# SUBSTRATE OXIDATION AND PLASMA ELIMINATION OF A LONG-CHAIN TRIGLYCERIDE EMULSION

## - studies in healthy individuals and patients with trauma

Wiveca Åberg

Karolinska Institutet

Stockholm 2011

From the Department of Molecular Medicine and Surgery, Karolinska Institutet,

Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

Lipid emulsions based on long-chain triglycerides (LCTs) are frequently used during total parenteral nutrition (TPN) and are usually administrated together with glucose. The metabolism of fat is determined by the underlying condition such as trauma, infection, malnutrition or age and also by substrate interaction when different nutrients are administrated simultaneously. The aim of this thesis was to evaluate plasma elimination rate of a lipid emulsion during a hypertriglyceridaemic- (HTG) clamp and the utilization of lipids as an energy source. An additional aim was to characterise to what extent the metabolism of lipids is affected by trauma, age and administration of insulin and glucose provided as an IG clamp.

Plasma elimination rate of a lipid emulsion and its relationship with lipoprotein lipase (LPL) activity and LPL mass were investigated before and after surgery. Postoperatively, the plasma TG elimination rate was 2.6 times higher. Infusion of lipids in the postoperative state was followed by a smaller rise in free fatty acids (FFA) in comparison with the preoperative situation. The postoperative basal fasting LPL activity was half of that in the preoperative state. The LPL activity rose during the first hour of lipid infusion and then remained at that level to the end of the infusion. LPL activity values were not significantly different during the clamps. The heparin-induced rises in LPL activity and LPL mass were similar before and after surgery.

Age-related effects on the plasma elimination rate of a lipid emulsion and substrate oxidation were examined by comparing elderly and young men. Plasma TG elimination rate was similar in the elderly and young subjects. This was evident also from the similar increments in plasma FFA in the two groups. Elderly subjects had lower energy expenditure in the basal state than young individuals. The proportion of active LPL was about three times higher in the young compared to the elderly individuals. LPL activity increased during the infusion and the rise was larger in the young than in the elderly individuals.

The effect of glucose and insulin on the plasma elimination rate and oxidation of a lipid emulsion was examined by using indirect calorimetry (IC) in conjunction with HTG and insulin/glucose (IG) clamp techniques. Administration of insulin and glucose in healthy man did not influence the plasma elimination rate of a lipid emulsion. Insulin and glucose did not significantly decrease FFA concentration and total lipid oxidation in this metabolic situation with an abundant supply of both carbohydrates and lipids.

In conclusion, using the HTG clamp we have confirmed an increased capacity for lipid clearance after trauma despite a lower basal LPL activity and a virtually unchanged LPL pattern during infusion of lipids. Elderly men have a capacity to intravenously hydrolyse a high TG load administered as a HTG clamp, which is not quantitatively different from the capacity of young men. This was evident also from the similar increments in plasma FFA in elderly and young individuals. Furthermore, our results show that the plasma TG elimination rate is not influenced by moderate increments of plasma glucose and insulin levels.

LIST OF PUBLICATIONS

This thesis is based upon the following papers:

I. Influence of trauma on plasma elimination of exogenous fat and lipoprotein lipase activity and mass.
    *Thörne A, **Åberg W**, Carneheim C, Olivecrona T, Nordenström J*
    Clin.Nutr. 2005;24:66-74

II. Fat oxidation and plasma removal capacity of an intravenous fat emulsion in elderly and young men.
    ***Åberg W**, Thörne A, Olivecrona T, Nordenström J*
    Nutrition 2006;22:738-743.

III. Combined hypertriglyceridemic- and insulin- glucose clamps for the characterization of substrate oxidation and plasma elimination of a long chain triglyceride (LCT) emulsion in healthy men.
    ***Åberg W**, Thörne A, Alvestrand A, Nordenström J*
    Submitted to Metabolism.

"In all maladies, those who are well nourished do best"
Hippocrates (400BC)

CONTENTS

LIST OF ABBREVIATIONS

BMI	Body mass index
BMR	Basal metabolic rate
DIT	Diet-induced thermogenesis
EE	Energy expenditure
FFA	Free fatty acid
FO	Fish oil
GERD	Gastroesophageal reflux disease
GPO	L-alpha-glycerol-phosphate oxidase
HDL	High-density lipoprotein
HL	Hepatic lipase
HTG	Hypertriglyceridaemic clamp
IC	Indirect calorimetry
IG	Insulin-glucose clamp
IV	Intravenous
IVFTT	Intravenous fat tolerance test
LCT	Long-chain triglyceride
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MCT	Medium-chain triglyceride
REE	Resting energy expenditure
RQ	Respiratory quotient
SD	Standard deviation
SEM	Standard error of mean
STG	Structured triglyceride
TG	Triglyceride
TPN	Total parenteral nutrition
VLDL	Very low density lipoprotein

1 INTRODUCTION

1.1 HISTORICAL SURVEY

The routine use of parenteral lipid emulsions in clinical practice began in 1962 with the development of a soybean oilbased lipid emulsion (1, 2). Fat emulsions based on long-chain triglycerides (LCTs) are frequently used during total parenteral nutrition (TPN), and are usually administrated together with glucose. However, the history of this field goes back more than 380 years.

The first landmark was the description of the circulation of blood by William Harvey in 1628, his discovery lead to the rational for intravenous (IV) infusions and injections. In 1665 Sir Christopher Wren published his studies on IV infusions of wine, ale and opiates in dogs. He found that alcohol given intravenously had the same effect as if it were given orally. Another pioneer in this field was William Courten, who in 1712 infused olive oil in a dog which, unfortunately, died. Studies to infuse fat continued and in 1873 Edward Hodder tried to infuse milk into three cholera patients. Two of the patients recovered from the disease but the third patient did not survive despite the milk infusion. It was later found that such milk infusions caused several adverse reactions and the method was abandoned at the end of the 1800s (3, 4, 5).

The first major step towards TPN, however, was the observation by Robert Elman in the 1930s that amino acids could be administered safely to humans (4). In the 1960s two other important steps towards TPN were developed: the so called "lipid system", which made it possible to infuse glucose and lipids by peripheral veins, and the "glucose system" introduced by Stanley Dudrick. Dudrick showed that a catheter in a central vein could be used to simultaneously administer a concentrated glucose solution and amino acids. This made it possible to cover a patient's total energy requirements with glucose alone. Between 1920-1960 many lipid emulsions of varying composition were developed and tested and most of them caused adverse reactions when given to humans (6).

1.2 DEVELOPMENT OF SAFE LIPID EMULSIONS

It was in the 1960s when it first became technically possible to provide adequate nutrition by vein. Schubert and Wretlind were the first to report, in 1961, on the use of a safe IV lipid emulsion including oil (soybean oil) and emulsifier (egg yolk phospholipids), later called Intralipid (2). Soybean oil emulsions have been commercially available since 1962 and are the usual source of lipid in parenteral lipid emulsions (6, 7, 8). Intralipid contains long-chained essential fatty acids which cannot be synthesised in mammals: mainly linoleic acid 52% (belonging to the omega-6 family) and linolenic acid 8% (belonging to the omega-3 family). The ratio between them is 6:1 (6). Over the last few decades long-chain triglyceride (LCT) emulsions have been modified in order to reduce the high amount of omega-6 fatty acids, in particular linoleic acid. These considerations led to the development of other lipids (8).

Since 1984, a mixture of medium-chain triglyceride (MCT) and LCT emulsions containing 50% MCT have been in clinical use (8, 9). Mixed MCT/LCT emulsions have been reported to possess advantages over pure LTC preparations. They are more quickly eliminated from the circulation and taken up by peripheral tissues (10, 11).
The structured triglyceride (STG) emulsion (medium- and long-chain fatty acids on the same glycerol molecule) was designed to provide both simultaneous delivery of LCT and MCT and a slower more controlled release of the MCT into the bloodstream than the physical mixture of MCT/LCT (12, 13, 14). Recent advances in new lipid emulsions containing MCT and soybean, olive and fish oil in order to reduce the ratio between omega-3 and 6 are proving to be safe and well tolerated without any adverse effects (8, 15, 16, 17, 18).

1.3 PLASMA ELIMINATION OF EXOGENOUS LIPIDS

The intravascular metabolism of fat emulsions involves two major steps, i.e. the lipolysis of emulsion TG by the endothelial-bound lipoprotein lipase (LPL), and the uptake of fat particles by various organs (liver, muscle and adipose tissue) when fat is eliminated from the bloodstream (9, 19).

Over the years many techniques have been used to characterise the metabolism of intravenous fat emulsions. The first method used was the oral fat tolerance test. With

this method different kinds of fats were administered orally, but this was found to be disadvantageous because it was difficult to evaluate the time course for exogenous TG removal from the blood stream. The blood levels during such a test depend not only on the rate of disappearance of exogenous fat from the blood, but also on various absorption processes. In order to bypass these problems an intravenous fat tolerance test (IVFTT) was developed (20, 21). With this method the plasma elimination rate is measured after an intravenous injection of a single bolus of fat. Hallberg found that fasting as well as operative trauma were associated with an increase in the elimination rate of intravenous fat from plasma (20). It is assumed that lipid emulsions are catabolised by mechanisms similar to those for endogenous triglyceride-rich particles such as chylomicrons and very low density lipoproteins (VLDL) (22, 23). Clearance from plasma does not necessarily mean that the fat emulsion is utilised to meet energy requirements. IVFTT is of limited value in the study of the energetics of lipid metabolism as it provides no information regarding the oxidation of intravenous fat emulsion. Using radioactively labelled fatty acids in fat emulsion makes it possible to determine if the infusion is readily used as an energy substrate (24). IVFTT measures mainly particle size (21) and therefore comparisons between different types of fat emulsions may not be valid. Particle size is a factor that varies between different fat emulsions as well as differences in molecular weights due to differences in fatty acid composition. LCT emulsions with a larger particle size are cleared faster from the blood with relative less hydrolysis than those of a smaller size (25).

An alternative approach to compare the metabolism of different types of fat emulsions as well as in patients under a variety of clinical conditions is to use a hypertriglyceride (HTG) clamp technique (26, 27). The principal of the technique is the maintenance of plasma TG concentrations at a fixed level during a certain period of time by controlling the rate of infusion of a fat emulsion. The infusion rate of lipids during the steady state period may thus reflect the plasma elimination rate of exogenous fat. The HTG clamp method has been shown to offer the potential of a more precise characterisation of lipid disposal than IVFTT as the plasma clearance rate corresponds to the lipid infusion rate during steady state plasma TG levels (28). Comparison between IVFTT and HTG clamp techniques regarding the plasma elimination rate of exogenous fat showed no discernible relationship (29).

