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POSTPRANDIAL PLASMA ACYLATION STIMULATING PROTEIN RESPONSE AND FAT METABOLISM IN POST-OBESE WOMEN

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I could not have done it with out you.

DEDICATION

My mother, my father, my sister and brother, all that

I am and will ever be I owe to you.

To you I dedicate this...

ABSTRACT

Acylation stimulating protein (ASP) is a plasma protein that significantly increases adipose tissue fat storage. *In vivo* and *in vitro* studies have suggested a role for plasma ASP in enhancing postprandial plasma triglyceride (TG) clearance. The primary objective of this study was to examine, for the first time, the postprandial response of plasma ASP and the fate of an exogenous fat source in 8 post-obese and 8 matched control women. This was done through following ^{13}C-labeled high fat breakfast meal (1062 Cal, 67 % fat) every 2 hours for 8 hours in 3 plasma pools and in expired breath CO_2. The 3 plasma pools were: TG fraction in triglyceride rich lipoproteins (TRL) with sedimentation factor $S_f > 400$ (referred to as chylomicron-TG), TG fraction in TRL with $S_f = 20 - 400$ (referred to as VLDL-TG), and plasma free fatty acid (FFA). The secondary objective was to examine fasting and postprandial resting energy expenditure (REE), thermic effect of food (TEF), carbohydrate to fat oxidation rate and insulin sensitivity, which are factors that have been implicated in the tendency of post-obese women to regain weight.

The post-obese and control women had similar fasting and postprandial plasma ASP concentrations as well as similar postprandial percent plasma ASP change from baseline. In addition, the two groups had similar fasting and postprandial REE, TEF, carbohydrate to fat oxidation rate, serum insulin concentration, and plasma FFA concentration. Moreover, there was no difference between the two groups in total plasma TG clearance, ^{13}C clearance from plasma VLDL-TG pool or percent recovered ^{13}C in breath CO_2. However, postprandially, the post-obese women had lower glucose

concentration and faster clearance of the TG fraction in plasma chylomicron and VLDL. The greatest difference between the two groups however was in the fractionation of the labeled FFA between plasma circulation and tissue uptake. Although, the post-obese women had a faster clearance of ^{13}C from plasma chylomicron-TG pool, ^{13}C concentration in plasma FFA pool remained similar at all postprandial time points to fasting non-enriched values, and was therefore lower than that in the control women whose plasma FFA ^{13}C concentration increased markedly.

This study casts light on a novel finding of metabolic differences that exist in the post-obese women. These differences are represented by an enhanced plasma clearance of the exogenous fat source, which is indicated by a faster hydrolysis of the circulating TRL accompanied by a faster tissue uptake of the generated FFA. These findings are compatible with an increased sensitivity to insulin and the ASP pathway.

RÉSUMÉ

Acylation stimulating protein (ASP) est une protéine plasmatique qui augmente significativement l'entreposage des lipides dans le tissu adipeux. Les études *in vivo et in vitro* ont suggéré que le rôle pour l'ASP plasmatique est d'augmenter le taux d'élimation des triglycérides (TG) plasmatiques. L'objectif primaire de cette étude est d'examiner, pour la première fois, la résponse post-prandiale d'ASP et l'utilization finale des lipides provenant d'une source exogène contrôlée en graisses chez 8 femmes post-obèse jumelées à 8 femmes contrôles. Suite à l'ingestion d'un déjeûner riche en graisses liées avec ^{13}C (1062 calories, 67% gras), l'étude suit l'évolution des lipides dans 3 fractions plasmatiques et dans le CO_2 expiré par la respiration, et ce chaque 2 heures durant 8 heures au total. Ces trois fractions sont: la fraction TG dans les lipoprotéines riches en TG (TRL) avec un facteur de sédimentation $S_f > 400$ (fait référence à chylomicron-TG), la fraction TG dans les TRL avec $S_f = 20 - 400$ (fait référence à VLDL-TG), et les acides gras libres plasmatiques (FFA). Le deuxième objectif est d'éxaminer différents facteurs impliqués dans la tendance à regagner du poids chez les femmes post-obèse comme: la dissipation d'énergie au repos (REE) à jeun et post-prandialement, l'effect thermique généré par la nouriture (TEF), le taux d'oxidation des lipides par rapport aux hydrates de carbon, ainsi que la sensibilité à insuline.

