight. After Estrogen administration a significant decrease of PHLPL activity was found in the 40 minutes' samples only. Triglyceride bloodlevels increased in this situation.

2.2.6 Chronic nonnephrotic renal failure

Gutman et al. (1973) measured PHLA in chronic nonnephrotic renal failure. The heparin dose was 10 U/kg body weight, blood was sampled 10 minutes after heparin injection. Low PHLA associated with hypertriglyceridemia was found.

2.3 Situations characterized by an increased plasma PHLPL activity. Correlation with triglyceride bloodlevels

2.3.1 Dietary influences

Cybulska et al. (1974) studied the influence of feeding soybean oil on plasma postheparin

lipolytic activity. Heparin dose was 10 U/kg body weight, blood was collected 10 minutes after heparin injection.
Isocaloric replacement of saturated fat by soybean oil in the diet for 10 days resulted in a significant increase of plasma PHLA while plasma TG levels decreased.

2.3.2 Obesity

Rafaeli-Eskhol and Diengott (1972) studied PHLA in obese patients.
The heparin dose was 10 U/kg body weight, blood was collected 10 minutes after heparin injection. PHLA was found to be significantly higher in obese patients when compared to values of control subjects. Total fasting resulted in a decrease of PHLA to normal values. These values remained normal after the patients were put on a low caloric diet for a few weeks. Triglyceride bloodlevels were not mentioned.

2.3.3 Hyperthyroidism

Plasma PHLA was found to be significantly increased in thyrotoxicosis (Nikkilä and Kekki, 1972). The heparin dose was 20 U/kg body weight, blood was collected 10 minutes after heparin injection. Triglyceride bloodlevels were slightly elevated.

2.3.4 Contraceptive steroids

Kissebah et al (1973) measured plasma PHLA in women taking contraceptives steroids. Women using either progesterone or megestrol showed a decrease in serum triglyceride concentration associated with an increased plasma PHLA. The heparin dose was 10 U/kg body weight, blood was collected 10 min. after heparin injection.

2.4 Discussion

Notwithstanding many methodological differences involved in the measurement of PHLA it is worth pointing out that there is a good agreement between the results from different authors.
Although the functional significance of PHLA is not well known, changes of PHLA have most frequently been accompanied by changes of the IVFTT in the same direction (see summary table).

Although it could be argued that a low PHLA is an artefact due to the fact that, when a high triglyceride concentration is present in the plasma, the enzyme could be relatively inactive towards added substrate in the in vitro test system, this is unlikely for the following reasons:

1 In the presence of hypertriglyceridemia a normal PHLA (Krauss et al., 1974) or even a high PHLA (Nikkilä and Kekki, 1972, Rafaeli-Eskhol and Diengott 1972) can be found.
2 The effect of adding chylomicrons or VLDL in vitro on the total plasma PHLA seems to be insignificant (Krauss et al., 1974b).

Consequently, it seems that in most situations, the PHLA can be regarded as a fairly reliable indicator of triglyceride removal efficiency.

SUMMARY TABLE

CLINICAL CONDITION	PHLA	IVFTT	TG-blood-level	References
High carbohydrate diets	↓	↓	↑	Mancini et al. 1973
High PUF[1] diets[2]	↑	↑	↓	Nestel and Barter 1973, Cybulska et al. 1974
Primary hypertriglyceridemia	↓	↓	↑	Adams et al. 1974
Obesity[2]	↑	↓	↑	Rössner et al. 1974, Rafaeli Eskhol and Diengott 1972
Diabetes mellitus	↓	↓	↑	Kissebah 1974
Hypothyroidism	↓	↓	↑	Nikkilä and Kekki 1972
Thyrotoxicosis	↑	↑	↑	Nikkilä and Kekki 1972
Alcoholic hyperlipemia[2]	↓	↓	↑	Krauss 1974 a, Chait et al. 1972
Chronic non-nephrotic renal failure	↓	?	↑	Gutman et al. 1973
Contraceptive steroids:				
1 Estrogens	↓	N	↑	Kissebah et al. 1973
2 Progesterone	↑	↑	↓	Kissebah et al. 1973
Oxandrolone	↑	↑	↓	Olsson et al. 1974, Glueck et al. 1973
Nicotinic acid, clofibrate	?	↑	↓	Boberg et al. 1970

1 PUF: Polyunsaturated fat
2 Results of PHLA and IVFTT taken from different sources
↑ increased activity
↓ decreased activity
N normal activity

Chapter II

Investigations and methods

1 Problem definition

Investigation of triglyceride clearing was performed in three different groups of children presenting with specific problems related to triglyceride metabolism.

1.1 Children with glycogen storage disease

Hyperlipemia in these children is a well known finding.
The pathogenesis of hyperlipemia in patients with a glucose – 6 – phosphatase deficiency seems to be as follows:
In the fasting state, the glycogenolyses of glycogen to pyruvate is increased because the conversion of glucose – 6 – phosphate to glucose is blocked.
Part of the excess pyruvate is converted into acetyl-COA and thence into fatty acids. Hypersecretion of pre-β-lipoprotein by the liver ensues. (Fernandes and Pikaar, 1969).
In patients with a deficiency of debranching enzyme or of the phosphorylase system, glycolysis is enhanced postprandially inducing liponeogenesis and hypersecretion of pre-β-lipoprotein by the liver. (Fernandes and Pikaar, 1969).
Although enhanced triglyceride production by the liver plays an important role in the pathogenesis of hypertriglyceridemia of glycogen storage disease, it may not be the only factor.
The question to be answered was the following: 'Does a decreased triglyceride elimination contribute to the development of hyperlipemia in these patients? '

1.2 Children with obesity

As many questions related to this condition in children are still unanswered we tried to give, at least a partial answer, to the following questions: is hyperlipemia a common finding in childhood obesity? Is the fractional Intralipid turnover rate abnormal?
Is the triglyceride production rate increased?
Is the PHLPL activity abnormal?
If this is the case is there a relation between PHLPL activity and increased insulin secretion?
Do the abnormalities disappear after caloric restriction?

1.3 Children treated by total parenteral nutrition (P.N.) for a prolonged period

In this last study we tried to answer the following questions.
Does the triglyceride clearing capacity of a patient change during PN?
Is PN with high fat doses feasible without causing hyperlipemia?
Is there an easy way to monitor the daily doses of fat emulsion during PN in such a way that Intralipid tolerance is not exceeded?

2 Methods

2.1 The IVFTT

The test procedure in our study has been as follows:

2.1.1 Subject procedure

All children were studied in the morning about 2 hours after a small standard carbohydrate breakfast.
The reason for this was that patients with GSD frequently cannot tolerate a much longer period of fasting.
As it has been shown that an acute glucose load does not affect the IVFTT value (Gibson et al., 1974) we assumed that a small carbohydrate breakfast would not affect Intralipid removal. A scalp vein infusion set was introduced into an antibrachial vein. A basal bloodsample was taken for the determination of plasma lipids and of plasma nephelometric blank value.
An Intralipid (Intralipid 20%) dose of 0.1 gr/kg body weight was injected intravenously within 45 to 60 seconds. The same Intralipid batch was used in all tests.
Bloodsamples of 0.1 ml were pipetted after 5, 10, 15, 20, 25, 30 and 35 minutes. The samples were directly transferred to test tubes containing 5 ml NaCl 0.9%, mixed by turning and taken to the laboratory.

2.1.2 Laboratory procedure

Intralipid standard curve

Standard solutions were prepared by diluting Intralipid 20% with saline as follows:
To six tubes, each containing 5 ml of saline were added 10, 20, 30, 40, 50 and 60 μl of Intralipid 20%. Intralipid concentrations in these tubes were 40, 80, 120, 160, 200 and 240 mg/100 ml.
From each tube 50 μl was pipetted and added to 5 ml saline (dilution $^1/_{100}$). The nephelometric readings of these diluted Intralipid emulsions were performed in a Vitatron photometer equipped with an adaptor for nephelometry. A saline blank was used to set the zero.
Standard solution 240 mg/100 ml (before dilution) was given an arbitrary value on the Vitatron scale. The nephelometric values of the other standard solutions were then measured.

It appeared that the standard curve was linear and included the origin. Therefore, when determining the Intralipid content of bloodsamples it is sufficient to measure the nephelometric value of only one standard.
In our experiments we compared the nephelometric readings of the bloodsamples to the value of the 240 mg/100 ml standard emulsion. With this standard, the Vitatron scale was set at 80, consequently all readings had to be multiplied by three in order to obtain the Intralipid concentration of the bloodsamples.

Measurement of bloodsamples turbidity

Bloodsamples (0.1 ml blood in 5 ml saline) were centrifuged at low speed (60 g) for 10 minutes. The supernatant was pipetted off and centrifugation was repeated. The supernatant was then transferred to tubes which were placed in the photometer for nephelometry.
The blood blank value was substracted from each reading.
Nephelometric readings were then converted to Intralipid concentration as described above.
Intralipid concentrations during the IVFTT were not corrected for hematocrit values. When Intralipid bloodlevels were determined in the course of longterm parenteral nutrition correction for hematocrit was performed, however.
The results of the IVFTT were expressed as follows:
The Intralipid concentrations were plotted on a logarithmic scale against time on a linear scale. The slope of the resulting straight line was determined by the method of least squares. The correlation coefficient of this line was calculated. The slope of the regression line represents the fractional removal constant which has been expressed as % removal/minute.

2.2 Calculation of triglyceride production rate

It has been shown (Rössner S. et al, 1974a) that a correlation exists between endogenous and exogenous (IVFTT) triglyceride clearance.
Consequently, by multiplying the basal plasma triglyceride concentration (mmoles/l) by the IVFTT value one gets a rough estimation of the relative plasma triglyceride elimination rate (mmoles/l plasma/minute). We assumed endogenous plasma triglyceride to be in a steady state just before performing the IVFTT. Consequently, the calculated plasma triglyceride elimination rate (mmoles/l plasma/min) is equal to the triglyceride production rate (mmoles/l plasma/min). The following limitations apply to this arbitrary measurement of triglyceride production rates.

1 Although a good correlation was found between endogenous and exogenous triglyceride elimination (Rössner et al., 1974a) in control patients and in patients with primary hypertriglyceridemia this might not be true for other situations.
2 As exogenous triglyceride (IVFTT) is eliminated more rapidly than endogenous triglyceride (Rössner S. et al., 1974) the triglyceride production rates calculated in this way most probably overestimate the real values for endogenous triglyceride production.

3 As plasma volumes of our patients were not measured the triglyceride production values could not be expressed in absolute terms but only as relative quantities produced/l plasma/min. Expression in this way, however, might be advantageous for the following reasons.

As children of different ages were investigated, comparison between these children was only possible by relating the triglyceride production to a quantifiable body measurement. The choice of plasma volume as quantifiable body measurement seems well indicated as the triglyceride production/1 plasma probably better defines the relationship between triglyceride production and triglyceride bloodlevel than absolute triglyceride production rates. Notwithstanding these limitations calculation of triglyceride production rates as performed in this study probably represents a valuable and simple way of investigating plasma triglyceride metabolism in children with different diseases associated with hypertriglyceridemia.

2.3 Measurement of plasma postheparin lipoprotein lipase activity

2.3.1 Subject procedure

In most children the PHLPL test was performed one hour after the IVFTT (see 2.1.1).
In patients with GSD, however, the two tests were performed on separate days in order to keep the fasting period as short as possible (see 2.1.1).
A scalp vein infusion set was introduced into an antibranchial vein. At zero time 100 U of heparin per kg body weight was injected intravenously. 2.7 ml of blood was taken for LPL activity analysis 5 and 40 minutes after the heparin injection and transferred into glass tubes containing 0.3 ml of 0.1 molar trisodium citrate. The bloodsamples were kept on ice. At the end of the test the bloodsamples were centrifuged and the plasma was frozen. LPL activity was determined within one month.

2.3.2 Laboratory procedure

When a choice has to be made between the many methods for the determination of PHLPL activity, a few points have to be considered, viz.:

1 The determination should be done in citrated plasma and not in serum. Apparently during the clotting process an inhibitor of lipoprotein lipase is released (Engelberg, 1955).
2 The triglyceride emulsion (substrate) should closely ressemble chylomicra, which are believed to be the physiological substrate. Intralipid seems to fulfill this requirement (Boberg and Carlson 1964, and Biale and Shafrir 1969).
3 The substrate triglyceride concentration should be sufficiently high to obtain a zero order reaction for at least one hour (Boberg and Carlson, 1964).
4 The triglyceride emulsion should be activated by incubation at 37° C with either high density lipoprotein (Korn, 1959) or by whole serum exactly as described by Jansen and Hülsmann, (1973a).
5 Relatively high concentration of albumin should be present in the assay mixture to bind released FFA (Kern et al., 1961). In the present investigation, mainly Jansen and Hülsmann's method has been followed with a few minor modifications.

Materials

1 *Glycerol-tri (oleate 1-C-14)* Amersham, circa 40 Mci/mMol

2 *Intralipid* (Vitrum, Stockholm) 10%

3 *0.2 M. Tris-buffer* pH 8.5

4 *10% solution of bovine serum albumin* (Cohn fract. V Serva Feinbiochemica, Heidelberg) in 0.2 M tris-buffer.

5 *Human inactive serum.* Citrated plasma was clotted at 37° for 30 min. by the addition of $CaCl_2$. The concentration of $CaCl_2$ was 0.025 M. After centrifugation the supernatant was dialysed at 4°C during 24 h. against 0.9% NaCl. By addition of dilute KOH the pH was brought to 8.5.

6 *Amberlite IRA-400 (OH).* Amberlite IRA-400 (Cl^-) was treated 7 times with a fivefold quantity of NaOH 5%. After washing with aq.dest. until neutral, water was removed by washing with isopropanol. Subsequently, the resin was washed with hexane and air dried. It was stored at -15°C.

7 *Dole-mixture:* 400 ml isopropanol, 100 ml heptane, 10 ml 2N H_2SO_4.

Method

A benzene solution of circa 3.5 μCi labelled tri-oleate was dried in a plastic tube in a nitrogen current. After addition of 1.1 ml Intralipid 10%, the mixture was sonicated in a Branson sonifier, setting 2, (8 times for circa 45", with intermittent cooling in ice).
Subsequently, 3.0 ml of the inactive serum (5)
6.0 ml of the albumin solution (4)
and 1.9 ml 0.2 M Tris-buffer (3) were added.
0.8 ml was introduced into glass-stoppered tubes and incubated for 40 min. at 37°C. 0.2 ml of the plasma samples were added and the reaction allowed to proceed for 35 min. at 37°C. 0.2 ml of distilled water served as a blank. The reaction was stopped by the addition of 5 ml Dole-mixture *). After shaking vigorously for circa 1 minute, 3 ml of the upper phase was introduced into a counting vessel containing 0.5 gr of Amberlite IRA-400 (OH^-).
These vessels were stirred from time to time. After 1 h. the supernatant was removed, the resin was washed 4 times with 5 ml of hexane. Subsequently, 1 ml of hyamine hydroxide solution (1M in methanol, Packard) was added and after circa 1 min. 10 ml of toluene with fluors.

*) Followed (after circa 2 min.) by 3 ml heptane and 2 ml water.