1.4 ENERGY EXPENDITURE AND SUBSTRATE OXIDATION

Knowledge of the metabolic and the thermogenic responses to nutrients is of clinical importance, e.g. for our understanding of nutritional requirements in patients with different diseases. Prescriptions are often based on weight, height (or derivatives of these) and on requirements related to a patient´s specific disease. Indirect calorimetry (IC) is a method which allows the non-invasive measurements of energy expenditure (EE). The calculation of an individual's predicted basal EE is based on sex, height, weight and age. By measuring oxygen consumption (VO_2), carbon dioxide production (VCO_2) and urinary excretion (N) it is possible to calculate overall substrate oxidation. The ratio between VO_2/VCO_2 referred to as the respiratory quotient (RQ) is different for each substrate. The complete oxidation of glucose gives an RQ value of 1.00 and 0.70 for lipids and 0.81 for protein. After an overnight fast an individual is expected to oxidise mainly lipids and to display an RQ close to 0.80. EE is defined as the energy expenditure obtained at rest in a lying position. EE also includes components due to physical or psychological stress, and variation in ambient or body temperature (30). Measurements of EE are especially useful in situations where its estimation is difficult, e.g. in critically ill and elderly patients (31). Changes in lipid oxidation usually occur in parallel with changes in plasma FFA levels and on tissue TG (32).

EE progressively decreases with age in healthy people (33), in part because of changes in body composition, fat mass increase and lean mass decrease in the elderly. Fat mass is less metabolically active than lean mass (34, 35, 36). Other causes could be a reduction in total caloric intake, (37), changes in lifestyle factors, and less physical activity (33). In elderly hospitalised patients one can assume that EE is elevated due to energy demanding processes such as inflammation, drugs, and fever that are provoked by the patient's disease. Earlier studies in sick elderly people have shown that EE does not increase, which can be due to reduced physical activity during disease and hospitalisation. When adjusted for differences in both body weight and fat-free mass, EE has been found to be similar in both healthy and sick elderly people (38). Although, women have a lower basal metabolism than men, which may due to that women generally have a higher percentage fat and less muscle mass than men. Hormone levels as well as fever affect the basal metabolism. EE decreases at starvation due to reduced body weight and loss of muscle mass

2 AIMS

The aim of this project was to evaluate the plasma elimination capacity and oxidation rates of a lipid emulsion (LCT) by using the HTG clamp technique and IC. We wished to identify to what extent the lipid metabolism is affected by trauma, age and glucose and insulin administration.

Specifically, the aims were:

- To examine the influence of a surgical trauma on the plasma elimination rate of exogenous triglycerides (TG) and its relationship with LPL activity and LPL mass.

- To examine age-related effects on the plasma elimination rate and substrate oxidation of a lipid emulsion.

- To examine the possible influence of glucose and insulin administration on the elimination rate and oxidation of a lipid emulsion.

3 SUBJECTS AND PROCEDURES

3.1 PATIENTS AND HEALTHY INDIVIDUALS

All studies were carried out in accordance with the Helsinki declaration and approved by the local ethical committee. Patients and healthy individuals were informed about the purpose and nature of the studies and written informed consent was obtained before entering them. All studies (I-III) were performed after an overnight (12 h) fast and the subjects arrived at the laboratory at 8 am. Prior to each study, all volunteers ingested their habitual diet containing at least 150 g carbohydrates per day, and in the postoperative state in study I the patients were given 125 g glucose intravenously as 5% glucose solution (2500 ml per day).

Nine patients (five men and four women) undergoing open abdominal surgery either operated by open procedures with semifundoplication/fundoplication due to gastroesophageal reflux disease – GERD – or with sigmoid/anterior resection due to adenoma/carcinoma in the colorectal region participated in study I (Table 1). All patients were weight stable and not on medication, except for GERD patients who were taking omeprazole. This treatment was withheld on the day of the studies. None of the patients had diabetes mellitus, lung or thyroid dysfunction or any other metabolic disorder.

Patients no.	Sex (M/F)	Age (yrs)	Weight (kg)	BMI (kg/m^2)	Diagnosis	Operation
1	F	74	66	24	Colon carcinoma (Dukes´ B)	Sigmoidresection
2	M	59	83	25	Rectum adenoma	Anterior resection
3	F	80	65	21	Colon carcinoma (Dukes´ B)	Sigmoid resection
4	M	44	84	27	Gastroesophageal reflux disease	Semifundoplication
5	M	50	74	24	Gastroesophageal reflux disease	Semifundoplication
6	F	58	74	27	Gastroesophageal reflux disease	Fundoplication
7	F	35	65	22	Rectum adenoma	Anterior resection
8	M	58	64	22	Colon carcinoma (Dukes´ B)	Sigmoid resection
9	M	63	70	22	Rectum carcinoma (Dukes´ B)	Anterior resection

Table 1 (study I). Demographic and clinical data

Ten elderly men, average age 73 years (range 70-78 years, BMI 21-27 kg/m^2), and 10 young men, average age 30 years (range 19-45 years, BMI 19-26 kg/m^2), were included in study II. Ten young men, average age 30 years (age 24-44 years, BMI 22-27 kg/m^2),

participated in study III. All volunteers responded to a health questionnaire and were found to be healthy, non-smokers, and with stable weight. None had diabetes mellitus, lung or thyroid dysfunctions or any other metabolic disorders and none were taking any medication. All the individuals were found to have normal blood chemistry, fasting blood glucose, liver function, lipids, electrolytes and creatinine.

The studies were performed between March 1 1991 and March 15 1992 (study I, Dnr:21/91); June 19 1995 and February 12 1996 (study II, Dnr: 146/95); December 21 1992 and May 27 1993 (study III, Dnr: 195/91). All three studies were performed at Karolinska University Hospital-Huddinge.

3.2 STUDY DESIGNS

3.2.1 Study I

In order to evaluate the plasma elimination rate of exogenous fat and a possible relationship with LPL activity and LPL mass in patients before and after a moderate surgical trauma, the elimination rate of exogenous fat was measured by a HTG clamp technique 24 h before and 48 h after operation (Fig. 1). The lipolytic capacity was determined as the change, i.e. rises in LPL activity and LPL mass, following a bolus dose of 100IU x kg BW^{-1} heparin sodium (Heparin ®, Fresenius Kabi, Sweden). The test was performed –4 days before and +3 days after surgery.
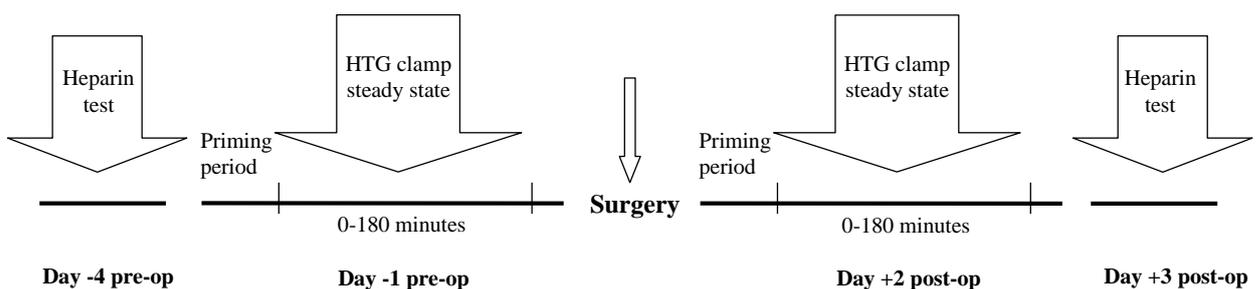

Figure 1 (study I). Nine patients underwent the heparin test and HTG clamp on two occasions: before and after surgery.

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3.2.2 Study II

This study evaluated the metabolic and thermogenic responses to nutrients in relation to age. Intravenous lipid elimination capacity as measured by HTG clamp with simultaneous determination of the potentially rate-limiting enzyme LPL, and substrate oxidation by IC was performed (Fig. 2).
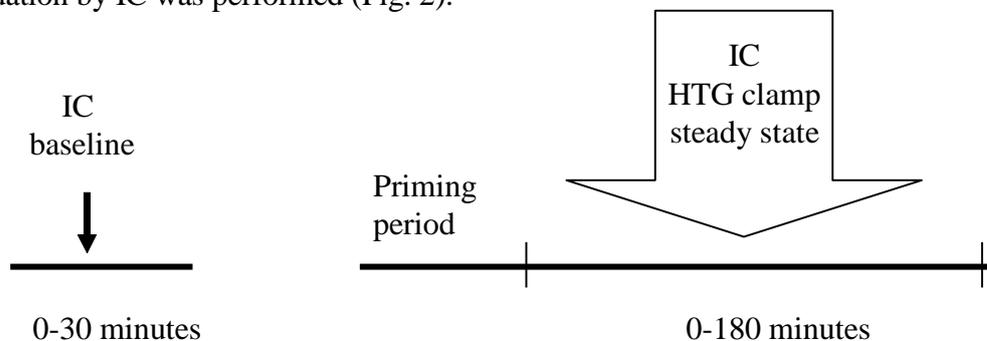

Figure 2 (study II). Ten healthy elderly and young subjects underwent a HTG clamp in combination with IC.

In order to evaluate if the effect of treatment was age dependent, 10 of the subjects were aged between 70 and 78 years (older) and 10 were between 19 and 45 years (younger).

3.2.3 Study III

In this randomised, open, crossover study the possible effect of insulin and glucose administration on the elimination rate and utilisation of fat emulsion was evaluated in 10 healthy men by measuring EE, HTG and insulin-glucose (IG) clamp techniques.