Les femmes post-obèse et contrôles ont des concentrations plasmatiques d'ASP à jeun et post-prandiales similaires, et un changement relatif (%) en ASP similaire par rapport aux niveaux à jeûn. Il n'y a pas de différence entre les deux groupes pour: REE à jeun et post-prandiale, TEF, le taux d'oxidation des lipides par rapport aux hydrates de carbone, les concentrations plasmatiques de FFA, et les concentrations sériques

d'insuline. En plus, il n'y a pas de différence pour le taux délimation dans le plasma des triglycérides totaux, l'élimination de ^{13}C dans la fraction plasmatique VLDL-TG, ainsi que pour le pourcentage recouvré de ^{13}C dans le CO_2 expiré. Par contre, dans la phase post-prandiale, les femmes post-obèse ont des taux de glucose inférieurs. A cela, s'ajoute un taux d'élimininination plus rapide de la fraction TG dans les chylomicrons et les VLDL. La plus grande différence est dans le fractionement de ^{13}C-FFA entre la circulation plasmatique et l'absorption par le tissu. Même si les femmes post-obèse ont un taux d'élimination plus rapide de ^{13}C dans la fraction plasmatique des chylomicrons-TG, les concentrations de ^{13}C dans les FFA plasmatiques demeurent constant mais augmentent dramatiquement chez les femmes contrôles.

Les données de cette étude mettent en lumière une découverte innovatrice sur les differences métaboliques qui existent chez les femmes post-obèse. Ces différences sont réprisentées par une augmentation de l'élimination des lipides provenant d'une source exogène, ce qui est indiquée par une hydrolyse rapide des TRL en circulation accompagnée d'une absorption plus rapide des FFA générés. Ces résultats sont compatibles avec une sensibilité accrue envers l'insuline et l'ASP et ses implications métaboliques.

LIST OF ABBREVIATIONS

The following abbreviations were used through out the thesis:

ASP	Acylation stimulating protein
AUC	Area under the 8 hours time curve
BMI	Body mass index
BSA	Body surface area
CHYLO	Chylomicron
FFA	Free fatty acids
FFM	Free fat mass
HDL-C	High density lipoprotein cholesterol
hr	Hours
Ht	Height
IR/MS	Isotopic ratio mass spectrometer
LDL-C	Low density lipoprotein cholesterol
LPL	Lipoprotein Lipase
min	Minutes
NIDDM	Non-insulin dependent diabetes mellitus
PDB	Pee Dee Belmnite
REE	Resting energy expenditure
SD	Standard deviation

SEM	Standard error of the mean
S_f	Sedimentation factor
TEF	Thermic effect of food
TG	Triglyceride
TRL	Triglyceride rich lipoproteins
VLDL	Very low density lipoprotein
W/H ratio	Waist/hip ratio
Wt	Weight

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I. 1. Literature Review

I. 1. 1. Obesity

I.1.1.1. Background

Obesity, defined as body mass index (BMI= weight/height^2) over 30 kg/m^2 is currently a common trait of Western countries [Hodge and Zimenet 1994]. Originally, obesity was never a health problem in the history of humankind nor has it been realistically plausible given the frequent food shortages. During episodes of famine and food scarcity, those who could effectively store energy were naturally selected as a consequence of the survival of the fittest. Genetic traits that favored obesity and promoted survival were selected too, particularly in the pregnant and nursing women. Fat storage, markedly around the lower abdomen would not only aid in the survival of the mother, but also is essential to that of her fetus, thus contributing to the preservation of the human species. In some rural cultures, "bride's fattening huts" still exist where brides spend days prior to their marriage consuming excess food so as to give them an earlier energy boost to last through the pregnancy period to follow [Ritenbaugh 1982].

Thus, obesity has its human biological and environmental roots in adapting to food shortage. However, with the advancement of civilization, food surplus and reliance on such energy saving inventions as motorized transport, lifts, escalators, computers and televisions, obesity has become an undeniable penalty of civilization. Progressively, humans have become one of the fattest species. This is graphically illustrated in that the Boeing's airplane designers have had to increase the assumed average passenger weight

by over 9 kg since its first flight. In the United States, the Second National Health and Nutrition Examination Survey (NHANES II) on 3838 men and 4173 women estimated the prevalence of obesity (BMI $\geq$ 30 kg/m^2) in 1976 – 1980 to be 12% in both men and women, an increase of 1.2 percent points from NHANES I in 1971-1974 [National Center for Health Statistics 1981]. In Canada, the prevalence of obesity in the 1981 Canada Fitness Survey on 4831 men and 5448 women was 9% in men and 8% in women [Hodge and Zimenet 1994]. In a more recent study in 1992 on 17,858 Canadians between the age of 18-74 years, the prevalence of obesity with a BMI $\geq$ 30 kg/m^2 was an alarming 15% in both men and women [Reeder et al. 1992]. Economic analysis attributes around 5% of the total health budgets of affluent countries to obesity. In the US, this has been estimated in the 1990's to be equivalent to an astonishing $69 billion, $45.5 billion of which are related to the direct cost of obesity associated disease [Wolf et al. 1994]. On the other hand, 2.4% of Canada's budget is spent on obesity related health problems [Birmingham et al. 1999].