After standing for at least 48 h. at 4°C and in the dark, the samples were counted. (Packard liquid scintillation counter).
If a μCi labelled tri-oleate (bμCi/mMol) has been used and the dpm counted was equal to c, then the lipolytic activity (μeq fatty acid/min/liter is:

$$\frac{C}{1000} \times \frac{3 \times \left(\frac{a}{b} + \frac{110}{0.884}\right) \times 1000}{a \times 2200 \times 0.2 \times 35 \times 0.956^{*} \times 0.75^{**} \times 0.9^{***}}$$

Precision of the method measuring PHLPL activity: the precision of the method used has been evaluated by calculating the coefficient of variation from 150 duplicate determinations.
The coefficient of variation was ± 3.7%.

*) 0.956 Efficiency of binding fatty acid to the resin
**) 0.75 Aliquot of the heptane supernatant
***) 0.9 Concentration of plasma in citrated plasma

Hepatic glycogenosis hyperlipidemia
postheparin lipoprotein lipase triglyceride

Triglyceride Clearing in Glycogen Storage Disease

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Extract

Peripheral uptake of triglyceride from plasma was investigated by intravenous fat tolerance tests and by postheparin lipoprotein lipase measurements in children with different types of glycogen storage disease.

The patients with a glucose 6-phosphatase deficiency were characterized by a significantly diminished triglyceride elimination rate (5.79 ± 2.78%/min) and 5-min postheparin lipoprotein lipase activity (40.2 ± 23 μEq fatty acid (FA)/liter/min). The patients with a deficiency of debranching enzyme showed a significantly diminished triglyceride elimination rate (4.84 ± 1.61%/min) whereas the 5-min postheparin lipoprotein lipase activities did not significantly differ from the control values (49.6 ± 27.7 μEq FA/liter/min).

The patients with a deficiency of the phosphorylase system showed neither a significantly diminished triglyceride elimination rate (7.34 ± 2.65%/min) nor a diminished 5-min postheparin lipoprotein lipase activity (61.7 ± 30.1 μEq FA/liter/min).

Triglyceride elimination rates were correlated positively with plasma lipoprotein lipase activities ($r = 0.55$, $P < 0.05$).

Speculation

The dietary treatment of hyperlipidemia in hepatic glycogenosis might be based on the outcome of the intravenous fat tolerance test, a low fat clearance being an indication for a low fat, high carbohydrate diet, a normal fat tolerance for a high fat, low carbohydrate diet.

Introduction

Little is known about the causes of hyperlipidemia in patients with glycogen storage disease (GSD) [4, 5, 8].

The highest lipid levels are found in patients with a glucose 6-phosphatase deficiency. They have the highest fatty acid cholesterol ratios and sometimes even a pre-β-band on the electropheretograms. *De novo* fatty acid synthetizing activity from citrate is high in liver biopsies of these patients [7]. The pathogenesis of the hypertriglyceridemia in patients with a glucose 6-phosphatase deficiency is presumed to be as follows: in the fasting state, conversion of glucose 6-phosphate to glucose is blocked, so that glycogen can only be degraded to pyruvate through the glycolytic pathway. Part of the excess pyruvate is converted into lactate, another part into acetyl-CoA, and thence into fatty acids, predominantly palmitate [4, 12].

Hyperlipidemia is less striking in children with a deficiency of debranching enzyme or of the phosphorylase system. In the latter deficiencies, the hyperlipidemia is characterized by a dense β-lipoprotein band, no pre-β-lipoprotein band being present on the elec-

tropheretograms; the glycolysis is enhanced postprandially, which leads to an increased lactate production and an increased liponeogenesis [4].

Although enhanced lipid production in the liver probably plays a causative role in the pathogenesis of GSD hyperlipidemia, it may not be the only factor. A pathologically low triglyceride clearance rate might also be an important factor. By measuring the triglyceride elimination rate and the serum postheparin lipoprotein lipase activity, we tried to gain more insight into the apparently complex causes of the hyperlipidemia.

Materials and Methods

The study comprised seven patients with a glucose 6-phosphatase deficiency, seven patients with a debranching enzyme deficiency, and six patients with a deficiency of the phosphorylase system. Enzymatic studies had confirmed the diagnosis in all patients (Tables I–III). Ten patients without hyperlipidemia in whom an intravenous fat tolerance test had been performed served as control subjects (Table IV). They all had a normal nutritional status. Informed consent was obtained in each case.

An intravenous fat tolerance test was performed in all GSD patients and control subjects. Heparin-released plasma lipoprotein lipase (LPL) activity was measured in most GSD patients and in some control subjects. Both tests were performed on consecutive days. The patients and the control subjects did not fast before the tests in order to prevent hypoglycemia in the patients. The meals during the 12-hr period before each test did not contain fat. The last meal was always given 2 hr before the test. The diet in the 2 weeks preceding the tests contained about 25% of total calories at fat, mainly polyunsaturated, for all patients.

The intravenous fat tolerance test [2] was carried out according to the method of Carlson and Rössner [3]. For all tests, a 20% Intralipid emulsion [15] was administered intravenously in a dose of 0.1 g/kg body wt. Capillary blood samples were collected every 5 min. The Intralipid concentration was determined by nephelometry. For each disappearance curve a minimum of four consecutive points was used. The logarithms of the Intralipid concentrations were plotted against time. Straight lines were obtained from which the correlation coefficients were calculated.

The LPL activities were measured after the intravenous injection of 100 U heparin/kg body wt in 5- and 40-min blood samples in most GSD patients, but in 5-min blood samples only in most control subjects. As we concur with Rössner *et al.* [13] that the 5-min postheparin LPL activity probably gives more information about fat elimination than the 40-min value, we have chosen the 5-min value as representative of LPL activity. The LPL activity was determined by the method of Kelly [11] as modified by Jansen and Hülsmann [9]. The following minor modifications were introduced: the incubation mixture consisted of 1.1 ml Intralipid emulsion containing approximately 3.5 μCi (^{14}C)trioleate, 3.0 ml human plasma freed of β-lipoproteins as described by Jansen and Hülsmann [9], 6.0 ml 10% solution of bovine albumin in 0.2 M Tris buffer pH 8.5, and 1.9 ml 0.2 Tris buffer, pH 8.5. This mixture was kept at 37° for 40 min. After this pretreatment, 0.8 ml incubation medium was mixed with 0.2 ml plasma to be investigated. The incubation time was 35 min.

Results

Intralipid Elimination Constant in Patients and Control Subjects

The Intralipid elimination constants of the patients with a glucose 6-phosphatase deficiency and of the patients with a debranching enzyme deficiency were significantly lower than those of the control patients ($P < 0.05$). For the patients with a deficiency of the phosphorylase system, the difference was not significant (Tables I–V).

Heparin-released LPL Activity in Patients and Control Subjects

The heparin-released LPL activity was higher in the 5-min sample than in the 40-min sample in the majority of GSD patients. Three out of six glucose 6-phosphatase-deficient patients, and one out of four debranching enzyme-deficient patients, however, showed 5-min activities lower than the 40-min activities. Most patients with a glucose 6-phosphatase deficiency showed lower 5-min LPL activities as compared with the control subjects, the difference between both groups being significant ($P < 0.05$). Some patients with a deficiency of debranching enzyme or a deficiency of the phosphorylase system showed decreased LPL activities as well, but the LPL activities of the latter two groups as a whole did not differ significantly from the LPL activities of the control subjects (Tables I–V).

Table I. Data for seven patients with a glucose 6-phosphatase deficiency[1]

Case	Sex	Age, yr	Cholesterol, mmol/liter	Triglycerides, mmol/liter	K_2, %/min[2]	r	Postheparin LPL activity, μEq FA/liter/min		Glucose 6-phosphatase in liver, nmoles P_i/min/mg protein
							5 min	40 min	
1. CC	F	4.2	4.87	3.12	2.46	0.9936	13.0	5.6	0.0
2. SS	M	3.5	3.53	1.75	5.20	0.9947	36.7	49.1	1.0
3. AR	F	2.9	5.78	8.55	3.00	0.9990	35.8	24.5	0.9
4. AW	F	29.2	9.02	13.00	6.22	0.9973	24.0	28.1	0.0
5. DS	M	1.9	3.86	0.79	4.90	0.9933	30.8	38.8	1.7
6. MB	F	3.2	3.35	2.47	9.00	0.9955	83.1		5.1
7. RS	M	3.0	6.30	2.56	9.79	0.9950	57.3	19.3	1.8
n			7	7	7		7		6[3]
m			5.24	4.60	5.79		40.2		
σ			2.29	4.46	2.78		23.0		

[1] F: female; M: male; LPL; lipoprotein lipase; FA: fatty acid.
[2] Intralipid elimination constant [3].
[3] Normal range 24.0–93.0.

Table II. Data for seven patients with a debranching enzyme deficiency[1]

Case	Sex	Age, yr	Cholesterol, mmol/liter	Triglycerides, mmol/liter	K_2, %/min	r	Postheparin LPL activity, μEq FA/liter/min		Debranching enzyme, nmol glucose produced from limit dextrin/min/mg protein	
							5 min	40 min	Leucocytes	Liver
1. PL	F	15.2	6.75	2.00	4.30	0.9897	98.0	68.2	0.00	
2. DL	F	14.0	5.50	2.27	3.75	0.9974	60.9	41.9	0.00	
3. AD	F	11.9			7.05	0.9855	51.4		0.00	0.00
4. JK	F	7.4	3.73	0.95	5.86	0.9050			0.09	0.13
5. JB	F	5.8	4.04	2.90	3.98	0.9929	38.2		0.00	
6. JZ	F	12.0	6.88	4.09	6.36	0.9760	22.6	37.7	0.00	0.20
7. JK	M	1.2	6.60	5.43	2.58	0.9906	26.4	18.0	0.20[2]	0.65[2]
Normal range									0.33–1.80	0.5–2.3
n			6	6	7		6		14	6
m			5.58	2.94	4.84		49.6			
σ			1.40	1.60	1.61		27.7			

[1] F: female; M: male; LPL: lipoprotein lipase; FA: fatty acid.
[2] The assay was modified and performed at a higher concentration of limit dextrin (normal range for leucocytes 2.07–5.04 (n = 8), for liver 2.13–9.09 (n = 3).

Levels of Triglyceride and Cholesterol in Blood in Patients and Control Subjects

The triglyceride levels were significantly higher ($P < 0.05$) in the three patient groups as compared with the control subjects. The cholesterol values were significantly increased in the patients with a debranching enzyme deficiency as compared with the control subjects (Tables I–V).

Correlations of Intralipid Elimination Constants, LPL Activities, and Levels of Triglyceride in Plasma of GSD Patients

The Intralipid elimination constants and the LPL activities 5 min after heparin injection showed a significant positive correlation ($r = 0.55$, $P < 0.05$, Fig. 1). The Intralipid elimination constants and the plasma triglycerides, on the one hand (Fig. 2), and the LPL activities 5 min after heparin injection and the plasma triglycerides, on the other hand (Fig. 3), were not significantly correlated.

Discussion

Much work has been done to find the cause of the deficient LPL activity in disturbances of the triglyceride elimination system. The synthesis of LPL activity in adipose tissue taken from rats appears to be under hormonal control. Insulin has a positive effect, whereas catecholamines, glucagon, growth-stimulating

Table III. Data for six patients with a deficiency of phosphorylase system[1]

Case[2]	Sex	Age, yr	Cholesterol, mmol/liter	Triglycerides, mmol/liter	K_1, %/min	r	Postheparin LPL activity, μEq FA/liter/min		Phosphorylase in leucocytes, nmol/min/mgprotein		Phosphorylase *b* kinase in leucocytes, U activated/min/mg protein
							5 min	40 min	−AMP	+AMP	
1. MT	M	2.8	5.37	3.69	3.66	0.9924	13.9		5.6	6.1	0.29
2. OT	M	1.4	5.30	2.87	5.50	0.9957	45.8	21.6	7.9	9.8	0.65
3. JH	M	7.1	4.84	1.08	11.10	0.9915	72.9	43.9	3.0	14.0	0.08
4. WT	M	9.1	5.67	0.91	6.50	0.9860	84.7	48.1			0.08
5. JI	M	6.0	4.26	1.87	8.90	0.9435	98.0	37.2	3.0	20.0	0.00
6. MR	M	7.2	6.24	0.83	8.39	0.9862	55.3	20.2	5.0	21.0	0.02
Normal range									15.8–47.9	18.0–50.1	0.37–0.65
n			6	6	6		6		16		8
m			5.28	1.87	7.34		61.7				
σ			0.68	1.17	2.65		30.1				

[1] M: male; LPL: lipoprotein lipase; FA: fatty acid.

[2] *Patients 1* and *2* (brothers) are phosphorylase deficient; phosphorylase activity in the liver 0.0 and 0.0 nmoles/min/mg protein, respectively; *patients 3–6* are phosphorylase *b* kinase deficient; liver biopsy, performed in one patient (*patient 4*), confirmed the diagnosis.

Table IV. Data for 10 control children[1]

Case	Sex	Age, yr	Cholesterol, mmol/liter	Triglycerides, mmol/liter	K_1, %/min	r	Postheparin LPL activity, μeq FA/liter/min	
							5 min	40 min
1. SE	M	3.1	4.57	0.45	11.59	0.9894		
2. KJ	F	1.5	4.38	0.52	8.01	0.9958	72.4	67.4
3. FJ	F	8.0	5.10	0.63	7.83	0.9791	102.7	
4. AD	F	4.2	4.60	0.93	5.70	0.9900		
5. PC	F	7.8	5.01	0.70	7.06	0.9981	42.5	
6. OM	M	4.2	4.35	0.68	8.66	0.9990		
7. VC	F	12.3	3.50	0.86	8.86	0.9896		
8. VS	F	8.1	4.23	0.60	7.11	0.9966		
9. OC	M	2.2	5.20	0.80	10.10	0.9820	113.7	
10. MR	M	9.9			9.20	0.9970		
n			9	9	10		4	
m			4.54	0.68	8.39		82.8	
σ			0.52	0.15	1.66		32.4	

[1] M: male; F: female; LPL: lipoprotein lipase; FA: fatty acid.

hormone, and adrenocorticotropic hormone have been shown to have a negative effect on the LPL activity [10]. Further studies on the factors regulating LPL activity were described in 1966 by Salamon and Robinson [14], who incubated intact epidymal fat bodies taken from rats that had been starved for 48 hr and in which the activity of LPL was low, in a medium designed to promote enzyme activity. They found that, as expected, the LPL activity increased to a level characteristic of the tissue of intact animals in the fed state. Glucose and insulin were important constituents of the incubation medium, insofar as the omission of either markedly reduced the increase in enzyme activity. Therefore, LPL synthesis in patients with a glucose 6-phosphatase deficiency may be impaired by the low levels of glucose and insulin in blood [6]. As for our patients with this enzyme defect, not only LPL activities, but also Intralipid clearance rates were significantly reduced as compared with the control patients. Our patients deficient in debranching enzyme or the phosphorylase system, however, in whom symptoms of hypoglycemia are mild and rare, showed LPL activities that did not differ significantly from those of the control patients. Nevertheless, the patients with a debranching enzyme deficiency showed Intralipid clearance rates significantly lower than the control subjects. We have no explanation for the discrepancy between the normal LPL activity and decreased fat elimination in debranching enzyme-deficient patients.