Randomisation was performed with consecutively sealed envelopes. The volunteers were studied on two occasions with an interval of at least one month. With IC, either the subjects received a HTG clamp alone and/or simultaneous HTG and IG clamps were given (Fig. 3).
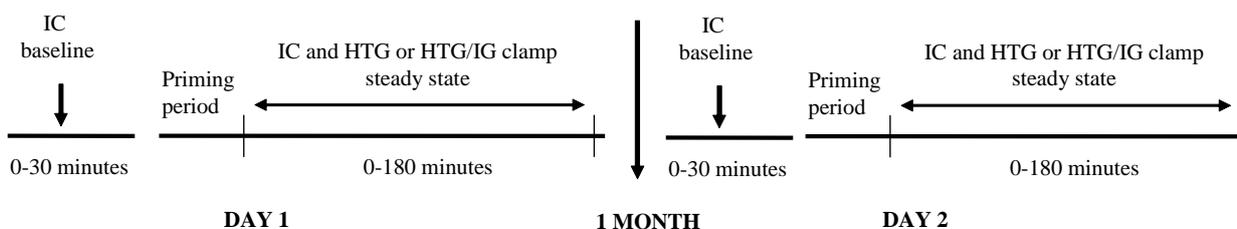

Figure 3 (study III). Ten healthy subjects underwent HTG and HTG/IG clamps in a randomised order in combination with IC.

3.2.4 HTG clamp technique (studies I-III)

The HTG clamp technique as described earlier by Carpentier et al was initially designed for the study of the changes in lipoprotein patterns over a relatively long infusion period (5 hours) and high TG steady state levels (6 and 11 mmol/L) during 4 hours and 1 hour, respectively (26). Furthermore, a combined infusion of glucose and amino acids was administrated in order to avoid endogenous metabolic changes, which would occur during a relatively long study period. We have modified the technique so that the serum TG concentration is kept as constant as possible at 4 mmol/L during 3 hours (28). This TG level was chosen based on clinical experience of TG values measured during infusion of fat emulsions. Another difference compared with the original description of the method is the use of an automated apparatus (Reflotron®) for the enzymatic measurement of TG.

The subjects were studied after an overnight fast. Indwelling catheters were inserted into each arm, one for infusing fat emulsion and the other for obtaining blood samples. After a control period of about 30 minutes basal blood samples were obtained. A constant infusion of a long-chain triglyceride emulsion (Intralipid®, 20%, Fresenius Kabi, Sweden) was administered using a volumetric pump (IVAC® AB, Sweden). A priming dose (13 mg TG x kg BW^{-1} x min^{-1}) was infused in order to rapidly increase the serum TG concentration to 4 mmol x L^{-1} . The priming period was the time calculated from the start of infusion until reaching steady state TG levels at 4 mmol x L^{-1}. This level was kept as constant as possible during 180 minutes by adjusting the fat infusion rate according to the TG values obtained from the Reflotron at the 10 minute measurements.

Blood samples were drawn from the arterialised hand vein (studies I and II) and from the radial artery (study III) in the basal state and at timed intervals throughout the study period (Fig. 4).

3.2.5 Indirect calorimetry (studies II and III)

The IC method was used to estimate energy expenditure. A commercial apparatus (Deltatrac®, Datex Instrumentarium Corp., Finland) was used. This device measures oxygen and carbon dioxide concentrations in the gas from the ventilated hood by a paramagnetic and an infrared analyser, respectively. The analysers were calibrated prior

to each study with air and precisely known gas concentrations (95.0% oxygen and 5.0% carbon dioxide) (39). IC was performed in studies II and III, but not in study I because the patients were unable to cope with the hood in the postoperative setting. Throughout the entire study period the participants remained in the decubitus position and at an ambient temperature of 22ºC. Oxygen consumption (O_2) and carbon dioxide production (CO_2) were continuously measured and average values were calculated over each 1 minute period using a ventilated-hood, under a canopy. Baseline energy expenditure calculations were based on gas exchange data from the last 30 minutes of the basal fasting period and then during the HTG and/or the HTG/IG clamps' steady state (0-180 minutes) (Fig. 4). Determination of the lipid and carbohydrate oxidation rates was obtained by using the table of Lusk (30) which is based on RQ values of 1.00 for 100% carbohydrate oxidation and 0.707 for 100% fat oxidation. Protein oxidation was assumed to be constant during the study and was not included in the analysis (30).

3.2.6 Heparin test (study I)

Heparin injection increases the activity of LPL and hepatic lipase (HL) (40). LPL is the principle enzyme for the hydrolysis of TG molecules in circulating lipoproteins as well as in artificial fat emulsions (23). The lipolytic capacity was determined as the change, i.e. rise, in LPL activity and LPL mass following a bolus dose of 100 IU x kg BW^{-1} heparin sodium (Heparin®, Fresenius Kabi, Sweden). In study I the patients underwent a lipolytic capacity test -4 days before and +3 days after operation.

After the initial blood sampling, an injection of heparin was given and venous blood for determination of post heparin plasma LPL activity and mass was drawn from the other arm 15 minutes after the heparin injection. The plasma samples were centrifuged immediately after collection at $+4^o$ C at 3000 RPM during 10 minutes and then stored in -70°. Samples were shipped on dry ice from Stockholm to Umeå, where the analyses were performed.

3.2.7 IG clamp technique (study III)

The IG clamp which was infused simultaneously with the HTG clamp in study III was performed as described by DeFronzo et al (41), but with minor modifications in order to mimic the situation during administration of total parenteral nutrition (TPN). Insulin (Actrapid® 100 IU/mL^{-1} Novo Nordisk A/S Bagsverde, Denmark) which was dissolved in 96 mL 0.9% sodium chloride (NaCl 154 mml/L^{-1}) and 4 mL of the subject's blood

(400 mU x m^{-2}) was infused as a bolus followed by 20 mU x m^{-2} x min^{-1}. The blood was added to prevent adherence of insulin to the plastic surface. Arterial blood samples were drawn every 5 minutes for determination of blood glucose levels using an automated glucose oxidase method (HemoCue AB, Ängelholm, Sweden). The glucose level was maintained at 7 mmol x L^{-1} during the steady state period by adjusting the infusion rate with a 20% glucose solution.

3.2.8 Sampling and analysis

An intravenous Teflon catheter (Venflon) was placed in an antecubital vein for infusion of a LCT emulsion (Intralipid® 20%, Fresenius Kabi, Sweden). Arterialised venous blood was collected from a Teflon catheter (Venflon) inserted in a dorsal hand vein. The hand was placed in a heated box (63^{0}C). No heparin was used to maintain catheter permeability (studies I and II). When insulin and glucose was infused simultaneously with LCT (study III) a second catheter was placed in the contralateral antecubital vein. In addition, a thin Teflon catheter was inserted into the arteria radialis for blood sampling and kept patient with an infusion of isotonic saline (Fig. 4).

Figure 4 (studies I-III). Schematic illustration of infusions and sampling during HTG clamp studies I and II, and HTG and/or HTG/IG clamps study III at baseline and steady state.

Key: s-FFA (free fatty acids), p-Glyc (glycerol), p-LPL (lipoprotein lipase), p-ß-OH (ß-OH-butyrate), s-Ins (insulin), p-Glu (glucose), s-Lactate, p-Adr (adrenaline), p-Noradr (noradrenaline), s-TG (triglyceride). TG concentrations were determined every 10 minutes and also analysed every hour during the clamp steady state in studies I-III. In addition, glucose was measured every hour during the clamp steady state and repeatedly every 5 minutes after the start of the glucose and insulin infusion in study III.

Serum concentrations of TG were determined enzymatically (Reflotron®, Boehringer Mannheim AG, Germany) every 10 minutes, which provided a value within 190 seconds. The TG concentration in serum was also measured at timed intervals by the routine method at the hospital: a L-alpha-glycerol-phosphate oxidase (GPO) method (studies I-III) (42). These methods determine free and bound glycerol and are therefore associated with a systematic error when measuring TG during the infusion of fat emulsions containing glycerol. Both methods, therefore, overestimate the true TG concentration in the samples. In a separate experiment we found an excellent correlation between the two methods (TG $_{Reflotron}$ = 1.09 TG $_{GPO}$ − 0.331; n=30; r^2 = 0.959) (28).

The fractional removal rate was calculated as the TG infusion rate at steady state divided by the pool size as previously described by using a regression equation based on height, weight and haematocrit values (43).

Respiratory gas exchange data were continuously measured and average values were calculated as mean values for every 1 minute period of oxygen consumption, carbon dioxide production, RQ and EE according to previously presented formulas. Baseline energy expenditure calculations were based on gas exchange data from the last 30 minutes of the basal fasting period. Carbohydrate and lipid oxidation rates were derived from tables by Lusk (30). We assumed protein oxidation to be stable in the resting condition and constant throughout the study period, and this was therefore not included in the analyses (30, 44).

Plasma glucose concentration was analysed enzymatically at timed intervals. Blood glucose was also analysed immediately after collection every 5 minutes, by the glucose oxidise method (HemoCue AB, Ängelholm, Sweden) (Fig. 4). All other samples were collected for later batch analyses. Serum insulin was analysed by radioimmunoassay (45). Serum FFA was determined by enzymatic colorimetric methodology (Wako Chemicals, GmbH, Nuess, Germany) and p-ß-OH-butyrate was analysed enzymatically by spectrophotometer (46). Plasma glycerol was analysed using ultrasensitive bioluminescence (47). Serum lipoprotein fractions (VLDL, LDL and HDL) and their content of TG and cholesterol were quantitated by a combination of ultracentrifugation and precipitation techniques (48,49). Plasma LPL was analysed by using antibodies to suppress hepatic lipase during the assay of LPL. LPL mass was determined by an ELISA technique, using a semi-purified preparation of human LPL as the standard (50). Plasma adrenaline and noradrenaline were determined by using a liquid chromatographic analysis with electrochemical detection (51). Serum lactate was measured fluorimetrically (52).

Serum samples were permitted to clot and centrifuged at $+4^{o}$ C during 10 minutes. Plasma samples were centrifuged immediately after collection. Samples were stored at -70°.

3.2.9 Statistical Methods

Statistical calculations were performed with Statview version 5.0 (SAS Institute Inc., Cary, NC, USA). The results in the text, tables and figures are shown as mean values (MV) ± standard error of the mean (SEM).

Repeated measures over time and comparison between treatments were tested by ANOVA. For other variables the statistical analyses were performed by means of Student´s paired t test (studies I and III) and Student´s unpaired t test (study II). Statistical significance was considered present if $p < 0.05$.

4 RESULTS AND DISCUSSION

4.1 STUDY I

In the present study the elimination rate was more than two times higher in the postoperative situation. This increment is much greater than the increments of 55% which have previously been reported in traumatised patients with IVFTT (24). When using the single bolus injection technique it is difficult to obtain sufficient data in all parts of the disappearance curve to adequately characterise both the maximal clearing capacity (K_1) and the fractional removal rate (K_2). The use of a constant infusion rate method, i.e. the HTG clamp method, obviates the problems with the IVFTT technique as the plasma clearance rate corresponds to the lipid infusion rate during steady state plasma TG levels. This technique therefore has the potential for a more precise characterisation of lipid disposal (28, 29).

In the postoperative state, the elimination rate of TG decreased from 0.756 ± 0.076 mmol TG x min^{-1} to 0.398 ± 0.025 mmol TG x min^{-1} (p<0.005) while the elimination rate before surgery was almost identical at the beginning and at the end of the clamp (0.201 ± 0.083 vs. 0.178 ± 0.088 mmol TG x min^{-1}; n.s.) (Fig. 5).
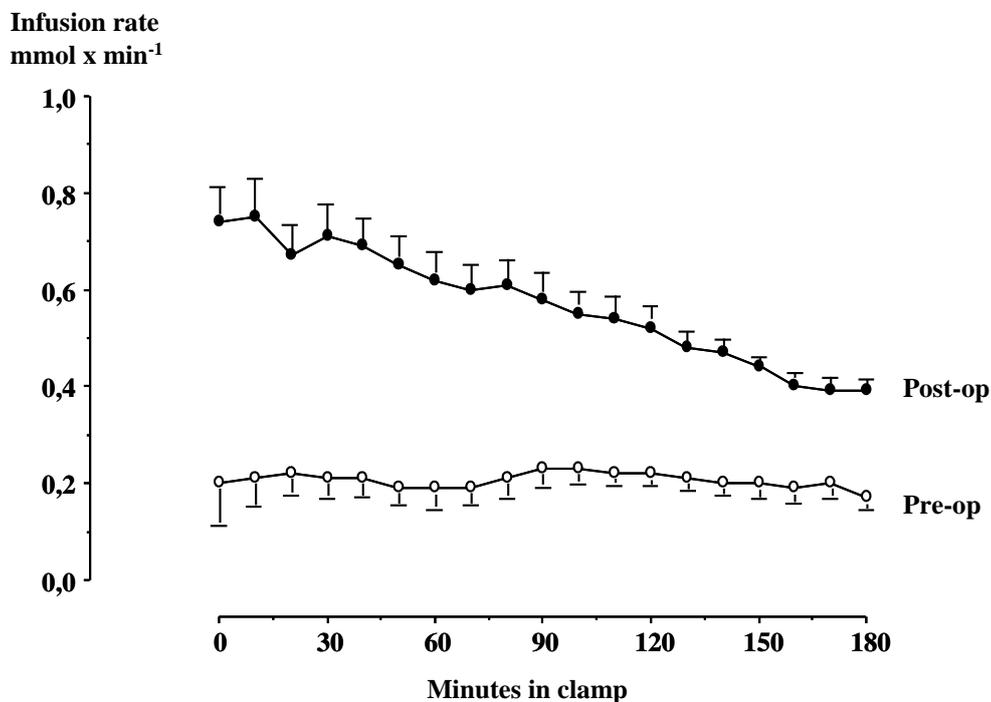
Figure 5. The infusion rate (plasma elimination rate) of a fat emulsion during the HTG clamp. Means ± SEM. *Significantly different from start of the clamp in the postoperative state p<0.005.*

Despite a similar plasma volume before and after surgery (2.86 ± 0.17 vs. 3.04 ± 0.16 L; n.s.), the average elimination rate of lipids was still higher in the postoperative state: 0.204 ± 0.018 vs. 0.080 ± 0.016 mmol x min^{-1} x L^{-1} ($p<0.001$).

The priming volume required to reach steady state TG levels was significantly higher in the postoperative state ($p<0.05$). This corresponds to a priming dose of $40 \pm 6\%$ pre-op and $34 \pm 3\%$ post-op (n.s.) of the total amount of infused lipids during the respective clamps. The priming period before steady state was obtained was not different before and after surgery: 64 ± 12 minutes, (10-120 minutes) vs. 69 ± 13 minutes, (20-140 minutes) (n.s.). During the steady state period, 465 ± 34 ml was infused post-op and 172 ± 33 ml was infused pre-op, respectively ($p<0.001$) (Fig. 6).
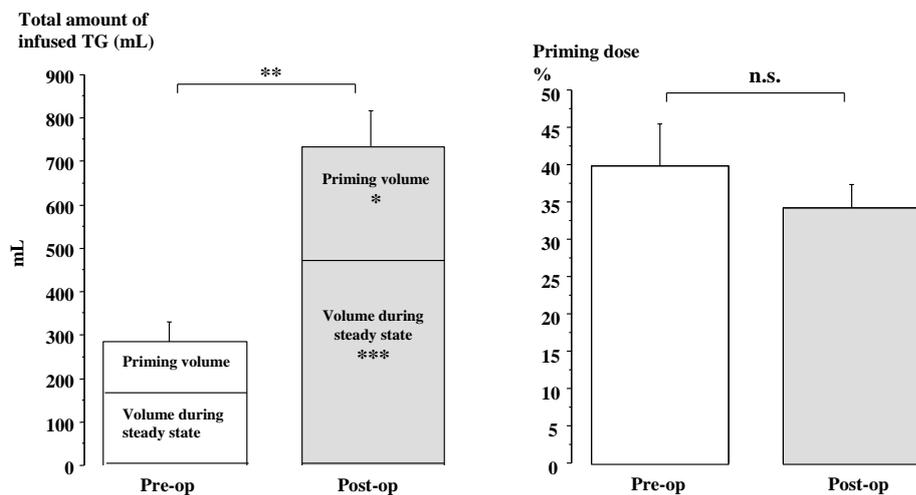
Figure 6. Total amount of infused TG (mL) during the respective clamps pre-op/post-op and percentage of priming dose of total amount of infused TG during the respective clamps. Means ± SEM.
*Significantly different *p<0.05;** p<0.01;*** p<0.001 pre-op vs. post-op.*

With the HTG clamp, however, there is no evidence that endogenous production of TG (i.e. production of hepatic TG) is suppressed. On the contrary, hepatic TG production may be expected to increase as Lewis et al (53) have shown that the infusion of Intralipid® and Heparin® increased VLDL-TG production by 180%. It is therefore likely that the observed 2.6-fold rise in the infusion rate of exogenous TG to maintain

steady plasma TG concentrations after surgery is a low estimate of the removal capacity. The hepatic TG production is of the order of 1.2 g/h (54) and the TG infusion rate post-op was of the order of 20-42 g/h. It is therefore evident that the error caused by endogenous TG production for the calculation of TG disposal with the HTG clamp must be quite small.

The fasting levels of FFA and glycerol were similar (n.s.) before and after operation. During the infusion of lipids the concentrations of FFA and glycerol increased in both pre- and postoperative states. The rise in FFA was less marked after surgery while the glycerol concentration rose more markedly in the postoperative state. The increased glycerol concentrations during lipid infusion were probably the result of the free glycerol content in the lipid emulsion rather than the result of increased TG hydrolysis (Fig. 7).
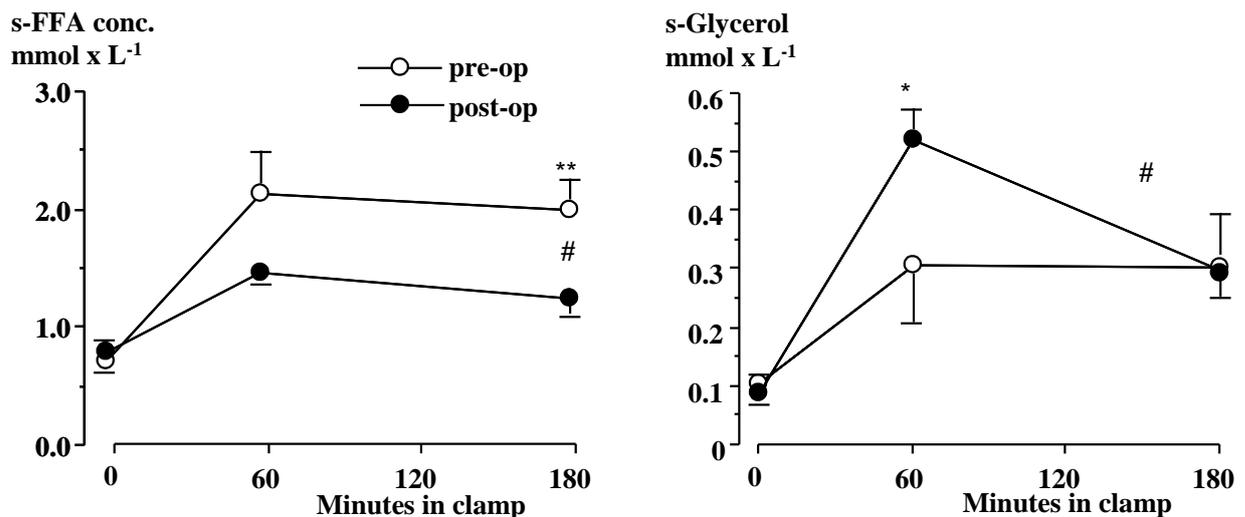
Figure 7. Serum concentration of FFA and Glycerol (mmol x L^{-1}) during the respective clamps pre-op/post-op. Means ± SEM.
*Significantly different *p<0.05;** p<0.01, pre-op vs. post-op.*
Repeated measures ANOVA for treatment time x intervention; # p<0.05

As expected, concentrations of glucose and insulin were higher in the postoperative state which may be due to insulin resistance in the postoperative state (55).

The TG concentrations in all lipoprotein fractions show a similar rise before and after operation. Total cholesterol concentration was significantly lower (p<0.05) postoperatively. The most pronounced effects of lipid clamping on the lipoprotein pattern included a 5-fold increase in VLDL-TG concentration, and a 2-fold rise in HDL-TG and VLDL-cholesterol concentrations. HDL-cholesterol showed a more pronounced decline in the postoperative state (p<0.01). It should be noted, however, that the method for the measurement of lipoprotein fractions did not ensure the removal of infused TG. Intralipid-TG has a similar density to that of VLDL so a considerable proportion of the marked increase in VLDL-TG that was observed is likely to be infused fat.