Thus, we may explain the hyperlipidemia of GSD

Table V. Intravenous fat tolerance test (IVFTT) and 5-min postheparin lipoprotein lipase (LPL) in glycogen storage disease patients and control subjects

	IVFTT K_2, %/min				5-min postheparin LPL, μeq FA[1]/liter/min			
	Glucose 6-phosphatase deficiency	Debranching enzyme deficiency	Phosphorylase deficiency	Control subjects	Glucose 6-phosphatase deficiency	Debanching enzyme deficiency	Phosphorylase deficiency	Control subjects
	2.46	4.30	3.66	11.59	13.0	98.0	13.9	
	5.20	3.75	5.50	8.01	36.7	60.9	45.8	72.4
	3.00	7.05	11.10	7.83	35.8	51.4	72.9	102.7
	6.22	5.86	6.50	5.70	24.0		84.7	
	4.90	3.98	8.90	7.06	30.8	38.2	98.0	42.5
	9.00	6.36	8.39	8.66	83.1	22.6	55.3	
	9.79	2.58		8.66	57.3	26.4		
				7.11				
				10.10				113.7
				9.20				
n	7	7	6	10	7	6	6	4
m	5.79	4.84	7.34	8.39	40.2	49.6	61.7	82.8
σ	2.78	1.61	2.65	1.66	23.0	27.7	30.1	32.4

[1] FA: fatty acid.

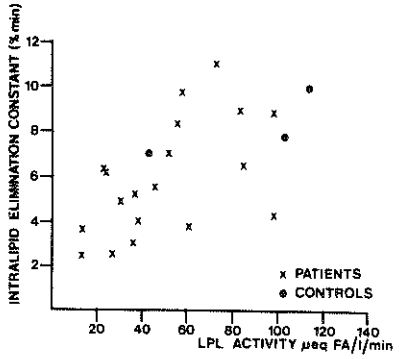

Fig. 1. Relation between lipoprotein lipase (*LPL*) activities measured 5 min after the intravenous injection of 100 U heparin/kg body wt and Intralipid elimination constants of patients with glycogen storage disease and control subjects. *FA:* fatty acid.

patients as follows. The hypertriglyceridemia of patients with a glucose 6-phosphatase deficiency is due to a combination of a decreased triglyceride elimination rate and increased endogenous liponeogenesis [7, 12], the former factor being the more important one. We did, indeed, observe that a high fat diet worsens the hypertriglyceridemia, which thus seems to be fat-induced (data not shown). The hyperlipidemia of patients with a deficiency of debranching enzyme or the phosphorylase system, however, is mainly caused by carbohydrate-induced liponeogenesis [4], as impaired fat elimination probably plays a significant role in debranching enzyme deficiency only. (Discrepant results of fat elimination tests in debranching enzyme deficients do not permit a firm conclusion.) The dietary implications should be that for children deficient

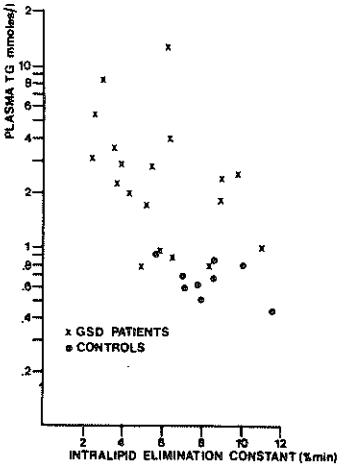

Fig. 2. Relation between Intralipid elimination constants and levels of triglyceride (*TG*) in plasma (log values) of patients with glycogen storage disease (*GSD*) and control subjects.

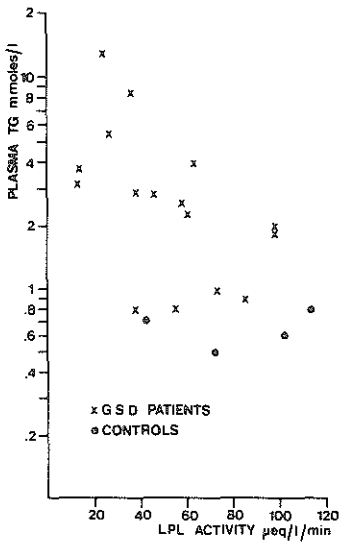

Fig. 3. Relation between lipoprotein lipase (*LPL*) activities measured 5 min after the intravenous injection of 100 U heparin/kg body wt and levels of triglyceride (*TG*) in plasma (log values) of patients with glycogen storage disease (*GSD*) and control subjects.

in glucose 6-phosphatase, a low fat, high carbohydrate diet is indicated, whereas for children with phosphorylase deficiency and possibly also those with a debranching enzyme deficiency, carbohydrate rather than fat restriction is indicated.

Summary

The hyperlipidemia of 20 patients with GSD was studied by the intravenous Intralipid tolerance test and the determination of the plasma postheparin lipoprotein lipase activity. Patients with a glucose 6-phosphatase deficiency were characterized by abnormally low results for both tests as compared with control children. Patients with a deficiency of debranching enzyme showed a decreased Intralipid elimination constant, whereas the LPL activities did not significantly differ from control values. Patients with a deficiency of the phosphorylase system showed normal results for both tests.

A positive correlation was found between the Intralipid elimination constant and the postheparin LPL activity in the patients.

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Chapter III (2)

Plasma triglyceride clearing in obese children [1],[2]

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ABSTRACT. In 13 obese children plasma triglyceride concentrations were found to be significantly elevated, while plasma cholesterol concentrations were normal. In the hypertriglyceridemic obese children, the plasma triglyceride removal, measured by the intravenous fat tolerance test, was significantly reduced. A few patients showed an increased triglyceride production. These abnormalities reverted to normal in 8 patients retested after weight loss. Plasma postheparin lipoprotein lipase activity was found to be increased and significantly related to the degree of obesity. As to carbohydrate metabolism, a decreased glucose tolerance and hyperinsulinemia were found. Hyperinsulinemia reverted to normal during dietary restriction, glucose intolerance did not.

Prospective and retrospective studies have shown the importance of childhood obesity as a forerunner of adult obesity (1, 2, 3). Mortality data from life insurance studies clearly demonstrate increased death rates among obese adults (4). Disturbances of carbohydrate and lipid metabolism can, among other factors, account for this increased mortality rate (4). Whereas carbohydrate metabolism has been fairly extensively studied in obese children (5, 6), surprisingly few studies of plasma lipids in childhood obesity are available. Plasma triglyceride and cholesterol levels in this condition have been reported to be elevated by some authors (7) and normal by others (8). The present investigation aimed primarily at studying plasma triglyceride clearing in a small group of obese children before and during weight reduction. As disturbances of plasma lipids in obesity often are related to disturbances of carbohydrate metabolism (9) the latter was also investigated.

Patients and methods

Patients

13 obese children (7 females and 6 males, ages ranging from 7 to 15 years) were studied. Relative weight has been used as a rough indicator of the degree of obesity (Table 1). It was calculated by dividing a patient's weight by his ideal weight for length taken from growth charts of normal Dutch children (10). Obesity was

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considered to be present when relative weight exceeded 1.2 (11). 3 Children had become obese before, the remainder after the age of 3 years. In all but one child obesity was present in first degree relatives. Age-matched children admitted to hospital for minor surgical problems were used as controls for plasma lipid values. Bloodsamples were always taken in the fasting state (12-14 hours). Control values for the intravenous fat tolerance test (IVFTT) were taken from a previous study (12). Control values for plasma postheparin lipoprotein lipase (PHLPL) activity were obtained from young healthy adults as control values for children were unavailable.

Experimental design

A detailed dietary history was obtained by a trained dietitian. Caloric content and composition of the diet was calculated (Table 2). During the short hospital admission which followed, the following investigations were performed.

On the morning of admission and 2 hours after a small standard fatless carbohydrate breakfast, bloodsamples were taken for the determination of basal plasma triglyceride and cholesterol concentration. Subsequently an IVFTT was performed as described previously (12). In short, 0.1 g/kg ideal body weight plus 0.05 g/kg excess weight (13) of Intralipid 20% (Vitrum, Stockholm) was injected i.v. within one minute. In the following half hour plasma Intralipid concentrations were determined by nephelometry at 5, 10, 15, 20, 25, and 30 minutes respectively. From the exponential Intralipid elimination curve obtained, the elimination constant was calculated and expressed as % removal/minute. Relative plasma triglyceride production rate was estimated by multiplying the IVFTT value by the plasma triglyceride concentration (mmoles/l plasma) as suggested by Rössner et al. (14). Results were expressed as mmoles/l plasma/minute. One hour after starting the IVFTT the plasma PHLPL activity was measured in bloodsamples taken 5 and 40 minutes after the i.v. injection of 100 U heparin/kg body weight. Plasma PHLPL activity was determined as described previously (12). On the second morning of hospital stay, after a fasting period of 12-14 hours bloodsamples were taken for the determination of basal free fatty acids (FFA), triglyceride, glucose and insulin levels. Then, an oral glucose tolerance test was performed. Capillary bloodsamples for glucose and insulin determinations were taken 30, 60, 90, 120, and 150 minutes after an oral glucose load of 1.75 g/kg ideal body weight. In the afternoon, the patients were sent home on a hypocaloric diet consisting of approximately 60% of the previous caloric intake (Table 2). Caloric distribution was as follows: Protein ± 30% of total calories, fat ± 25% of total calories, carbohydrate ± 45% of total calories. After a diet period varying from 6 weeks to 5 months, 8 patients were readmitted to hospital. The weight loss varied from 1-9 kg. The same investigations as described above were repeated. For clinical characteristics see Table 1.

Analytical methods

Blood glucose was determined by the glucose oxidase method (15). Serum triglycerides concentrations were determined according to the method of Schmidt and von Dahl (16). Serum

cholesterol concentrations were determined according to the method of Carr and Drekter (17). For the determination of serum FFA we used the extraction method described by Royer (18) followed by titrimetric measurement according to Korovina (19). Plasma insulin levels were determined according to the method of Maingay (20) with modifications according to Herbert et al. (21).

Statistical methods

The two-sample test of Wilcoxon, based on rank-order, was used for calculating the significance of differences between means of obese and control patients. Paired data analysis was performed when comparing pre- and post-weight reduction results. Simple correlation coefficients were calculated by Kendall's distribution free test. Regression analysis was performed for calculating the relationship between the logarithm of plasma triglyceride concentration and the Intralipid elimination constant.

Table 1 Clinical and laboratory data in 13 obese children before and during (8 patients) weight reduction [1]

Case	Sex and age (yr)	Weight (kg)		Height (cm)	Relative weight [2]		Fasting cholesterol concentration mmoles/liter		Fasting FFA [1] concentration maeq/liter		Fasting triglyceride concentration mmoles/liter		K_2 [3] %/min		Relative triglyceride production rate mmoles/l plasma/min		Postheparin LPL [1] activity μeq FA [1] /l/min 5 min		40 min	
		B.	A.	B.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.
1	F, 7	39	32	126	1.69	1.23	4.25	4.30	0.66	1.20	0.52	0.38	9.0	8.9	4.68	3.38	181.8	63.9	232.2	142.1
2	F, 8	49	40	142	1.48	1.14	5.10	3.84	–	1.25	0.83	0.30	4.6	13.3	3.81	3.99	115.2	61.9	249.8	159.4
3	F, 11	56	49	152	1.40	1.20	6.07	6.60	0.62	–	1.46	0.53	8.9	7.0	12.99	3.71	46.6	50.1	163.8	126.6
4	M, 13	65	60	148	1.75	1.60	4.31	4.00	0.85	1.04	1.11	0.45	4.3	8.7	4.77	3.92	89.3	100.7	110.7	192.6
5	F, 8	43	40	136	1.43	1.21	5.26	5.02	0.97	0.75	0.97	0.67	3.8	7.3	3.68	4.89	53.6	94.8	56.5	138.1
6	M, 12	61	60	148	1.75	1.64	5.64	4.90	0.97	0.84	2.10	1.21	3.3	6.3	6.93	7.62	105.1	182.5	198.1	209.1
7	F, 7	42	37	131	1.50	1.32	5.32	4.05	1.18	–	0.96	0.51	6.9	9.9	6.62	5.05	109.8	115.5	222.6	150.6
8	M, 11	52	48	145	1.48	1.34	5.97	4.47	1.12	0.72	0.34	0.42	11.5	10.8	3.91	4.93	103.0	84.2	167.0	104.1
9	F, 10	55	–	146	1.58	–	4.71	–	1.05	–	0.50	–	11.0	–	5.50	–	115.7	–	170.0	–
10	F, 11	63	–	131	2.33	–	5.77	–	–	–	3.47	–	4.6	–	15.96	–	116.4	–	235.7	–
11	M, 14	60	–	156	1.46	–	4.85	–	0.69	–	1.05	–	4.6	–	4.83	–	63.3	–	109.5	–
12	M, 15	90	–	169	1.60	–	5.28	–	–	–	2.12	–	6.9	–	14.62	–	69.5	–	167.6	–
13	M, 13	71	–	164	1.54	–	6.50	–	0.73	–	1.76	–	4.0	–	7.04	–	–	–	–	–
Mean		–	–	–	–	–	5.31	4.64	0.87	0.94	1.32	0.55	6.4	9.0	7.33	4.68	97.4	94.15	173.6	152.8
S.D.		–	–	–	–	–	0.67	0.89	0.21	0.20	0.86	0.28	2.8	2.3	4.29	1.34	36.7	41.92	58.8	34.2
Controls																				
Mean							5.17		0.94		0.53		8.3		5.59		51.4		129.3	
S.D.							0.77		0.30		0.12		1.6		1.38		18		31	
n							(19)		(13)		(22)		(10)		(9)		(7)		(5)	

[1] LPL: lipoprotein lipase; FFA: Free fatty acids; FA: fatty acid; B; before weight reduction; A; after weight reduction.
[2] Patient's weight divided by ideal weight (see text)
[3] Intralipid elimination constant (see text)

Table 2 Dietary history of obese patients

Case	Sex	Age, yr	Weight kg	Daily caloric intake			Caloric distribution		
				total	per kg body weight	per kg ideal body weight[1]	CHO	% Fat	Protein
1	F	7	39	1500	39	66	52	28	19
2	F	8	49	2000	41	61	44	44	12
3	F	11	56	1600	29	40	56	23	22
4	M	13	65	1800	28	48	47	35	17
5	F	8	43	1900	44	64	44	39	17
6	M	12	61	3100	51	90	47	41	12
7	F	7	42	1900	45	68	47	41	12
8	M	11	52	1900	36	54	54	33	13
11	M	14	60	2600	43	63	55	35	10
12	M	15	90	3100	34	55	39	43	17
13	M	13	71	2500	35	54	51	32	17

[1] See under patients and methods.

Results

Plasma triglyceride, cholesterol and FFA concentrations in obese patients before and during diet therapy

When compared to values obtained in age-matched control children, the plasma triglyceride concentration of obese patients were initially significantly increased ($p < 0.001$, Table 1). During diet therapy plasma triglyceride levels of obese patients decreased to normal values (Fig. 1). No significant changes of plasma cholesterol and FFA concentrations occurred (Table 1).

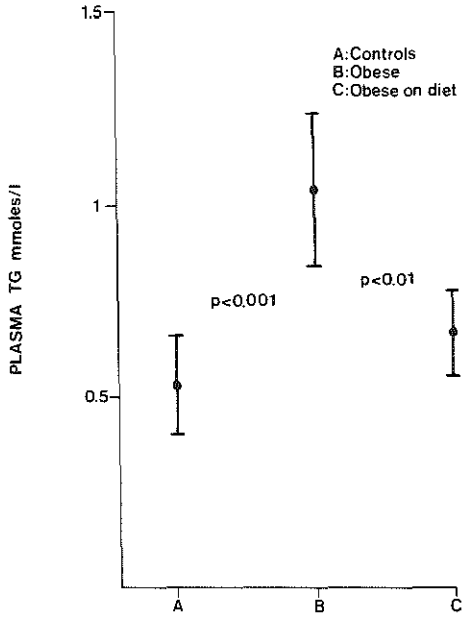

Fig. 1
Plasma triglyceride concentrations (means ± SEM)
A. 22 control patients
B. 8 obese patients
C. the same 8 obese patients during diet therapy

Intralipid elimination constant in obese patients before and during diet therapy

10 out of 13 obese children were found to have fasting triglyceride concentrations above the range of our control children (higher than mean + 2s of control children). The mean Intralipid elimination constants of these 10 obese hypertriglyceridemic children was significantly lower than that of control children ($p < 0.002$). 8 Children could be retested after weight reduction. 4 Of these showed initially decreased Intralipid elimination constants. All 8 children showed normal results when retested (Table 1).

Relative triglyceride production rate in obese patients before and during diet therapy

3 Out of the 13 obese patients showed triglyceride production rates exceeding the range found in control children (Table 1). Of these 3 children, only 1 could be retested during diet therapy and showed then a normal result (Table 1). Normal triglyceride production rates were found in the other 7 children retested during diet therapy (Table 1).

Postheparin lipoprotein lipase activity of obese patients before and during diet therapy

When compared to values obtained in 7 young adults, a significant elevation of 5 minutes PHLPL activity was found in obese patients initially ($p < 0.01$). No significant elevation of 40 minutes PHLPL activity was found (for values see Table 1). The slight decreases of 5 and 40 minutes PHLPL activities which occurred during diet therapy were not significant.

Plasma triglyceride concentration and Intralipid elimination constant

The relationship between the logarithm of plasma triglyceride levels and the Intralipid elimination constant is shown in Fig. 2. A significant negative correlation was present in the obese patients before diet therapy ($r = 0.66$, $p < 0.02$) and in the 8 patients retested after weight reduction ($r = 0.80$, $p < 0.02$).

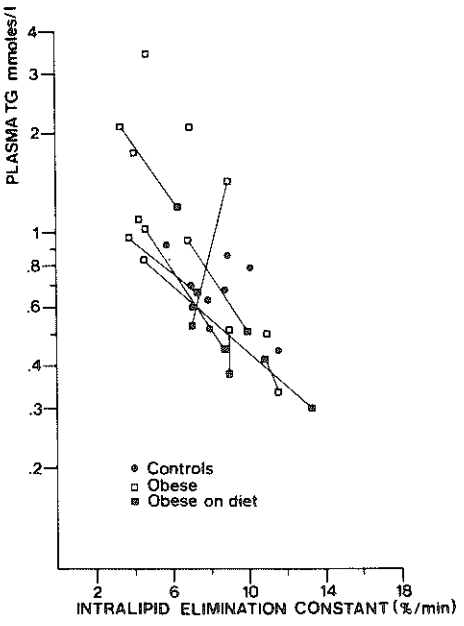

Fig. 2
Relationship between plasma triglyceride concentration (log-value) and the Intralipid elimination constant in 9 control patients, in 13 obese patients before and 8 obese patients after weight reduction (Straight lines connect individual patients before and after values).

Plasma triglyceride concentration and postheparin lipoprotein lipase activity

No correlation was found between plasma triglyceride concentration on the one hand and 5 or 40 minutes PHLPL activity on the other.

Oral glucose tolerance test in obese patients before and during diet therapy

A diminished glucose tolerance was found in obese patients both before and during diet therapy (Fig. 3). Control values were taken from Paulsen et al. (6). Only the fasting values decreased significantly during diet therapy (Fig. 3).

Plasma insulin levels before and during oral glucose tolerance tests in obese patients before and during diet therapy

Elevated insulin values were present initially in obese patients (Fig. 3). The significant decrease to normal values (6) during diet therapy is shown in Fig. 3. The insulin area under the insulin curve showed a significant decrease from 337 ± 70 (SEM) to 166 ± 20 ($p < 0.05$).

Correlation between fasting and stimulated insulin release and other metabolic parameters

No significant correlation was found between fasting insulin levels, peak insulin levels or area under the insulin curve on the one hand and glucose tolerance (peak glucose, 2 hours glucose, area under the glucose curve), triglyceride

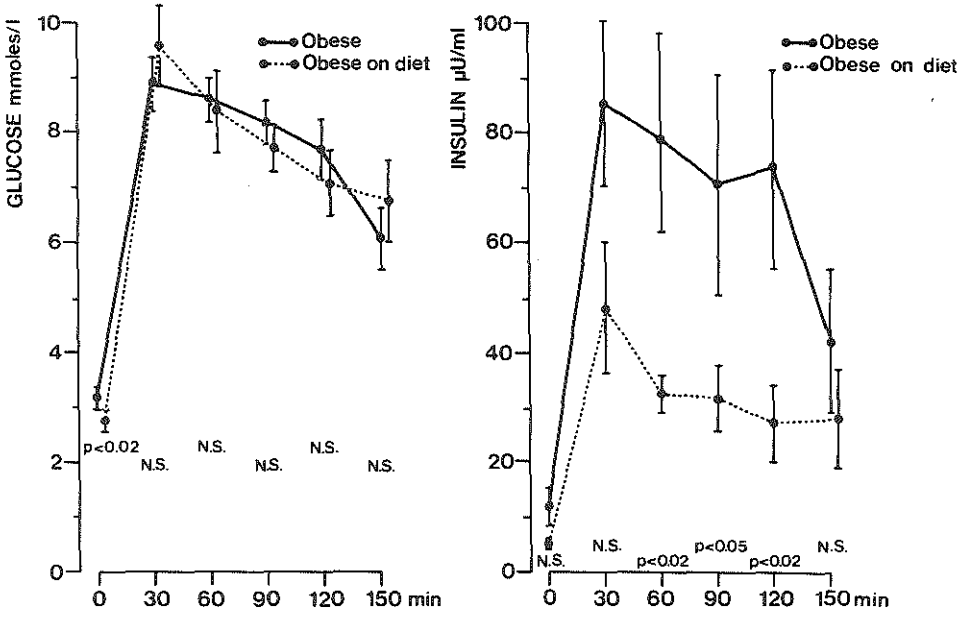

Fig. 3
Glucose and insulin curves during oral glucose tolerance tests in 8 obese patients before (●—●) and during (●···●) weight reduction (data are given as means ± SEM).
N.S.: difference non significant

bloodlevels, triglyceride production rate, Intralipid elimination constant and PHLPL activities on the other hand.

Correlations between relative weight as a measure of obesity and metabolic data

A significant positive correlation was found between relative weight and 5 minutes PHLPL activity in the 12 obese patients before diet therapy ($p < 0.05$, Fig. 4). This correlation was found once more in the 8 patients retested after weight loss ($p < 0.05$), Fig. 5). No significant correlation could be found between relative weight and any of the other metabolic parameters measured.

Discussion

Before discussing the results obtained one should point out that when retested after weight loss our patients were still on diet therapy. Consequently, when looking at the metabolic effects of diet therapy we cannot dissociate between the effects of the diet itself and those of weight loss. As shown in Table 2 dietary history revealed the caloric intake of most of our obese patients to be normal. This is in agreement with findings of others (22, 23) and supports the idea that obesity is more a problem of energy expenditure than of energy intake.

The significant increase of plasma triglyceride levels found in our obese children is in agreement with the data of Aikawa M. (7), although, contrary to the findings of this last author, plasma cholesterol levels were found to be normal in our patients. The findings of normal fasting free fatty acid levels in our obese children is in agreement with litterature data (6).

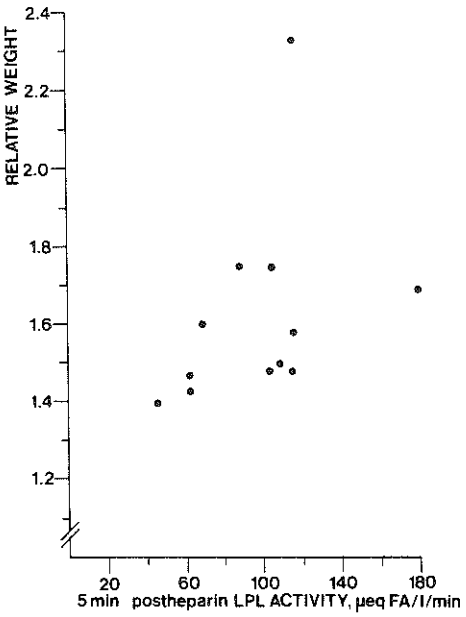

Fig. 4
Relationship between relative weight and plasma 5 min PHLPL activity in 12 obese patients before diet therapy

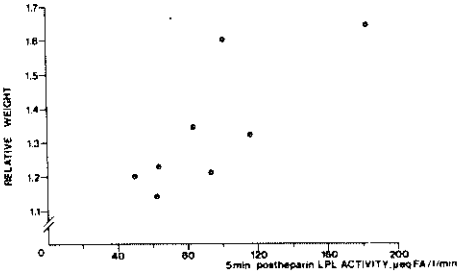

Fig. 5
Relationship between relative weight and plasma 5 min PHLPL activity in 8 obese patients after weight loss.

Plasma triglyceride removal (measured by the IVFTT) was significantly decreased in hypertriglyceridemic obese patients. Plasma triglyceride production rates were calculated (see methods) and appeared to

* increased in some

be * patients (3 out of 13). These results point to a defective removal mechanism as the main causative factor of hypertriglyceridemia in obese children, elevated triglyceride production rates playing a contributory role in some children. Hypertriglyceridemia associated with a decreased Intralipid removal constant has been reported in obese adults (14). Different authors using the radio-glycerol technique for measuring plasma triglyceride turnover reported divergent results. Some investigators found triglyceride turnover to be elevated in obese patients (9) while others reported no increase and pointed to a defective plasma triglyceride removal as the probable cause of hypertriglyceridemia in these patients (24). We found a significant negative correlation between plasma triglyceride levels and Intralipid elimination constants (Fig. 2). This most probably reflects the close dependency of triglyceride bloodlevels on removal efficiency in obese children.

We found increased 5 minutes plasma postheparin LPL activities in our obese children when compared to values obtained from 7 young healthy adults. Although such a comparison between the data of two different age groups can only be made with reservation, our results are supported by those of others who reported elevated plasma PHLPL activities in adult obese patients (25). This is not very surprising as it is known that fat cell volume is increased in obese children (26) and that fat cell LPL content is positively correlated with fat cell volume in normal man (27). Thus, an increased body fat mass could lead to a higher LPL content of adipose tissue. The significant positive correlation found between relative weight (as a measure of obesity) and 5 minutes postheparin LPL before (Fig. 4) and after weight loss (Fig. 5) seems to support this hypothesis.

The finding in our patients of high plasma PHLPL activities associated with low IVFTT values could be explained as follows. In normal man only 13% of circulating Intralipid is removed by adipose tissue (28). Consequently a normal to high Intralipid fractional removal in adipose tissue of our patients could be compatible with a low IVFTT value if other quantitatively more important removal sites were blocked. As in our patients plasma PHLPL activities were high and related to the degree of overweight (relative weight) we suggest that triglyceride removal in adipose tissue of obese children is increased above normal while removal at other extrahepatic sites is markedly decreased. This hypothesis is supported by the findings of Björntorp and Sjöström who showed that large fat cells in vitro are metabolically more active than small fat cells (29) and consequently that in obesity fat cell triglyceride turnover is increased. The striking decrease of plasma insulin levels during the second oral glucose tolerance test (Fig. 3) has been frequently reported in adult obese patients after weight loss (30, 9). While a low caloric, low carbohydrate diet could lower the insulin response to an oral glucose load, the same diet is known to result in glucose intolerance (31). Consequently, the persisting glucose intolerance we found, could be due to the fact that a preparatory diet, as advised by Conn (31), was not used in our patients before performing the second glucose tolerance test.

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Chapter III (3)

ENHANCEMENT OF FAT ELIMINATION DURING INTRAVENOUS FEEDING

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ABSTRACT. Forget, P. P. F. X., Fernandes, J. and Haverkamp Begemann, P. (Department of Paediatrics, Sophia Children's Hospital and Neonatal Unit, Erasmus University, Rotterdam, the Netherlands). Enhancement of fat utilization during prolonged intravenous feeding. Acta Paediatr Scand, 63:750, 1974.—An 8-year-old girl with severe underweight caused by anorexia nervosa was treated with total intravenous nutrition for 4 weeks. During this period the Intralipid dose was stepwise increased, the doses of Vamin and glucose were kept constant. The Intralipid dose was monitored by the determination of the serum Intralipid levels. The fat utilization was investigated by intravenous fat tolerance tests and the estimation of postheparin lipoprotein lipase activity of the plasma. The Intralipid elimination constant increased from 7% to 22%/min, the postheparin lipoprotein lipase activity increased from 50 to 317 μEq fatty acid/min/l. These data enabled us to increase the Intralipid dose from 3 g fat/kg/per day to 8 g fat/kg/per day, without an increase of the triglyceride blood levels. We may conclude that lipoprotein lipase is an inducible enzyme. It is not clear which component of the hypercaloric intravenous regime causes this induction.

KEY WORDS: Parenteral feeding, postheparin lipoprotein lipase, intravenous fat tolerance test

Little is known about the utilization and tolerance of fat emulsions in paediatric patients.

An Intralipid (Vitrum) dose of 4 g/kg body weight per day has been used by some authors with acute side effects (6) and by others without any toxic effects (1). In the former studies the daily dose was infused over a period of 6 hours, in the latter over a period of 24 hours. It is tempting to infer that acute toxicity arises whenever the dose/time ratio is too high leading to high Intralipid blood levels.

It has been shown by Hallberg (4) that fat tolerance tests with high dosage yield elimination curves, which are biphasic. At the point where one phase passes into the other, lipoprotein lipase (LPL), the enzyme responsible for triglyceride elimination, becomes saturated. An Intralipid dose which exceeds the maximal LPL clearing capacity is probably slowly eliminated by aspecific ways such as phagocytosis by RES cells and liver (6). The plasma Intralipid levels must therefore not exceed a critical concentration of approximately 100 mg/100 ml, when LPL becomes saturated.

The Intralipid blood level depends on the one hand on the patient's clearing capacity for fat and on the other hand on the Intralipid dose.