Increased LPL activity has been reported in traumatised patients (56) but the possible relationship for the observed increased fat clearance in this class of patients is unclear. Plasma LPL activity and mass measured before the HTG clamp decreased by about 50% post-operatively. Decreased amounts of LPL mass in plasma after surgery may reflect a decreased production of the enzyme in adipose tissue. Plasma LPL activity values showed a non-significant increase during the HTG clamp with no differences between the pre- and postoperative clamps. Previous studies have indicated that traumatised patients exhibit increased LPL activity in adipose tissue but no change in skeletal muscle LPL activity (56). Injection of heparin resulted in a large rise in both LPL activity and LPL mass. The values reached were similar before and after operation. Earlier studies indicate that heparin releases mainly or only the active form of the lipase (57, 58).

	LPL activity (mU/mL)		LPL mass (ng/mL)	
	Basal	**Post-heparin**	**Basal**	**Post-heparin**
Heparin test				
pre-op (day –4)	2.9 ± 0.6	338 ± 95**	132 ± 24	1205 ±332**
post-op (day +3)	3.7 ± 1.2	307 ± 73**	69 ± 11##	1223 ±240**
HTG clamp				
pre-op (day –1)	4.2 ± 0.9		133 ± 27	
post-op (day +2)	2.3 ± 0.5		64 ± 11##	

Table 2 (study I). LPL activity and mass before and after operation (n=9)
*Basal vs. post-heparin: ** p<0.01*
Pre- vs. post-op: ## p<0.01

The post-heparin activity was similar before and after surgery (Table 2). Post-heparin LPL activity should reflect the amount of lipase available for emulsion metabolism at vascular surfaces throughout the body. Hence, the increased rate of emulsion clearance can not be ascribed to increased LPL action. In accordance with this, plasma FFA levels were lower, not higher, during the fat clamp after surgery (Fig. 7). Some route of lipid clearance other than LPL mediated is thus implicated. Hultin et al compared the metabolism in rats of chylomicrons and a labelled emulsion, similar to those used for parenteral nutrition (59). Their data indicate that a large fraction of the emulsion droplets was removed from plasma with little or no preceding lipolysis. The present study suggests that the capacity for this type of removal is markedly increased after surgery. Moreover, the data indicate that the mechanism is saturable since as the clearance rate slowly decreased during the infusion period (Fig. 5).

From this study we can conclude that a moderate surgical trauma is accompanied by a greater than two-fold rise in the plasma elimination rate of exogenous fat despite a lower basal LPL activity and a virtually unchanged LPL pattern during the infusion of lipids. Trauma may substantially enhance the fat elimination capacity but a significant proportion of the infused fat is not utilised for metabolic purposes.

4.2 STUDY II

The average infusion rate of lipid emulsion during the steady state clamp period, i.e. the plasma elimination rate of exogenous fat, was similar in both elderly and young individuals (n.s.) (Fig. 8). The plasma volume was also similar in the elderly and young individuals (n.s.). The fractional elimination rate per litre of plasma was not influenced by age (n.s.).
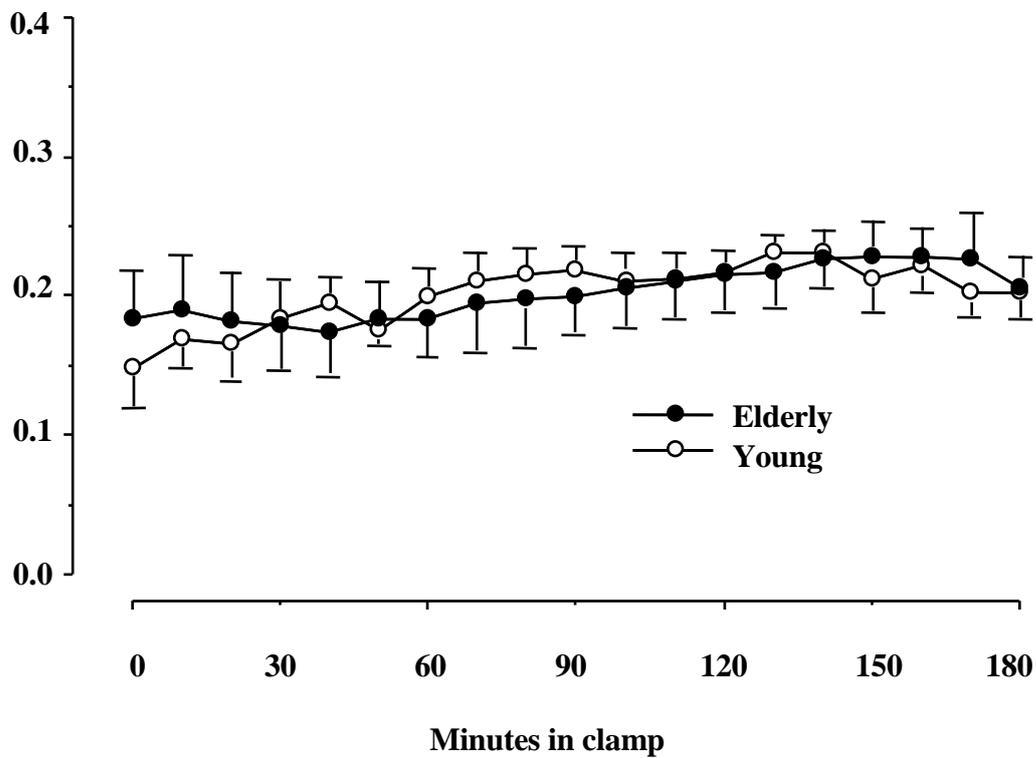
Figure 8 . The infusion rate (plasma elimination rate) of a lipid emulsion during the HTG clamp. Means ± SEM.

The priming volume required to reach steady state TG levels was significantly higher in the elderly group (p<0.05). This corresponds to a priming dose of 47 ± 4% in the elderly and 41 ± 3% in the young individuals (n.s.) of the total amount of infused lipids during the respective clamps. The priming period before steady state was obtained was significantly longer in the elderly vs. younger men: 65 ± 6 minutes, (50-110 minutes) vs. 37 ± 6 minutes, (10-80 minutes), p<0.01. During the steady state period, 161 ± 21 vs. 161 ± 12 ml was infused in elderly and young men (n.s), respectively (Fig. 9).
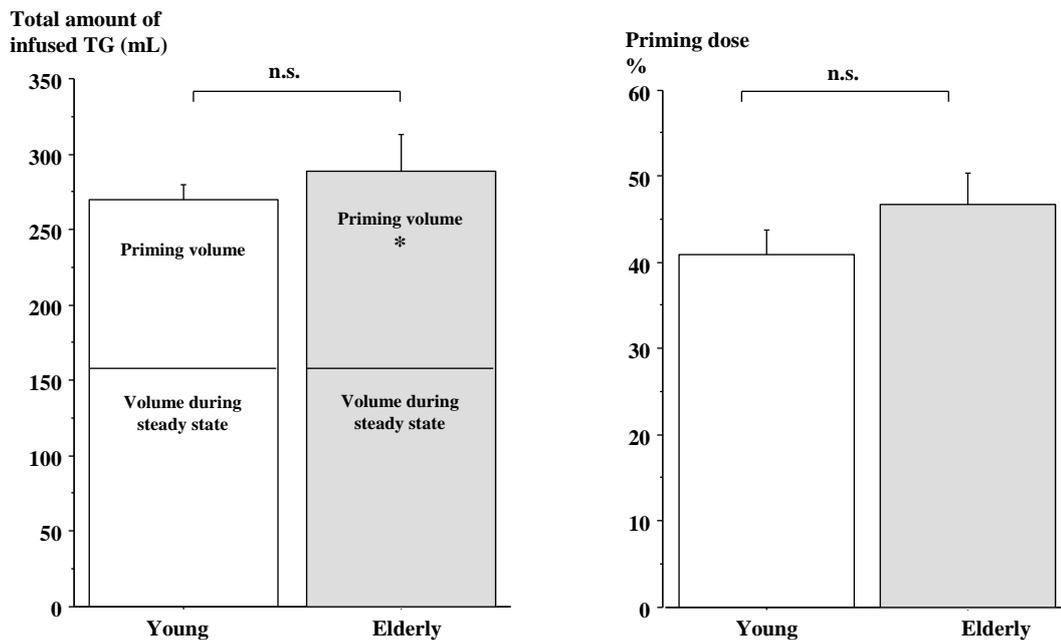
Figure 9. Total amount of infused TG (mL) during the HTG clamp in young and elderly subjects and percent priming dose of total amount of infused TG during the clamp. Means ± SEM. *Significantly different * p<0.05; young vs. elderly.*

In the basal state, the concentrations of serum FFA were higher in the elderly than in the young subjects (p<0.05). The capacity to hydrolyse the load of exogenous triglycerides supplied during the HTG clamp was thus unaffected by age. This was also evident from the similar increments in plasma FFA in the two groups (Fig. 10).
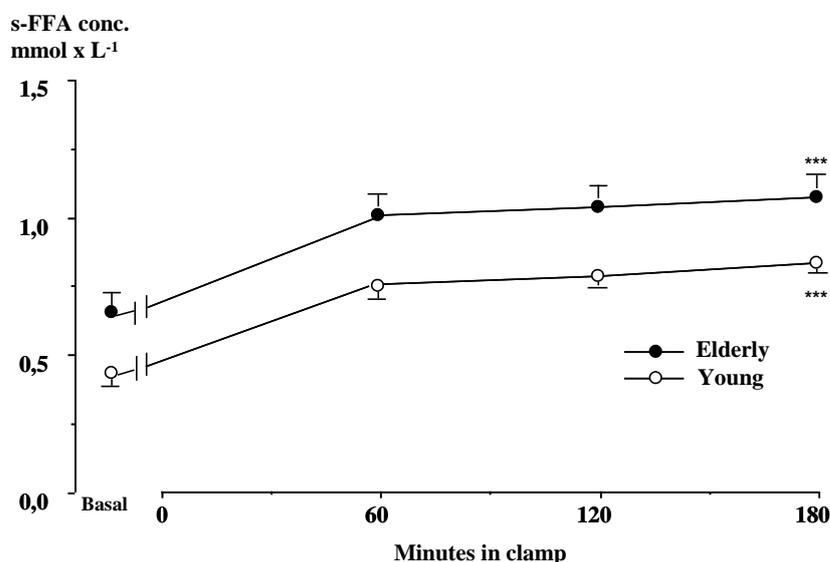
Figure 10. Serum concentration of FFA (mmol x L^{-1}) during the HTG clamp in young and elderly individuals. Means ± SEM.