Recently we had the opportunity to study a patient, who had to be fed parenterally for a prolonged period. By determining this patient's clearing capacity at frequent intervals and by the daily investigation of Intra-

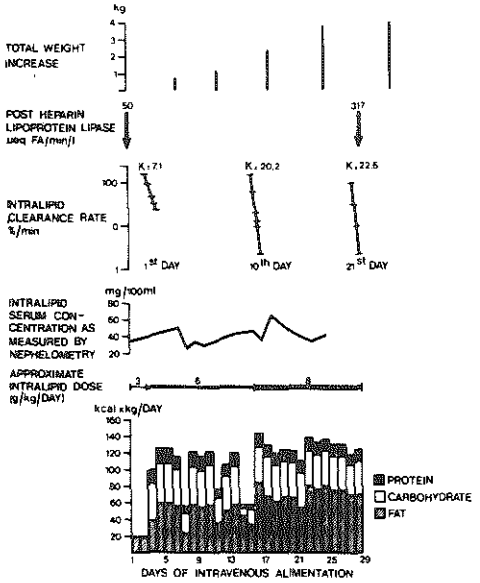

Fig. 1. Complete parenteral nutrition for 4 weeks in an 8-year-old child. The diagram shows the amount of fat (20% Intralipid), amino acids (Vamin) and glucose given. The changes in body weight, postheparin LPL, Intralipid clearance rate and Intralipid serum concentration are also recorded.

lipid blood levels, we tried to get some more insight in the mechanism of fat clearing during parenteral feeding.

MATERIAL AND METHODS

The patient was an 8-year-old girl with a long history of anorexia. Her height was just under, her weight far below, the third percentile. No evidence of organic disease was found. In close cooperation with our psychiatrists we decided to replace oral by parenteral feeding for 4 weeks.

We administered Intralipid 20% (Vitrum), Vamin (Vitrum) and glucose from separate bottles simultaneously, at a constant rate around the clock. Caloric intake was stepped up from 60 to 120 calories/kg/day (Fig. 1). Vitamins and minerals were given orally.

The intravenous fat tolerance test (IVFTT) was carried out according to Carlson & Rössner (2). For all tests a 20% Intralipid emulsion was administered intravenously in a dose of 0.1 g fat/kg body weight, within one minute. Capillary blood samples were collected every 5 minutes for 30 minutes. The Intralipid concentrations were determined by nephelometry. For each disappearance curve a minimum of four consecutive points was used. The logarithms of the Intralipid concentrations were plotted against time. Straight lines were obtained from which the correlation coefficients were calculated. Steady state plasma Intralipid concentrations were estimated by nephelometry as well.

The postheparin LPL activities were measured 5 minutes after the intravenous injection of 100 U of heparin/kg body weight. The measurement of postheparin LPL activity has been described previously (5).

RESULTS

The Intralipid blood levels remained relatively constant during the whole period of intravenous feeding. Initial value for IVFTT was 7.1%/min. End value was 22.6%/min (Fig. 1). Correlation coefficients of the IVFTT curves were higher than 0.99 for each curve. Initial value for postheparin LPL was 50 μEq FA/min/l, end value 317 μEq FA/min/l (Fig. 1).

The triglyceride and cholesterol blood levels were determined before and after the intravenous feeding period and at the maximum Intralipid infusion rate. The triglyceride levels were always within the normal range, though slightly higher during the infusion period as compared with the prae and post infusion levels.

The cholesterol levels were slightly lower during the infusion period as compared with the prae and post infusion levels. The patient did not develop acetonuria, acidosis, or coagulopathy and the liver function tests remained normal. The weight of the patient increased 4 kg in 4 weeks.

DISCUSSION

The concentration of Intralipid in the plasma at any given time during constant infusion represents a balance between the rate of entry into the plasma and the rate of removal. At a constant removal rate, the Intralipid blood levels depend on the rate of entry into the blood. The observation in our patient that the plasma levels remained rela-

tively constant when the infused fat dose was brought up from 3 g to 8 g/kg body weight/day has to be explained by a rising rate of removal. This has been confirmed in our patient by the increase in postheparin LPL activity parallel to the increased fat removal as measured by the IVFTT.

It is not clear which component of the intravenous feeding regimen caused this enzyme induction. Although no hormonal studies were performed in our patient hyperinsulinaemia has been observed during intravenous alimentation (3). Enhanced insulin production could be responsible for the anabolic response to parenteral feeding. Induction of LPL synthesis could be an aspect of this anabolic response.

As for our patient, the change of fat tolerance observed during parenteral feeding, would have made the use of a standard fat infusion dose quite arbitrary. By controlling lipid blood levels during the intravenous feeding period it was possible on the one hand to avoid hyperlipidaemia and on the other to take maximum benefit of the high caloric value of fat emulsions.

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Chapter III (4)

UTILIZATION OF FAT EMULSION DURING TOTAL PARENTERAL NUTRITION IN CHILDREN

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ABSTRACT. Forget, P., Fernandes, J. and Haverkamp Begemann, P. (Department of Paediatrics, Sophia Children's Hospital and Neonatal Unit, Erasmus University, Rotterdam, the Netherlands). Utilization of fat emulsion during total parenteral nutrition in children. Acta Paediatr Scand, 64: ■■■, 1975.—Tolerance for Intralipid fat emulsion during total parenteral nutrition (PN) was studied in 6 children. The Intralipid dose was monitored by the daily determination of plasma Intralipid levels. Fat removal was investigated at the start of and during the PN period by the intravenous fat tolerance test (IVFTT) and by determining the plasma postheparin lipoprotein lipase (LPL) activity. When the plasma Intralipid levels exceeded a value of 100 mg/100 ml, hyper pre-β lipoproteinaemia, hypertriglyceridaemia, hypercholesterolaemia and hyperphospholipidaemia appeared. During PN most patients showed marked increases of postheparin LPL. Return to normal values occurred after discontinuation of PN. Maximal LPL activities were found to correlate significantly with total daily caloric intake ($r=0.95$, $0.05<p<0.01$). The Intralipid elimination constant hardly changed during PN, with the exception of patient 6, who showed a marked increase (from 7 to 22 %). Conclusions of this study are as follows: First a high caloric intake during PN leads to a marked increase of postheparin LPL activity. Second, by monitoring plasma Intralipid levels at 100 mg/100 ml approximately, it is possible to adjust the Intralipid dose in order to prevent hyperlipaemia and to take maximal benefit from rising fat tolerance. Thirdly the IVFTT appeared to be of little value to estimate the child's fat elimination capacity.

KEY WORDS: Parenteral nutrition, hyperlipaemia, postheparin lipoprotein lipase, intravenous fat tolerance test

During the last few years, total parenteral nutrition has been used on a wide scale in paediatric patients.

The more generalised use of fat emulsions in intravenous feeding programs (1, 12) has made it possible to infuse solutions through peripheral scalp veins for prolonged periods. The use of small peripheral veins instead of a "central line" via a catheter located into a major calibre vein has well-known advantages. Phlebitis can early be detected and the risk for catheter-related complications can be avoided. Apart from the practical advantage of including fat emulsions in parenteral nutrition there are also theoretical reasons to justify its use.

1. The presence of fat in the intravenous fluid prevents the development of an essential fatty acid deficiency which otherwise develops rapidly during PN (16).

2. It facilitates hypercaloric feeding because of the high calorie/volume ratio of fat emulsions.

3. It precludes infusion of unphysiologically high amounts of carbohydrates and aminoacids, otherwise needed to maintain a calorically adequate regimen.

Both the advantages mentioned and the very low incidence of clinical side effects associated with the use of Intralipid (Vitrum) fat emulsion in adult patients (11) make its use in children

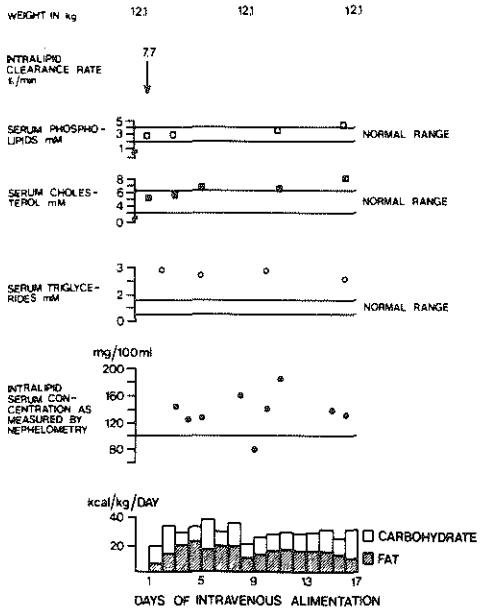

Fig. 1. Total intravenous alimentation of a girl of 2 years with encephalitis. Aminoacids were not administered because it was necessary to restrict the volume intake.

attractive. However, higher caloric needs expressed on a weight basis in children could lead to the use of a higher fat dose/kg body weight than in adults. Are these high doses devoid of clinical side effects? Is it possible to determine the fat tolerance of an individual child in order to adjust the quantity to be infused to the quantity he can tolerate. Little is known about the utilization and tolerance of fat emulsions in paediatric patients. The same Intralipid dose per kg body weight per day has been used by some authors with acute side effects (20) and by others without toxic effects (1). As we have stated earlier (7) toxicity seems to be associated with high Intralipid blood levels. High blood levels are found when the capacity of the enzyme lipoprotein lipase for fat elimination is exceeded. This happens when the Intralipid blood level exceeds a concentration of 100 to 150 mg/100 ml (10).

At this point when the enzyme (LPL) is saturated with its substrate (Intralipid), excessive fat doses are no longer eliminated by the physiological enzymatic removal mechanism but by aspecific uptake in RES and liver (20).

In our preliminary study (7) in one patient we found that LPL is an inducible enzyme. The present paper reports the study of six patients, the first one included. Elimination capacity for fat was frequently checked during the PN period. Intralipid blood levels were checked daily and the Intralipid dose adjusted in order to obtain an Intralipid blood level of 100 to 150 mg/100 ml (LPL saturation level). Frequent controls of serum triglycerides, cholesterol concentrations, and of liver function tests were made. Free fatty acids and ketone bodies serum levels were followed in 2 patients.

MATERIAL AND METHODS

Patients and infusion method

Six patients were studied. We administered Intralipid 20% (Vitrum), Vamin (Vitrum), and glucose from separate bottles simultaneously, at a constant rate around the clock.

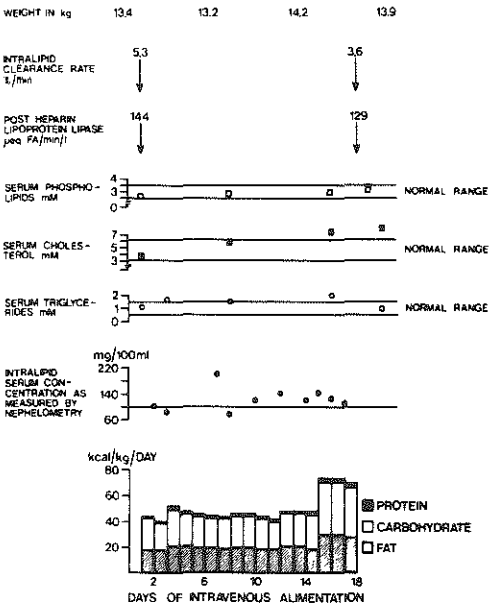

Fig. 2. Total intravenous alimentation of a girl of 4 years with cerebral trauma.

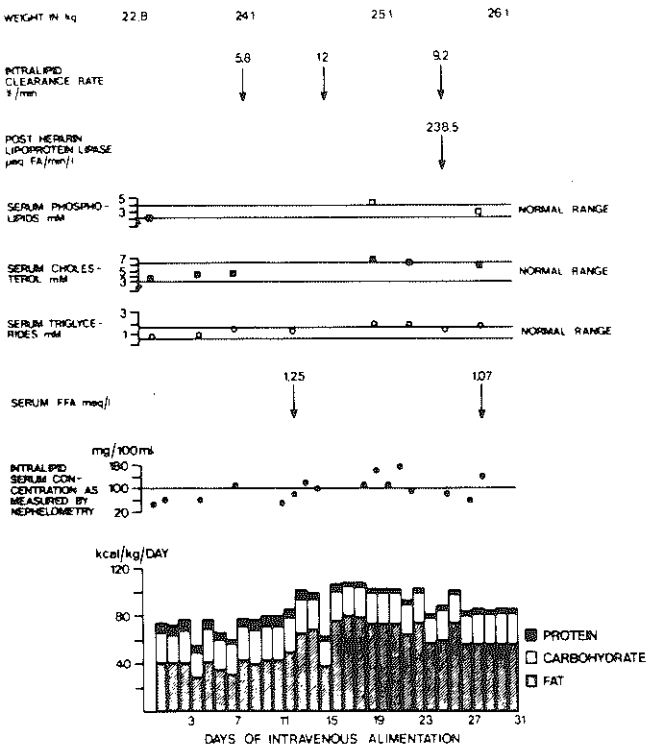

Fig. 3. Total intravenous alimentation of a boy of 8 years with encephalitis.

Vitamins and minerals were added in adequate amounts (12). All solutions were infused in a peripheral vein of the child.

Laboratory methods

The intravenous fat tolerance test (IVFTT) was carried out as described by Carlson & Rössner (3). For all tests a 20% Intralipid emulsion was administered intravenously in a dose of 0.1 g fat/kg body weight, within one minute. Capillary blood samples were collected every 5 minutes for 30 minutes. The Intralipid concentrations were determined by nephelometry. For each disappearance curve a minimum of four consecutive points was used. The logarithms of the Intralipid concentrations were plotted against time. Straight lines were obtained from which the correlation coefficients were calculated. Steady state plasma Intralipid concentrations were estimated by nephelometry. These levels were slightly overestimated, no allowance being made for the fasting plasma nephelometric value which was unknown during the infusion of fat emulsion.

The postheparin LPL activities were measured 5 minutes after the intravenous injection of 100 U heparin/kg body weight. LPL activities were measured according to the method of Kelly (13) with minor modifications (8).

Serum triglycerides concentrations were determined *ad modum* Schmidt & von Dahl (22). Serum cholesterol concentrations were determined *ad modum* Carr & Drekter (4). Serum phospholipids concentrations were determined *ad modum* Zilversmit & Davis (23). Lipoprotein electrophoresis was performed *ad modum* Postma & Stroes (17). For the determination of serum free fatty acids we used the extraction method described by Royer (21) followed by titrimetric measurement according to Korovina (14). Serum ketone bodies were only qualitatively tested (Ketostix, Ames).

RESULTS

The results of PN in patients 1–6 are shown in the corresponding Figs. 1–6. Other data, not shown in the figures, are as follows.

Liver function tests, performed weekly, remained normal in all patients. Coagulopathy did not develop in any patient. Qualitative examinations for the presence of ketone bodies in the blood were frequently performed in most patients, with negative results, irrespective the levels of blood lipids. In patients 1 and 2 hyperlipaemia occurred, associated with the

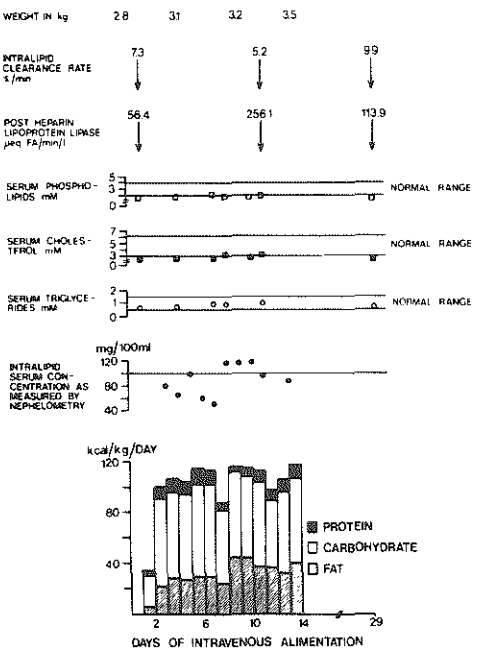

Fig. 4. Total intravenous alimentation of a female infant of 22 days with intractable diarrhea.

appearance of an increased pre-β lipoprotein band on electropherograms. In patient 3 hyper pre-β lipoproteinaemia appeared only on the 20th and 21st day of the PN period. In patients 5 and 6, hyperlipaemia did not occur, even during high Intralipid doses. In both patients 5 and 40 min postheparin LPL activities were measured; the 40 min values were equal to or lower than the 5 min values.

DISCUSSION

It can be seen from the results shown above that individual children widely differed in their tolerance for Intralipid. During PN some patients (1 and 2) developed hyperlipaemia while receiving low quantities of fat whereas other patients (3, 4, 5, and 6) tolerated very high fat doses and thus seemed to be very resistant to the development of hyperlipaemia. Is it possible to predict, and thus avoid the development of hyperlipaemia using as parameters either the postheparin LPL activity, the IVFTT, or the Intralipid blood levels?

The relation between fat tolerance and the postheparin LPL activity should be discussed first. The postheparin LPL activity is of mixed origin as LPL has been found in extracts of many tissues such as adipose tissue, muscle, heart, spleen, lung, kidney medulla and lactating mammary gland (19). Moreover, a significant fraction of postheparin LPL seems to originate from the liver (15). The functional significance of postheparin LPL activity is therefore not exactly known. It does, however, probably give an overall indication of the organism's capacity to eliminate triglyceride from the blood.

Patient 1 in whom LPL was not determined, and patient 2 in whom no rise of LPL was found, tolerated only very small fat doses. Patients 3, 4, 5, and 6 showed very high postheparin LPL activities during PN and tolerated very high fat doses. Why did the postheparin LPL rise in some patients and not in others? To answer this question we looked for a correlation between the quantities of nutrients administered intravenously and maximal postheparin LPL activities. We first calculated the means of the daily quantities of fat, carbohydrate and protein given to the patients in the 3 days preceding the enzyme determinations. No correlation could be found between the intake of either fat, carbohydrate, protein or a combination of two of these nutrients on the one hand and postheparin LPL activity on the other hand. A good correlation coefficient ($r=0.95$, $0.05<p<0.01$) was found, however, between total caloric intake and postheparin LPL activity (Fig. 7). As it is known that LPL decreases with starvation, one could wonder whether the values at the start of the PN period could have been subnormal and have risen to normal during PN. This is not the case, since the initial values in our patients fall within the normal range found in our laboratory (82.8 ± 32.4, $n=4$)and secondly because high LPL values during PN in patients 4 and 5

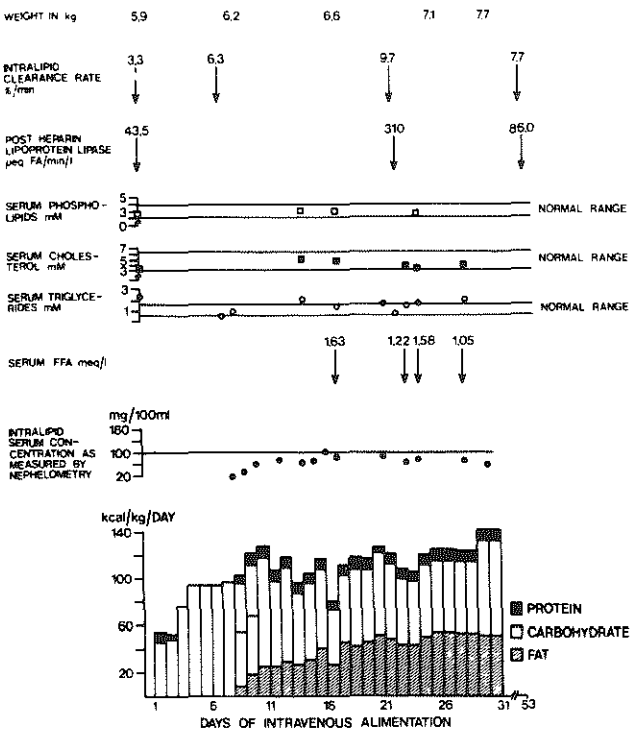

Fig. 5. Total intravenous alimentation of a girl of 13 months with intractable diarrhea.

decreased to the normal initial values after complete oral re-alimentation. A high caloric intake (probably accompanied by changes in insulin production) thus seems to induce synthesis of more enzyme. As no correlation could be found between fat intake and postheparin LPL, it seems that the increased enzyme synthesis does not result from a specific fat inducement but is part of a more generalised anabolic effect of high-caloric PN.

The second question to be discussed concerns the relation between fat tolerance and the outcome of the IVFTT. With the exception of patient 6 who showed a threefold rise of fat elimination as measured by the IVFTT, all patients, while tolerating very different fat doses and some showing substantial rises in postheparin LPL activity, only showed slight deviations of IVFTT within the normal range for children (8.39 ± 1.66, $n = 10$). Thus, the IVFTT did not give a good evaluation of the organism's total fat removal capacity. One reason for this could lie in the fact that the fat dose used in the IVFTT was too low. By giving such a low dose one probably only makes use of the small, directly available fraction of the total LPL pool that might be located on capillary endothelium "receptors" as suggested by Robinson & Wing (18). This small fraction probably does not rise during PN. Support for the supposition that the IVFTT did not rise because of the low fat dose used in the test may be found in a recent study of Intralipid elimination in preterm and small-for-date babies (9). With a low fat dose (such as we used) Intralipid elimination curves were not significantly different in the two groups of patients. When a higher dose (0.5 g/kg body weight) was used,

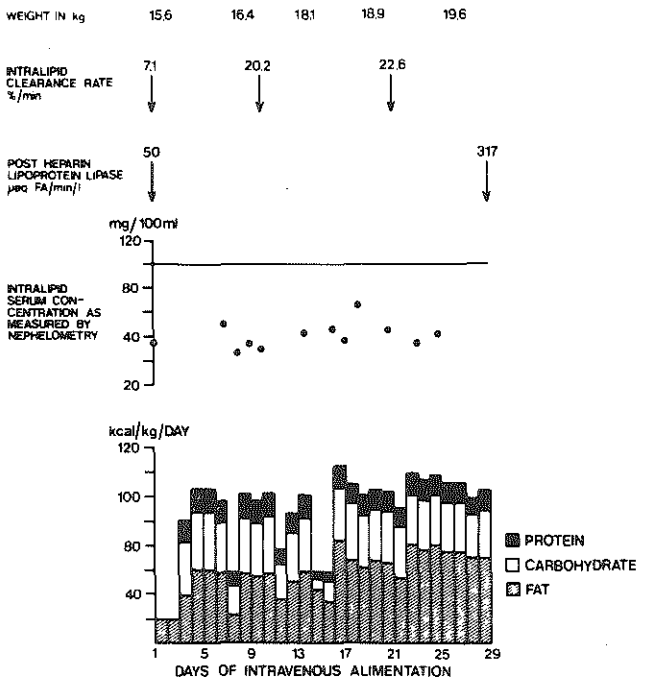

Fig. 6. Total intravenous alimentation of a girl of 8 years with anorexia nervosa.

small-for-date babies showed a decreased fat tolerance, as compared with the preterm infants.

The last question to be discussed concerns the relation between fat tolerance and the Intralipid blood levels. In our patients, hyperlipaemia developed whenever Intralipid blood levels exceeded a value of about 100 mg/100 ml. This level corresponds with the LPL saturation level as described by Hallberg (10). It is noteworthy that the hyperlipaemia which occasionally developed was not limited to triglycerides but involved cholesterol and phospholipids, too. At the same time, hyper pre-β lipoproteinaemia could be demonstrated on electropherograms. This can be explained by our hypothetical model of fat elimination during PN.

Fat elimination, when no saturation of the LPL system occurs, is depicted in Fig. 8. Circulating Intralipid can be eliminated in two different ways. First, by the well-known physiological removal mechanism involving the LPL enzyme system probably located in the capillary endothelium of peripheral tissues (19). This enzyme splits triglyceride molecules into glycerol and free fatty acids. Most of the fatty acids that are liberated are deposited in adipose tissue after local resynthesis into triglycerides. The rest remains in the blood stream, is taken up by the liver and secreted

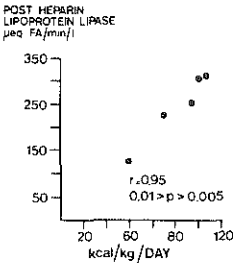

Fig. 7. Correlation between the (highest) 5 minutes' post-heparin LPL activity and total caloric intake of 5 patients during PN.

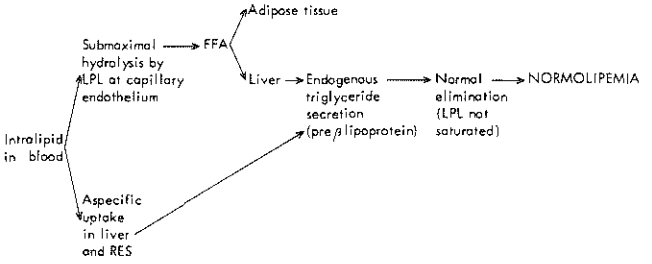

Fig. 8. Hypothetical model for Intralipid metabolism during PN when LPL is not saturated.

back into the blood as pre-β lipoprotein. LPL being unsaturated, the latter is normally eliminated and no hyperlipaemia ensues. The second elimination mechanism involves direct aspecific uptake of fat in RES (5) and liver (20). Direct liver uptake probably plays only a minor physiological role, as it has been demonstrated that there is very little net uptake of intact chylomicrons by the liver in rats (6). Here again fat is transformed by the liver into pre-β lipoprotein, which is secreted into the blood and normally eliminated because LPL is unsaturated.

Fat elimination when saturation of LPL occurs is depicted in Fig. 9. Surplus fat exceeding the LPL-related removal mechanism is slowly eliminated by the second aspecific mechanism for fat removal. Endogenous triglyceride secreted by the liver cannot be efficiently eliminated LPL being saturated. Hyper pre-β lipoproteinaemia develops accompanied by a rise of its three lipid components, viz. triglycerides, cholesterol and phospholipids. This model assumes the existence of a common saturable triglyceride removal mechanism for both exogenous and endogenous triglycerides. Evidence for this may be found in a recent study of hyperlipaemic patients (2). The presence of high serum free fatty acid levels in patients 3 and 5 is in agreement with the hypothetical model. It should be pointed out that ketosis did not occur. The presence of elevated serum free fatty acid levels unaccompanied by ketosis can be ascribed to the fact that glucose was infused simultaneously.

The following conclusions may be drawn from this study of Intralipid utilization in children. First, by controlling Intralipid blood levels daily and keeping these below 100 mg/100 ml during total PN one can adjust the Intralipid dose to the individual child's tolerance and thus take maximum advantage of the

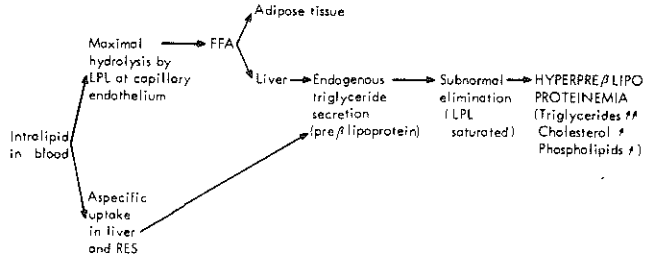

Fig. 9. Hypothetical model for Intralipid metabolism during PN when LPL is saturated.

high caloric value of fat emulsions without the risk of inducing hyperlipaemia. Second, as caloric restriction seems to be incompatible with a normal-to-high fat elimination capacity, one should be very cautious in the use of fat emulsions in children who for whatever reason receive a limited caloric intake during PN.

As a general rule, it seems advisable to start PN with a low daily fat dose and relatively high carbohydrate and protein intake. Then, Intralipid blood levels being kept below 100 mg/100 ml by daily control, fat doses can be raised progressively. Subsequently, the majority of patients will develop an increasing postheparin LPL activity coupled with an increased tolerance for fat, till, in the end, they tolerate daily fat doses of 4–6 g/kg body weight.

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Addendum I

The relative triglyceride production rate of patients with glycogen storage disease

After publication of our manuscript entitled 'Triglyceride clearing in glycogen storage disease' more work on the IVFTT seemed to justify its use for the calculation of relative triglyceride production rate. These calculations have been performed retrospectively in the same way as set forth in chapter 2. They are presented and discussed in this addendum. Triglyceride production rate appears to be elevated in most patients with GSD when compared to values of control subjects (Fig. 1). The highest values were found in patients with a glucose-6-phosphatase deficiency.

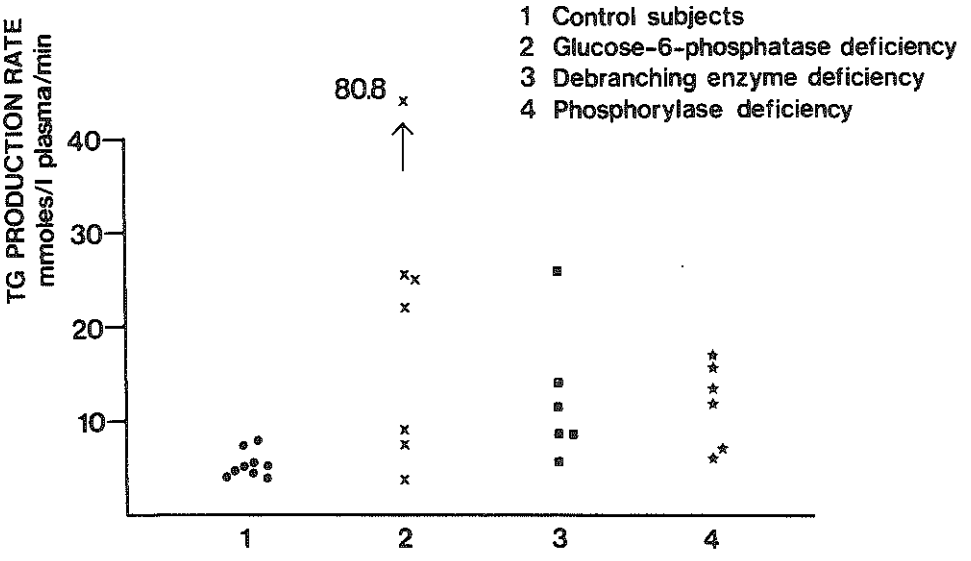


Fig. 1
Triglyceride production rate in children with GSD and in control subject.

When the results of triglyceride production and triglyceride removal of patients with GSD are put together the following can be concluded. The hypertriglyceridemia of patients with a deficiency of glucose-6-phosphatase or a debranching enzyme can be ascribed to both an increased production (Fig. 1) and a decreased fractional removal of plasma tri-

glycerides (see paper 1). The hypertriglyceridemia of patients with a deficiency of the phosphorylase system is mainly due to increased plasma triglyceride production. Considering these results and the fact that a diet rich in polyunsaturated fat is known to improve triglyceride removal (Nestel and Barter, 1973) and increase plasma PHLPL activity (Cybulska et al., 1974), the effects of different diets on plasma lipids in children with GSD can be defined as follows: A high-carbohydrate low-fat diet in patients with a glucose-6-phosphatase deficiency can be expected to lower the triglyceride production rate (by decreasing the enhanced fasting liponeogenesis and by lowering lipolysis) and lower TG removal concomitantly. The reverse holds for a high-fat low-carbohydrate diet. The resulting plasma TG concentration depends on which of these two regulating processes is most affected. As these children do better on a high-carbohydrate diet it can be presumed that in these patients increased TG production plays the predominant role in the development of hypertriglyceridemia.