*Differences from baseline values are denoted by asterisks; *** p<0.001.*

Repeated measures ANOVA for treatment time x intervention; n.s.

At baseline, plasma LPL activity was almost three times higher in the young than in the elderly subjects: 0.65 ± 0.09 vs. 0.23 ± 0.02 mU x mL^{-1}, (p<0.001), respectively. This presumably reflects the larger and more active muscle mass in the young. Coppack and co-workers showed that in man there is a release of active LPL from forearm muscle, whereas they found no release of LPL activity from adipose tissue in the fasting state and only a small release after feeding (60). These studies show that in man, skeletal muscle is probably the main contributor to plasma LPL activity. LPL activity increased during the infusion (61, 62), and the increase was larger in the young than in the elderly individuals (Fig. 11).
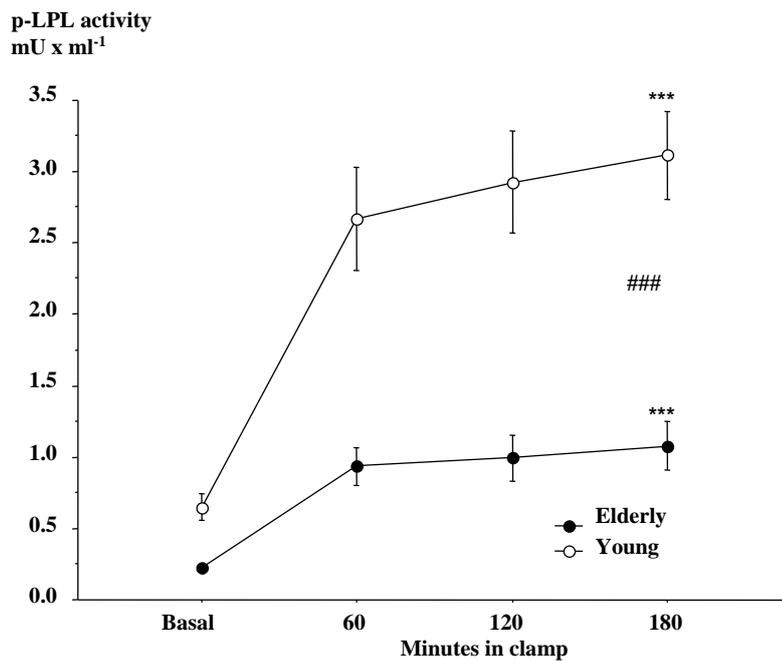
Figure 11. Plasma concentration of LPL activity (mU x mL^{-1}) during the HTG clamp in young and elderly individuals. Means ± SEM.

Differences from baseline values are denoted by asterisks; *** p<0.001.

Repeated measures ANOVA for treatment time x intervention; \#\#\# p<0.001.

This indicates that the difference in the amounts released was due to a larger amount of extractable LPL in the young individuals, and not because of differences in the mechanisms by which the emulsion increased the release of the lipase into plasma.

The basal measurement of energy expenditure and RQ was performed after a 12 hour fast. Our study demonstrated that elderly subjects had lower energy expenditure in the basal state than young individuals (64 ± 3 vs. 79 ± 2 kcal x hour^{-1}; p<0.001) (63). RQ-values of 0.78-0.80 indicated that the subjects at baseline had a metabolic situation where fat was the prominent oxidative fuel. The administration of large amounts of fat during the clamp could therefore not be expected to change this situation and fat continued to be the dominant supplier of energy. During the clamp, energy expenditure increased both in the elderly and the young individuals. However, the increase in energy expenditure was not statistically significantly greater in the elderly than in the young individuals (9.0 ± 1.3 % vs. 6.0 ± 1.8 %; n.s.) (Fig. 12).
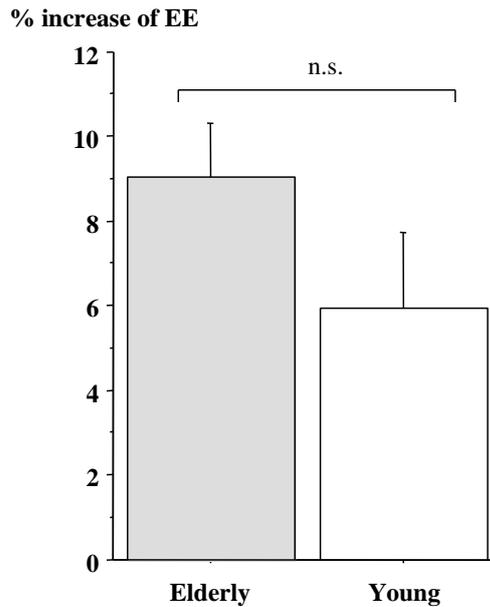
Figure 12. Percentage increase of EE in 10 elderly and young individuals during the HTG clamp. Means ± SEM.

The increase in lipid oxidation was similar in the elderly and in the young individuals (15.1 ± 4.9 % vs. 23.9 ± 7.0 %; n.s.). This indicates that elderly subjects are capable of oxidising fat as readily as young individuals. During the HTG clamp there was an approximately 14.8 ± 14.0 % decrease in carbohydrate oxidation in the young vs. 3.2 ± 12.1 % in the elderly individuals. This difference did not reach statistical significance (Fig. 13). In one study by Al-Jaouni R et al comparing substrate oxidation during cyclic TPN in elderly and middle-aged patients, the elderly subjects had a significantly higher lipid oxidation as well as plasma FFA levels and lower carbohydrate oxidation vs. younger patients in response to TPN infusion. However, in contrast to our study, these investigations were done in malnourished patients who had been receiving cyclic TPN for at least 2 weeks (64).
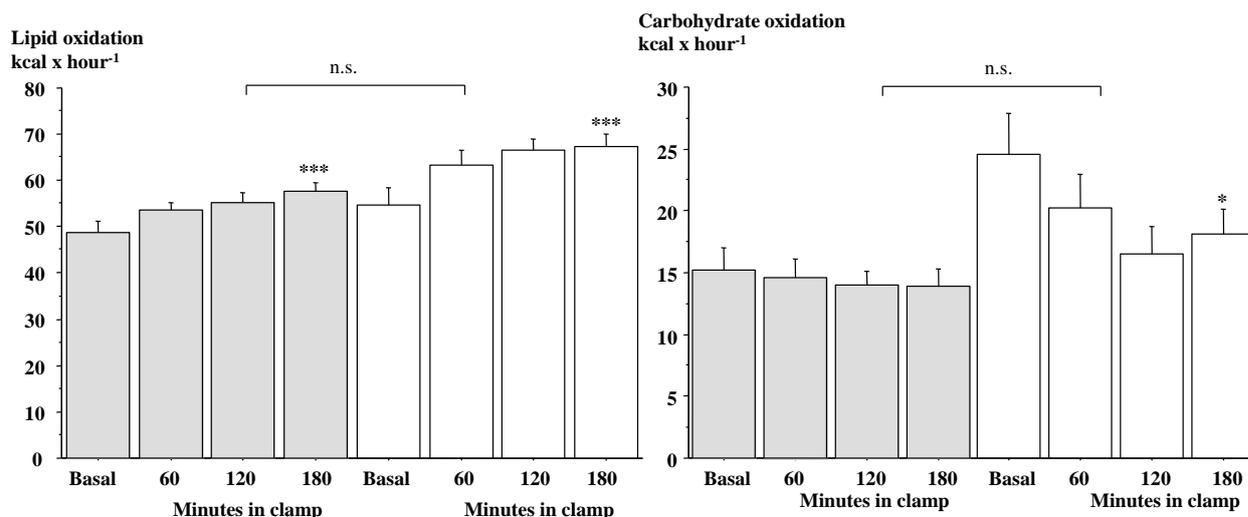
Figure 13. Lipid and carbohydrate oxidation (kcal x hour^{-1}) in 10 elderly (shaded bars) and young (open bars) individuals during the HTG clamp. Means ± SEM.
Differences from baseline values are denoted by asterisks; *p<0.05, *** p<0.001. Repeated measures ANOVA for treatment time x intervention; n.s.*

Studies by others have reported that the capacity to release FFAs from endogenous fat stores is similar in both elderly and young individuals (65). However, there are also reports indicating that the elderly may have a reduced capacity to mobilise and oxidise fat (66, 67). The reasons for these opposing findings are not clear.

Results from this study suggest that elderly men have a capacity to intravenously hydrolyse a high TG load administered as a HTG clamp, which is not quantitatively different from the capacity of young men. Furthermore, the elderly have a similar capacity to oxidise the administered fat, as have young individuals.

4.3 STUDY III

In this study we wanted to investigate the influence of glucose and insulin administration on the plasma elimination and oxidation rates of a lipid emulsion in healthy men. The subjects were studied on two occasions either with administration of a HTG clamp alone or with HTG and IG clamps in parallel.

We found that the infusion rate required to maintain stable serum TG concentrations at 4 mmol x L^{-1} did not differ between the HTG and combined HTG/IG clamps (Fig. 14). Furthermore, the plasma TG fractional elimination rate was similar (0.084 ± 0.008 vs. 0.075 ± 0.006 mmol x min^{-1} x L^{-1} ;n.s.) during the HTG and HTG/IG clamps. Our results thus show that the plasma TG elimination rate is not influenced by moderate increments of plasma glucose and insulin levels, which is in accordance with the findings of Iriyama K et al (68).
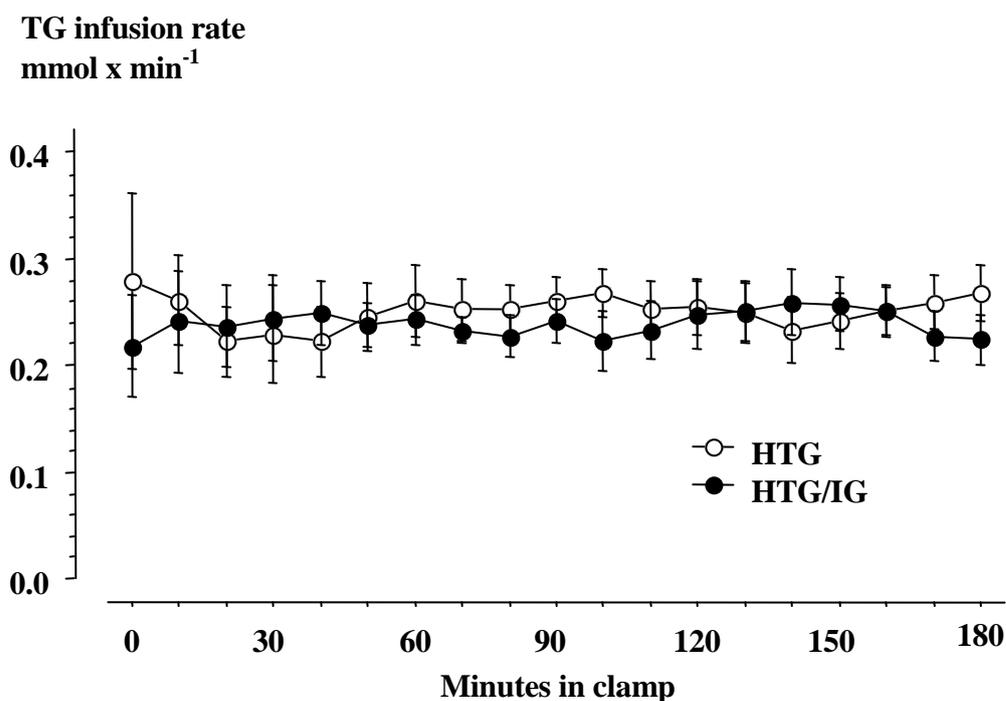
Figure 14. The infusion rate (plasma elimination rate) of a fat emulsion during the HTG and HTG/IG clamps. Means ± SEM.