In patients with a debranching deficiency or a phosphorylase deficiency, a high-fat diet will lower TG production (by decreasing enhanced postprandial liponeogenesis) and increase TG removal. The reverse occurs on a high-carbohydrate low-fat diet. The synergistic effect of diet therapy on both processes regulating plasma TG levels in the latter two groups of children explains why a low-carbohydrate high-polyunsaturated-fat diet influences favourably their plasma lipid abnormalities.

Addendum II

Plasma postheparin lipoprotein lipase activity during total parenteral nutrition before and after introduction of Intralipid

In paper 4 we showed that plasma PHLPL activity rose during total PN in some patients, whereas in other patients it did not. The following investigations were performed to study more closely the mechanisms involved.

The patient was an 11 year old boy suffering from anorexia nervosa who was treated by total parenteral hypercaloric nutrition for a few weeks. During the first 9 days of PN no fat was infused. From the 10th day onwards Intralipid was introduced in the intravenous regime. The daily dose was increased stepwise as described in paper 4. Plasma PHLPL

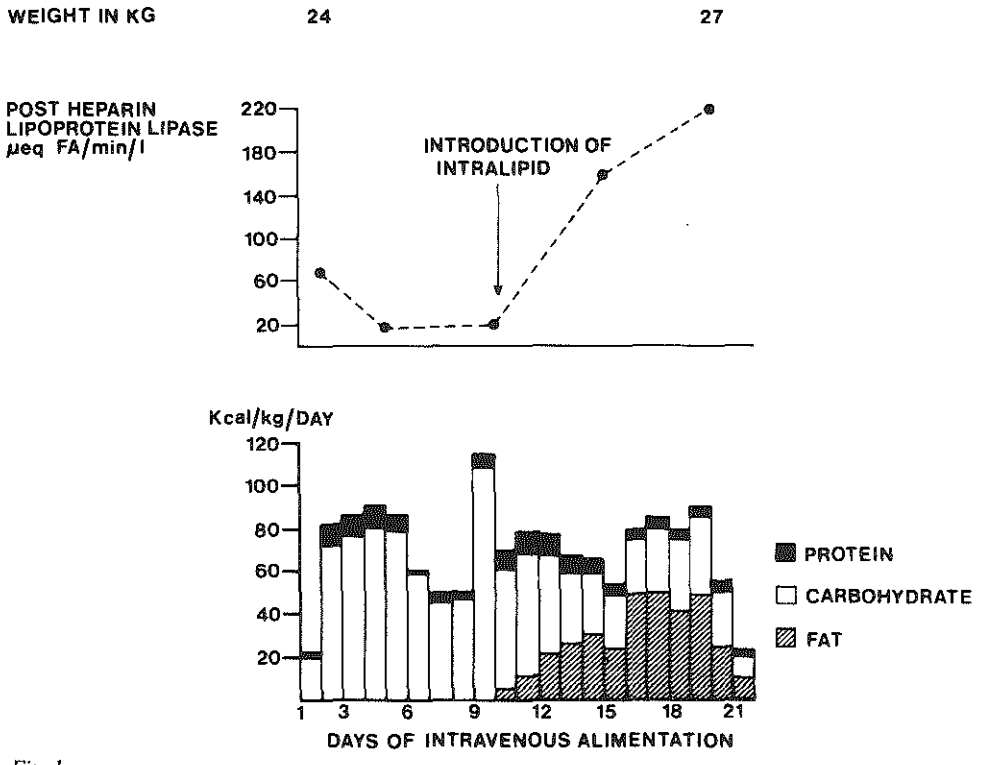


Fig. 1
Total intravenous alimentation of a boy of 11 years with anorexia nervosa.

activity was measured several times during the whole period. As we suggested that the insignificant rise of the IVFTT value during total PN could be due to the low Intralipid test dose used (paper 4), the IVFTT was modified and a higher fat dose of 0.3 g/kg body weight was administered. Serum triglyceride levels were followed for 2 hours after the fat emulsion had been injected.

Plasma PHLPL activity decreased to abnormally low values while the patient received fat-less high-carbohydrate hypercaloric intravenous nutrition (Fig. 1). The activity rose to high values after fat introduction (Fig. 1). Results of plasma triglyceride curves and lipoprotein electrophoresis before and on the 19th day of PN are shown in Fig. 2, 3, and 4 (Intralipid standard curve being non linear at high concentration nephelometry was not performed). The TG curve performed before PN (low PHLPL activity) showed high TG bloodlevels (Fig. 2) while lipoprotein electrophoresis which was normal at the start showed an increased pre-β band and a chylomicron (Intralipid) band 2 hours after fat injection (Fig. 3). The TG curve performed on the 19th day of PN was flat (Fig. 2). Lipoprotein electrophoresis at the start and 2 hours after fat injection were both normal (Fig. 4).
The dependence of PHLPL activity on fat intake has been documented earlier (Fredrickson et al., 1963). Mancini et al. (1973) further showed that a high-carbohydrate low-fat diet given to adults fed orally resulted in a significant decrease of PHLPL activity. In paper 4 we showed the dependence of PHLPL activity on caloric intake. Results obtained in the present investigation make it very likely that the PHLPL activity during total PN

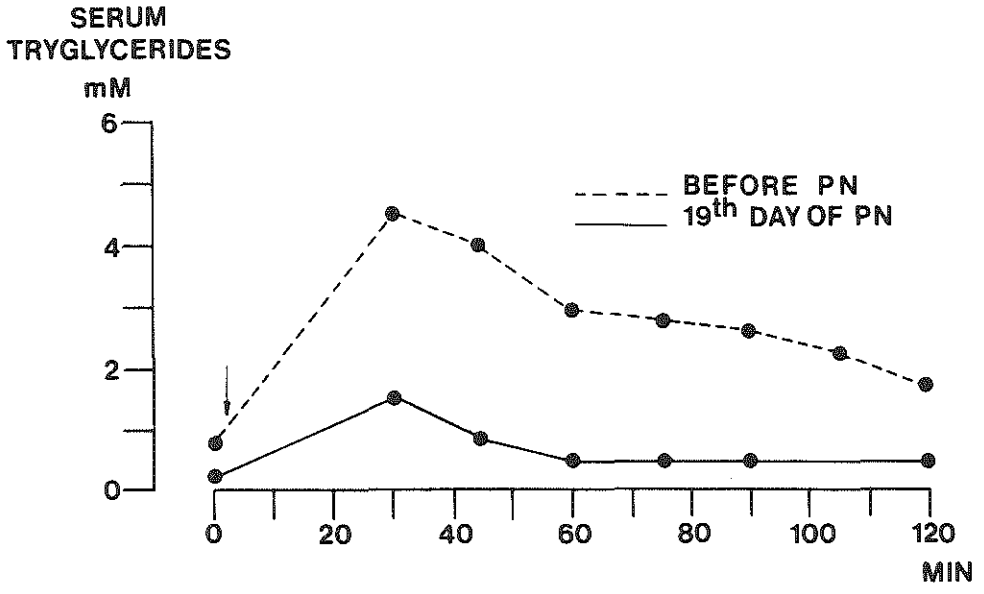


Fig. 2
Serum triglyceride curves obtained after the i.v. injection (arrow) of Intralipid (0.3 g/kg body weight).

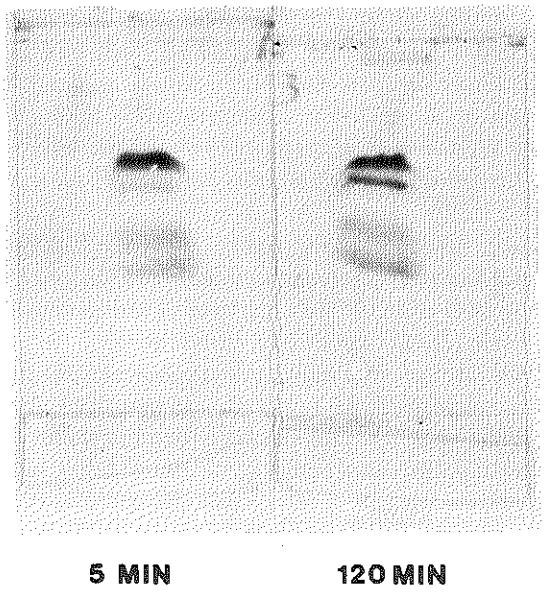


Fig. 3
Lipoprotein electrophoresis before (5 min) and 120 min after Intralipid injection (before total PN).

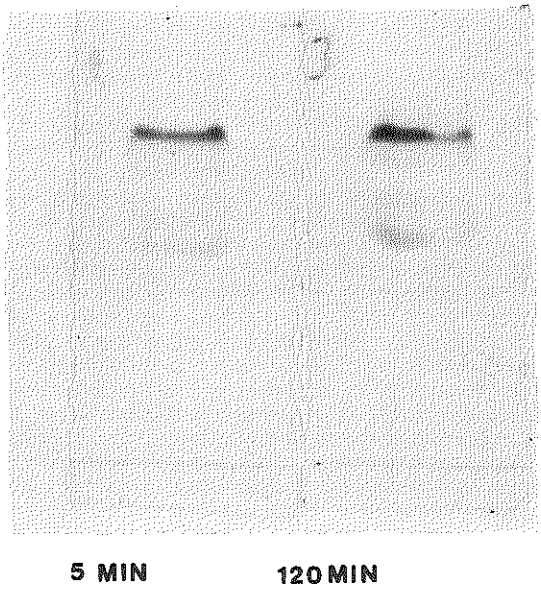


Fig. 4
Lipoprotein electrophoresis before (5 min) and 120 min after Intralipid injection (19th day of total PN).

depends on both caloric and fat intake. Results of fat tolerance tests show that high PHLPL activities are associated with a better fat removal. This is best illustrated in Figures 2, 3, and 4. Postheparin plasma contains lipolytic enzymes of hepatic and extra-hepatic origin (Krauss et al. 1973, Jansen et al., 1973) b. During total PN both fat removal and PHLPL activity increase. The following assays performed in plasma samples of this patient point to the extrahepatic tissues as main contributors to increased plasma PHLPL activity in this condition.

a. On the basis of immunological differences between hepatic and extra-hepatic PHLPL an antiserum against human hepatic lipase has been developed by Jansen (1973b). Using this antiserum it was found that 94% of the PHLPL increase of our patient's plasma during PN was of extrahepatic origin (Jansen and Forget, unpublished).

b In our patient's postheparin plasma the ratio of triacylglycerolhydrolase activity to palmitoyl-CoA hydrolase activity was calculated and found to increase after the introduction of fat in the PN regime. In the rat a high ratio has been found to reflect a high extrahepatic contribution to plasma postheparin lipolytic activity (Jansen and Hülsmann, 1974). Extrapolating from rat to man, it is likely that the extrahepatic tissues are the main contributors to the increased PHLPL activity during total PN.

Chapter IV

General discussion and speculation

In order to interpret the results as a whole, we will present in this chapter a hypothetical model of plasma triglyceride metabolism and show how our results fit into it. The model presented is based on two assumptions.

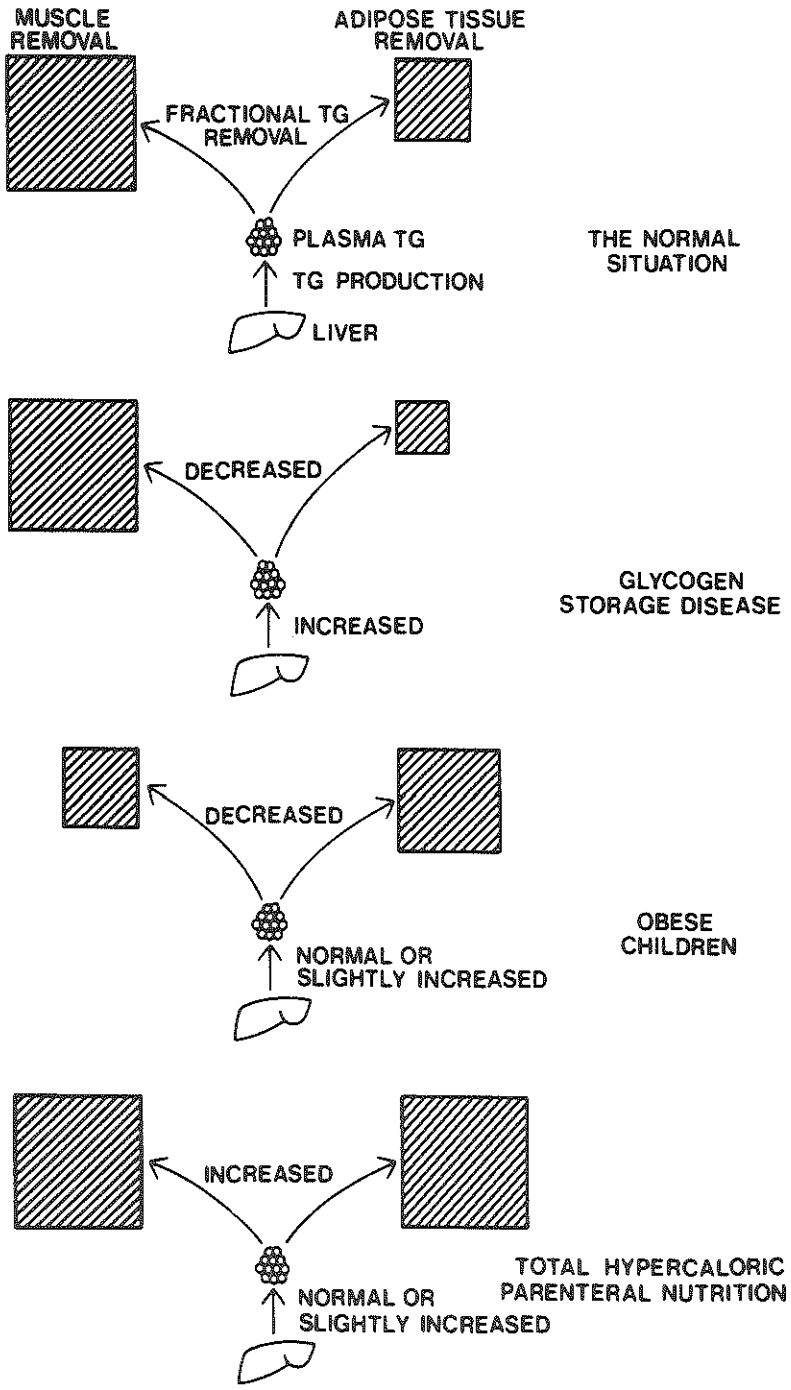
1 Muscular tissue plays the predominant role in plasma triglyceride removal, as has been found by Kaijser and Rössner (1974), who studied the 'extraction of exogenous triglycerides in human forearm muscle and subcutaneous tissue'.