The priming volume required to reach steady state TG levels was significantly higher with the combined HTG/IG clamps than with the HTG clamp (p<0.05). This corresponds to a priming dose of 43 ± 3% for the HTG/IG and 34 ± 2% for the HTG clamp (p<0.05) of the total amount of infused lipids during the respective clamps. The higher priming volume could be due to a two times longer priming period in the HTG/IG group, 62 ± 8; range 70 (20-90 min) vs. 29 ± 3; range 30 (10-40 min) in the HTG group (p<0.01). During the steady state period, 185 ± 18 ml was infused during the HTG/IG clamps and 200 ± 21 ml was infused during the HTG clamp, respectively (n.s.) (Fig. 15).
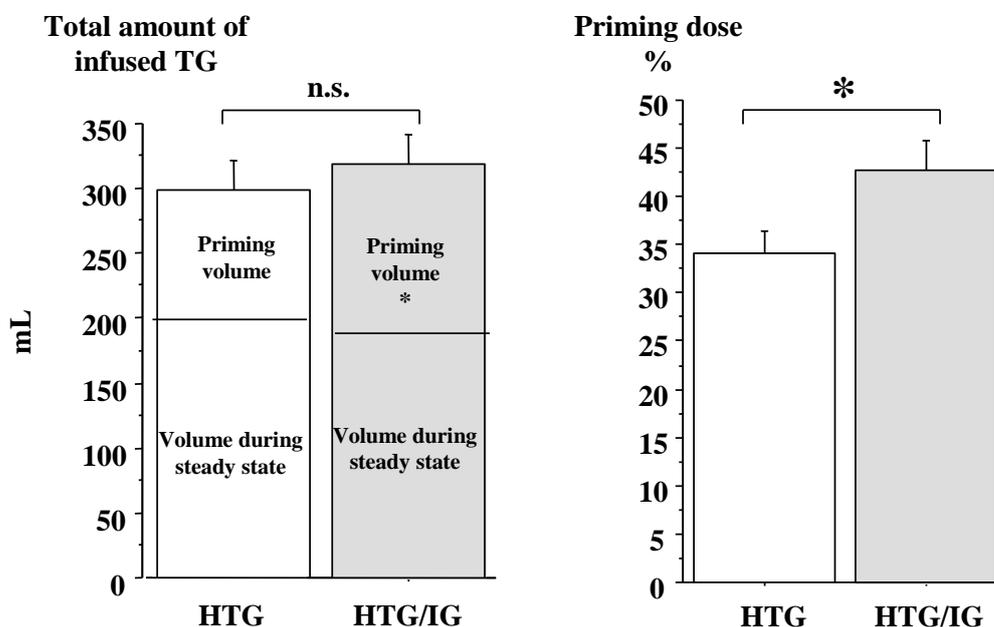
Figure 15. Total amount of infused TG (mL) during the respective clamps and priming dose percentage of total amount of infused TG during the respective clamps. Means ± SEM.

*Significantly different * p<0.05: HTG vs. HTG/IG.*

The infusion of insulin and glucose during the insulin-glucose clamp resulted in physiological increments in serum insulin concentrations and moderate hyperglycaemia, (36.3 ± 3.0 vs. 7.0 ± 0.5 mmol x L^{-1}) whereby lipid oxidation and serum FFA remained virtually unchanged despite the simultaneous infusion of lipids (Fig. 16).
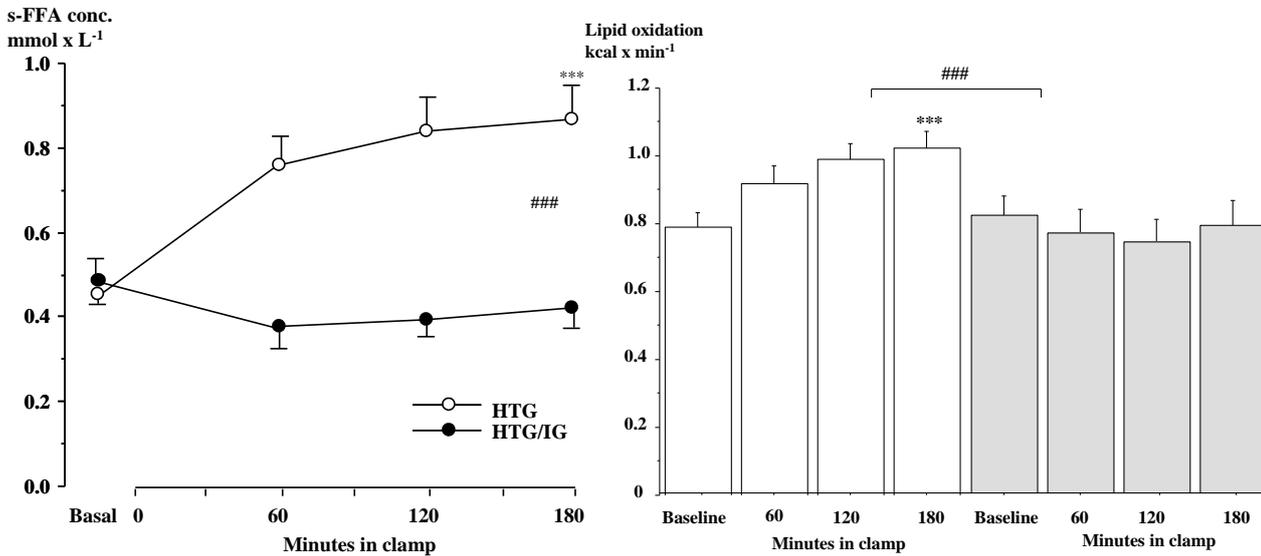
Figure 16. Serum concentration of FFA (mmol x L^{-1}) and lipid oxidation (kcal x min^{-1}) during the HTG (open bars) and HTG/IG (shaded bars) clamps in 10 healthy individuals. Means ± SEM. *Differences from baseline values are denoted by asterisks; *** p<0.001. Repeated measures ANOVA for treatment time x intervention; ### p<0.001.*

This suggests an increased channelling of FFA, presumably toward TG synthesis rather than oxidation. Under these circumstances carbohydrate oxidation increased significantly (Fig. 17).
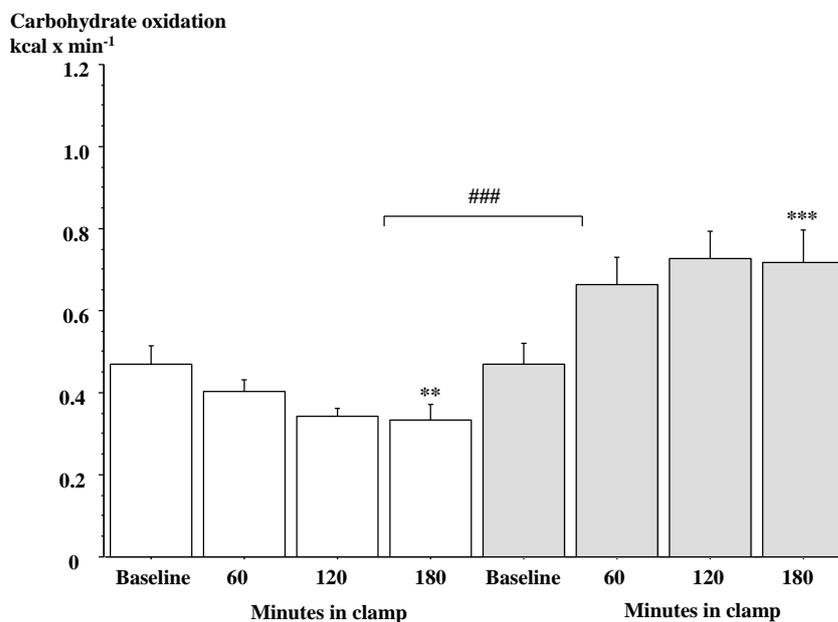
Figure 17. Carbohydrate oxidation (kcal x min^{-1}) during the HTG (open bars) and HTG/IG (shaded bars) clamps in 10 healthy individuals. Means ± SEM.
Differences from baseline values are denoted by asterisks;**p<0.01, *** p<0.001. Repeated measures ANOVA for treatment time x intervention; ### p<0.001.

The mechanism involved could either be that insulin suppressed lipid oxidation directly by inhibiting the mobilisation and/or oxidation of intracellular lipid stores, or that the inhibition of lipid oxidation was secondary to a stimulation of glucose oxidation by insulin (the Randle cycle). The design of the present study does not allow an elaboration on which of these mechanisms plays the greater role, but Groop et al have demonstrated that FFA oxidation is primarily determined by the serum FFA concentration. Our observation of a close correlation between lipid oxidation and plasma FFA concentration supports their findings (69). This suggests that factors other than the FFA concentration are contributing to lipid oxidation and that differences in FFA concentration are not driving any differences in lipid oxidation between the trials.