2 Adipose tissue contributes much more than muscular tissue to plasma PHLPL activity. Although there is, at present, no firm evidence to prove this, the following data give some support to this hypothesis.
   a. Total starvation in obese patients has been shown to bring about a 50% decrease of PHLPL activity (Huttunen, 1974) and a decrease of adipose tissue LPL activity (Persson, 1970). Still, during total starvation of these obese patients Intralipid removal was found to increase slightly (Huttunen, 1974). This author suggested increase of triglyceride removal by muscle to compensate for decrease of triglyceride removal by adipose tissue. If in this situation muscle LPL activity is increased it is apparent that this did not prevent a decrease of total PHLPL activity during total starvation. Consequently, while muscle triglyceride removal probably compensates for decreased triglyceride removal by adipose tissue, it does not seem to compensate for decreased plasma PHLPL activity. This could mean that the quantitative contribution of muscle to plasma PHLPL activity is small.
   b In obese children we found a significant correlation between the degree of adiposity and plasma PHLPL activity. This again, suggests that adipose tissue is the main contributor to plasma PHLPL activity.

If these 2 assumptions are correct, the following conclusions can be drawn: while the IVFTT measures the fractional triglyceride removal as a whole, the plasma PHLPL activity mainly reflects that part of the removal process which takes place in adipose tissue. The quantitative importance of the latter would be less than that of muscular tissue according to Kaijser and Rössner, 1974.

Taking these two assumptions into account the following model for plasma triglyceride metabolism can be presented (see Figure). The line connecting the liver to the plasma TG pool represents TG production (intestinal production, being minimal in the fasting state,

MODEL OF PLASMA TRIGLYCERIDE METABOLISM



is not represented in the Figure). The greater 'muscle' area indicates the predominant function of muscle in plasma triglyceride removal. It should be pointed out that the muscle and adipose tissue areas shown in the Figure only reflect the fractional triglyceride removal of these tissues and do neither represent the masses nor the total TG removal of these respective tissues. It should be stressed that, assuming steady state plasma TG levels, TG production is equal to total TG removal. Consequently, a decreased fractional TG removal is compatible with a normal or increased total TG removal (not represented in the Figure) in situations where TG production is either normal or increased. How the results of our few investigations fit into this model is shown in the Figure.

The conclusions of our first study (paper 1) performed in patients with *glycogen storage disease* can be summarized as follows. In GSD, plasma TG production being increased, total plasma TG removal is increased accordingly (paper 1, and addendum 1). The low PHLPL activities found indicate, according to our second assumption, a low adipose tissue LPL content. Low plasma insulin concentrations, such as found in glucose-6-phosphatase-deficient children (Lockwood et al., 1969) could be responsible for this low adipose tissue LPL content as insulin is known to regulate adipose tissue LPL (Borensztajn, 1973). On the other hand, high plasma glucagon levels, as might be present in these children could increase muscle LPL (Borensztajn, 1973). A low adipose tissue, high muscle LPL content would be compatible with a low total TG removal in adipose tissue compensated by a high total TG removal in muscle.

Results of paper 2 performed in *obese children* can be summarized as follows. Triglyceride production has been found to be normal or (more rarely) increased. Triglyceride fractional removal in hypertriglyceridemic obese children was significantly decreased, plasma PHLPL activity was increased, and significantly correlated with the degree of obesity. This situation is depicted in the Figure. Triglyceride production is shown to be normal or slightly increased. Triglyceride fractional removal is shown to be increased in adipose tissue and markedly decreased in muscle. Consequently, the IVFTT value is decreased. The lack of correlation between the IVFTT values and plasma PHLPL activities in these children suggests that in this situation removal blocks at other sites (probably muscle) than adipose tissue most probably account for the decreased IVFTT values. The high plasma PHLPL acitivity probably reflects high adipose tissue LPL content (assumption 2). Consequently, triglyceride removal by adipose tissue is probably increased.

The following litterature data give some support to this hypothesis. Schade (1974) reported obesity to be characterized by an increased insulin and a decreased glucagon secretion. According to studies performed in the rat (Borensztajn, 1973), this could result in a high adipose tissue, low muscle LPL content. Similar findings, in obese children, could be responsible for a low muscle, high adipose tissue triglyceride removal.

Results of our last study performed in *total parenteral nutrition* (papers 3 and 4, addendum 2) showed that, during total parenteral nutrition with fat emulsion, plasma PHLPL activity rose as well as triglyceride removal capacity (higher tolerance) in those

children receiving a hypercaloric regime. This situation is depicted in the Figure. Triglyceride production is shown to be slightly increased.
This might result from the high plasma FFA found during total PN (see paper 4). Triglyceride fractional removal is shown to be normal in muscle and probably greatly increased in adipose tissue. Consequently, the fractional triglyceride removal is increased. We think this conclusion to be warranted in spite of the fact that we could not demonstrate a 'significant' rise of the IVFTT values in these children. The very high plasma PHLPL activities found in some patients are compatible with a high LPL content of adipose tissue (see assumption 2). This results in an increased triglyceride removal in adipose tissue

Summary

This thesis aimed at investigating some aspects of plasma triglyceride metabolism in children. In the *introduction* general aspects of plasma triglyceride metabolism are presented.
Chapter 1 reviews recent litterature data on the intravenous fat tolerance test and on plasma postheparin lipoprotein lipase activity (PHLPL) in different situations associated with abnormal plasma triglyceride concentration.
Chapter 2 defines the subjects of investigation. They are:
1 Plasma triglyceride metabolism in children with glycogen storage disease.
2 Plasma triglyceride metabolism in obese children.
3 The use of Intralipid fat emulsion during total parenteral nutrition. The methods used in these investigations are described. They are the intravenous fat tolerance test (IVFTT) and the measurement of plasma PHLPL activity. A method for calculating relative triglyceride production rate is also presented.

In *chapter 3* the following papers are presented.

1 Triglyceride clearing in glycogen storage disease

The results point to a relative inefficiency of plasma triglyceride removal in patients with glucose-6-phosphatase deficiency or debranching enzyme deficieny. Patients with a deficiency of the phosphorylase system showed normal results. After publication of this paper, more work on the IVFTT seemed to justify its use in the calculation of relative triglyceride production rates. These calculations have been performed retrospectively and are presented in an addendum (I). In short, triglyceride production rates were found to be elevated in all three groups of patients with glycogen storage disease investigated. Our conclusions are as follows:
The hypertriglyceridemia of patients with a deficiency of glucose-6-phosphatase or of debranching enzyme can be ascribed to both increased production and decreased removal of plasma triglycerides. The hypertriglyceridemia of patients with a deficiency of the phosphorylase system is due to increased plasma triglyceride production only.

2 Plasma triglyceride metabolism in obese children

In 13 obese children plasma triglyceride concentrations were found to be significantly increased above normal while plasma cholesterol concentrations were normal. In the hypertriglyceridemic obese children, the plasma triglyceride removal, measured by the intravenous fat tolerance test was significantly decreased. A few patients showed an increased triglyceride production. These abnormalities reverted to normal in all patients retested after weight loss. Plasma PHLPL activity was found to be increased and significantly related to the degree of obesity. As for carbohydrate metabolism a decreased glucose tolerance and hyperinsulinemia were found. Hyperinsulinemia reverted to normal during dietary restriction, glucose intolerance did not.
These results are further discussed.

3 Enhancement of fat elimination during intravenous feeding

It is shown in this paper that a rise in plasma PHLPL activity occurred during longterm parenteral nutrition with fat emulsion in a patient with anorexia nervosa. This rise was accompanied by a rising tolerance for fat emulsion and a rise of the IVFTT value. This preliminary work prompted further study of this problem in more patients.

4 Utilization of fat emulsion during total parenteral nutrition in children

The rise of plasma PHLPL activity accompanied by a rise in fat tolerance has been confirmed in most children investigated. The IVFTT value did not increase invariably, however. Interpretation of these results is presented. It is further shown that when Intralipid tolerance is exceeded hyperlipemia ensues. Tolerance is shown to be exceeded when a 'critical' Intralipid bloodlevel is surpassed. By controlling Intralipid bloodlevels during total parenteral nutrition it is possible to adapt the daily fat dose infused to an individual child's tolerance. Studies performed in another patient are presented in addendum II. It is shown that total PN without Intralipid resulted in a decrease of plasma PHLPL activity. Introduction of Intralipid induced a very significant rise of plasma PHLPL activity while fat tolerance increased simultaneously. Arguments are presented, which point to the extrahepatic tissues as site of origin of the augmented plasma PHLPL activity during total PN.

In *chapter 4* the results are discussed in a general context. A model of plasma triglyceride metabolism is presented. It is shown how the results obtained in our different investigations fit into this model.

Samenvatting

Enkele aspecten van de plasma triglyceride stofwisseling vormen het onderwerp van dit proefschrift. Algemene aspecten van de plasma triglyceride stofwisseling worden besproken in de *Introductie.*

Hoofdstuk 1 geeft een litteratuuroverzicht over de intraveneuze vettolerantie test (IVFTT) en de plasma postheparine lipase (PHLPL) activiteit in verschillende situaties die gekenmerkt zijn door verhoogde plasma triglyceride concentraties.

Hoofdstuk 2 beschrijft de verschillende onderdelen van deze studie.
Deze zijn:
1 Plasma triglyceride stofwisseling bij kinderen met glycogeenstapelingsziekte.
2 Plasma triglyceride stofwisseling bij kinderen met vetzucht.
3 Het gebruik van Intralipid vetemulsie gedurende volledige parenterale voeding. De gebruikte methoden zijn beschreven. Deze bestaan uit de IVFTT en het meten van de plasma PHLPL activiteit. Een methode voor het berekenen van de relatieve plasma triglyceride productie is beschreven.

Hoofdstuk 3 bevat de volgende publicaties:

1 Triglyceride clearance in glycogeen stapelingsziekte

De resultaten wijzen erop dat patiënten met een glucose-6-phosphatase of een debranching enzym deficiëntie een verlaagde plasma triglyceride eliminatie hebben. Normale resultaten werden verkregen bij patiënten met een deficiëntie van het phosphorylase systeem. Nadat deze studie gepubliceerd was, maakte recente literatuur over de intraveneuze vettolerantie test het mogelijk om deze laatste te gebruiken voor het berekenen van de relatieve triglyceride productie. Retrospectief zijn deze berekeningen gedaan, en weergegeven in addendum I. Samenvattend, werd er een verhoogde relatieve triglyceride productie gevonden in alle drie bestudeerde patiëntengroepen met glycogeen stapelingsziekte. Onze conclusies zijn als volgt: Hypertriglyceridemie bij patiënten met deficiëntie van glucose-6-phosphatase of van debranching enzym wordt veroorzaakt door zowel een verhoogde triglyceride productie als een vertraagde triglyceride eliminatie. Hypertriglyceridemie bij patiënten met een deficiëntie van het phosphorylase systeem wordt uitsluitend veroorzaakt door een verhoogde triglyceride productie.

2 Plasma triglyceride stofwisseling bij kinderen met vetzucht

Wij vonden bij 13 zeer dikke kinderen een significante verhoging van de plasma triglyceride concentraties. De plasma cholesterol concentraties waren normaal. Er werd een significante verlaging van de plasma triglyceride eliminatie (gemeten door middel van de IVFTT) gevonden bij dikke kinderen met hypertriglyceridemie. De triglyceride productie was slechts verhoogd bij enkele dikke kinderen. Deze afwijkingen normaliseerden na vermagering. De plasma PHLPL activiteit was verhoogd en bleek significant gecorreleerd met de mate van vetzucht. Wat betreft de koolhydraat stofwisseling, werden een verminderde glucose tolerantie en hyperinsulinemie gevonden bij dikke kinderen. Na vermagering werd de insuline spiegel weer normaal, de glucose intolerantie bleef echter onveranderd. Deze resultaten worden besproken.

3 Verhoging van de veteliminatie gedurende volledige intraveneuze voeding

Deze publicatie laat zien dat de PHLPL activiteit omhoog ging gedurende totale parenterale voeding van een patiënt lijdende aan anorexia nervosa. Deze verhoging ging gepaard met een verhoging van de tolerantie voor Intralipid vetemulsie en van de IVFTT constante. Deze preliminaire studie leidde tot verder onderzoek van dit phenomeen in andere patiënten.

4 Gebruik van vetemulsie bij kinderen gedurende volledige intraveneuze voeding

In de meeste kinderen steeg de plasma PHLPL activiteit en dit ging gepaard met een toenemende tolerantie voor vetemulsie. De verhoging van de IVFTT constante werd niet bij alle patiënten gevonden. Deze verschillen worden verder besproken en er wordt verder op gewezen, dat wanneer de Intralipid tolerantie werd overschreden er hyperlipemie ontstond. Deze Intralipid 'intolerantie' ontstond wanneer de Intralipid bloedspiegels hoger waren dan de zogenaamde kritische concentratie. Door het dagelijkse controleren van Intralipid bloedspiegels gedurende totale parenterale voeding is het mogelijk de dagelijks te infunderen vetdosis aan te passen aan de individuele tolerantie van het kind. Onderzoek bij een laatste patiënt is gepresenteerd in addendum II. Er wordt hierin gedemonstreerd dat parenterale voeding zonder Intralipid resulteerde in een daling van de plasma PHLPL aktiviteit. Completering van volledige intraveneuze voeding met Intralipid induceerde een zeer significante verhoging van de plasma PHLPL activiteit en een verhoging van de tolerantie voor Intralipid. Verder worden argumenten naar voren gebracht die de hypothese steunen dat de verhoogde PHLPL die gevonden werd tijdens totale parenterale voeding afkomstig is van de extra-hepatische weefsels.

De resultaten van deze verschillende onderzoekingen worden besproken in *hoofdstuk 4.* Modellen van de plasma triglyceride stofwisseling in de door ons onderzochte situaties worden weergegeven.

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Curriculum vitae

De promovendus werd geboren op 24 november 1943 te Thijsville. Het diploma klassieke Grieks-Latijnse humaniora behaalde hij in 1961 te Brussel (college St. Michel).
Hij studeerde geneeskunde te Namen en daarna te Brussel en legde daar het artsexamen af in 1968.
Hierna werd hij opgeleid tot kinderarts in de kinderafdeling van het St. Annadal Ziekenhuis te Maastricht en daarna in het Sophia Kinderziekenhuis te Rotterdam, alwaar dit proefschrift tot stand kwam.
Vanaf januari 1973 werkt hij als wetenschappelijk medewerker op het gebied van de kindergastroënterologie in het Sophia Kinderziekenhuis.

Verantwoording

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Prof. Dr. H.K.A. Visser, hoofd van de afdeling Kindergeneeskunde van het Sophia Kinderziekenhuis, waar dit proefschrift bewerkt werd.

Mevr. Dr. P. Haverkamp-Begemann heeft de lipoproteine lipase bepalingen verricht, haar adviezen waren van grote waarde.

De medische en paramedische staf van het Sophia Kinderziekenhuis hebben hun zeer gewaardeerde medewerking verleend.

De heer Strik heeft zijn expert statistische adviezen gegeven.

Mej. N. de Kort heeft de lipiden bepalingen verricht.

Mevr. M. Verbraaken is verantwoordelijk geweest voor het typen van dit proefschrift.

De audiovisuele dienst van het Sophia Kinderziekenhuis verzorgde alle grafiekwerk en tekeningen.

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