During the HTG/IG clamp, EE increased by $14 \pm 2\%$ ($+0.19 \pm 0.01$ kcal x min^{-1}; $p<0.001$) and this was a significantly greater increase ($p<0.01$) than the $6 \pm 2\%$ ($+0.08 \pm 0.01$ kcal x min^{-1}; $p<0.05$) observed during the HTG clamp (Fig. 18). The corresponding increase in EE above basal EE in relation to administered energy during the clamp (DIT) was virtually of the same magnitude 4.5 ± 0.5 % and 3.4 ± 0.8 % (n.s.) during the HTG/IG and HTG clamps, respectively.
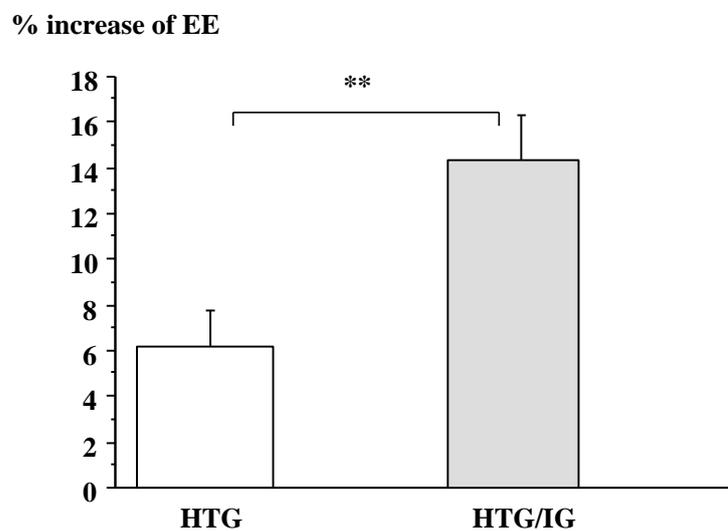
Figure 18. Percentage increase of EE during the HTG and HTG/IG clamps in 10 healthy individuals. Means ± SEM.
*Significantly different ** p<0.01: HTG vs. HTG/IG.*

RQ values of 0.82 to 0.81 indicated that, at baseline, the subjects had a metabolic situation in which fat, rather than carbohydrates, was the prominent oxidative fuel. As expected, administration of fat during the HTG clamp did not change this situation. RQ increased during the combined administration of insulin-glucose together with fat. RQ was significantly higher at steady state in the HTG/IG (0.85 ± 0.01) than in the HTG clamp (0.79 ± 0.01), $p<0.01$.

The data obtained by indirect calorimetry showed a markedly increased energy intake of fat above basal energy expenditure during the HTG (+2.2 kcal/min) and the combined HTG/IG clamp (+2.1 kcal/min from fat and +2.0 kcal/min from glucose) only marginally influenced the ratio of lipid and carbohydrate oxidation. At baseline, lipid and carbohydrate oxidation provided 63 and 37%, respectively, of total EE. At the end of the clamps, lipid oxidation provided for 73% during the HTG and 52% of total EE during the HTG/IG clamp, respectively (Fig. 19). This indicates that despite a markedly increased carbohydrate and lipid intake a high degree of lipid oxidation was maintained.
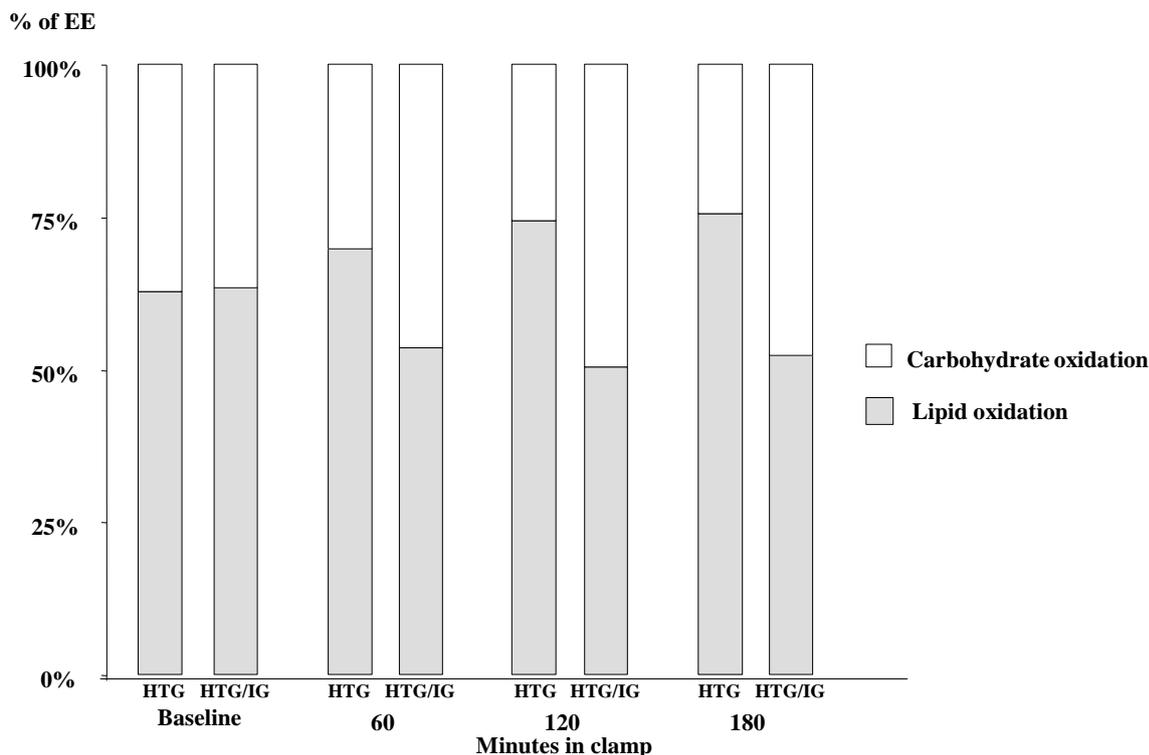
Figure 19. Percentage of lipid and carbohydrate oxidation of total EE during the HTG and HTG/IG clamps in 10 healthy individuals. Mean.

The significant increase in serum ß-OH-butyrate concentration during the combined infusion HTG/IG clamp suggests that increased glucose and insulin availability does not shut off hepatic fatty acid oxidation, although the elevated plasma insulin concentrations would, at a certain degree, be expected to limit FFA-induced ketogenesis (70). This is in line with our findings showing that elevated insulin concentrations in the HTG/IG clamp are associated with smaller increases in plasma ß-OH-butyrate concentrations than in the HTG clamp (Fig. 20).
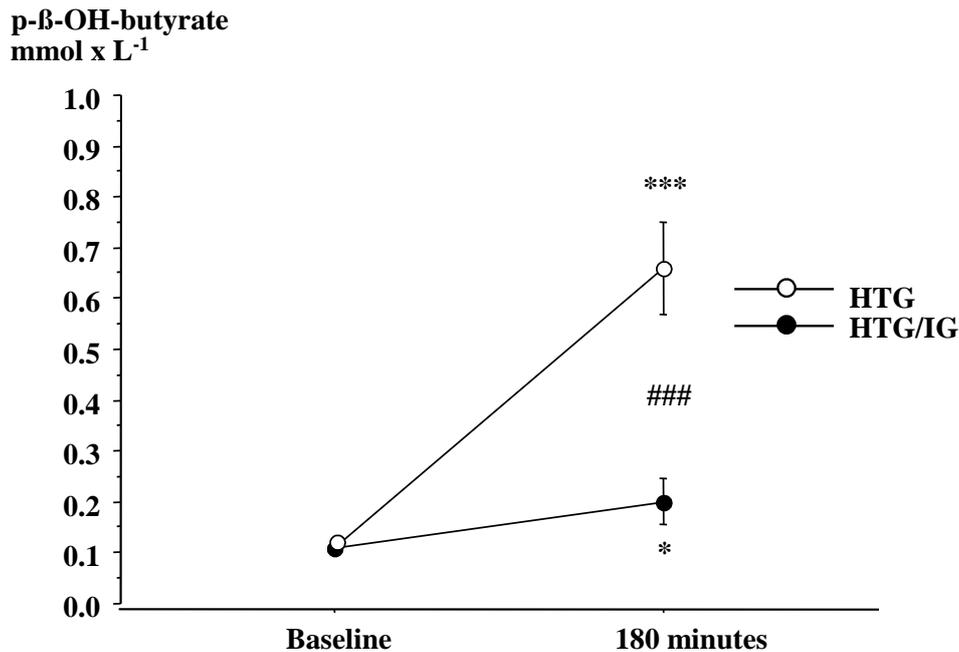
Figure 20. Plasma ß-OH-butyrate concentrations during the HTG and HTG/IG clamps in 10 healthy individuals. Means ± SEM.

Differences from baseline values are denoted by asterisks;$*p<0.05$, $*** p<0.001$. *Repeated measures ANOVA for treatment time x intervention;* $### p<0.001$.

In conclusion, the administration of glucose and insulin provided as an IG clamp in healthy volunteers did not influence the plasma elimination rate of a fat emulsion determined by a HTG clamp. Furthermore, the modestly decreased FFA levels and augmented glucose oxidation noted during the combined HTG/IG clamp were accompanied by a high rate of lipid oxidation. ß-OH-butyrate increased during both clamps, indication of an ongoing hepatic fatty oxidation despite the administration of glucose/insulin.

5 CONCLUSIONS

The design of the studies in this thesis has allowed a characterisation of the plasma elimination capacity and oxidation rates of a long chain triglyceride emulsion under various physiological and pathophysiological conditions by use of a HTG clamp technique and IC. The results of these studies can be summarised as follows:

1. Moderate surgical trauma was accompanied by a greater than two-fold rise in the plasma elimination rate of exogenous fat despite a lower basal LPL activity. However, a significant proportion of the infused fat was not utilized for metabolic purposes.

2. Elderly men had a capacity to intravenously hydrolyse a high TG load, administered as a HTG clamp, which was not quantitatively different from that of young men. This was evident also from the similar increments in plasma FFA during fat administration. The increase in energy expenditure was similar in the elderly and the young indicating that elderly did not have an impaired capacity to oxidise intravenously administered fat.

3. Glucose and insulin provided as an IG clamp did not influence the plasma elimination rate of a fat emulsion. Furthermore, the modestly decreased FFA levels and augmented glucose oxidation noted during the combined HTG/IG clamps were accompanied by a high rate of lipid oxidation. ß-OH-butyrate increased during both clamps indicating an ongoing hepatic fatty oxidation despite the administration of glucose/insulin.

6 ACKNOWLEDGEMENTS

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