I.1.1.2. Health Risks

There are many causes for the development of obesity ranging from the purely genetic (e.g. Prader-Willi syndrome) through the purely environmental (e.g. pastry cooks and sweet shop owners) to the purely behavioral (e.g. Sumo wrestlers) [Prentice 1997]. Though treatment should therefore be tailored to the cause, obesity in most of its forms shares the same detrimental health risks. Obesity is associated with decreased longevity and increased morbidity from a variety of disorders and disease.

Non-Insulin Dependent Diabetes Mellitus (NIDDM): It has long been recognized that obesity is a risk factor for NIDDM, and the World Health Organization

(WHO) study group has named obesity as the single most important factor in the development of NIDDM [World Health Organization 1985]. The risk of NIDDM increased with BMI above 30 kg/m^2 by 93-fold in women and 42-fold in men [Cham et al. 1994, Colditz et al. 1995]. Like obesity, NIDDM is associated with increased mortality and increased risk for various disorders, including hyperlipidemia, atherosclerosis of coronary, cerebral and peripheral vessels, and hypertension [Kissebah et al. 1989]. Approximately 80% of NIDDM individuals are obese and many obese individuals have NIDDM or impaired glucose tolerance [for review Kissebah and Krakower 1994].

Cardiovascular Disease: A number of cardiovascular risk factors are influenced by obesity including hypertension, impaired glycemic control, impaired haemostatic factors and dyslipidemia. Dyslipidemia is characterized by elevated plasma total cholesterol, triglyceride (TG), LDL cholesterol (LDL-C) and decreased HDL cholesterol (HDL-C), and elevated apolipoprotein B (ApoB) [Dattilo and Kris-Etherton 1992, for review Kissebah and Krakower 1994]. Moreover, cerebrovascular disease is common in the obese [Shinton et al. 1991]. It was believed that only severe obesity was associated with an increased risk of coronary heart disease. Recent findings, however, show a clear association with even the modest weight gain. In a recent 14-year prospective study on 114,281 female nurses aged 30-35 years old, who did not have NIDDM, coronary heart disease, stroke or cancer, Willet and colleagues showed that, compared to women with BMI < 25 kg/m^2, the risk of coronary heart disease was increased 2-fold in those women with BMI 25-28.9 kg/m^2 and 3.6-fold in those with BMI of greater than 29 kg/m^2 [Willet et al. 1995]. In males a 10% increase in weight will increase the risk of coronary heart

disease by 38% whereas a 20 % rise corresponds to an increased risk of 86% [for review Jung 1997].

Other Related Disease: Obesity is also associated with a number of other health complications. A large American study has shown that the mortality rate for cancer was increased by obesity, manifested as colorectal and prostate cancers in men and endometrium, gallbladder, cervix, breast and ovary cancers in women [Garfinkel 1985]. Digestive problems are also elevated with obesity particularly liver abnormalities and gall bladder disease which increases 2.7 fold with obesity [Rimm et al. 1975, Klain et al. 1989]. In addition, endocrine changes in the obese are associated with a higher risk of obstetric complications, infertility and respiratory problems resulting in sleep apnea, hypoventilation, arrhythmias and eventually cardiac failure [for review Jung 1997].

Perhaps one aspect that is frequently missed when dealing with the detrimental consequences of obesity is the negative psychological effect. Obese individuals sustain chronic psychological stress. They suffer a lifetime of bullying and social castigation and isolation which result in prejudice and impaired opportunities in education, employment, marriage and even medical services. Surveys in the US during the 1970's showed that obesity rated worse than a criminal conviction for rape as an undesirable feature in a potential marriage partner. Fortunately, these attitudes are now changing, partially because most people have at least one family member who is obese [Prentice 1997]. Nevertheless, although the stress is reduced, this chronic psychological stress further adds to the deteriorating health of the obese and decreases their quality of life.

I. 1. 2. Weight Loss

I. 1. 2. 1. Health Benefits

Weight loss decreases the prevalence of morbidity and mortality in the obese. Recent evidence indicates that weight loss of more than 9 kg in overweight women is associated with a 25% reduction in all causes of mortality (diabetic, cardiovascular and cancer). If the obese individual has already developed a weight associated disease then intentional weight loss of any amount has been shown to reduce mortality by 20% [Williamson et al. 1995].

Non-Insulin Dependent Diabetes Mellitus (NIDDM): In the nurses' study of 114,281 healthy women, those who gained 8 to 11 kg increased their risk of developing NIDDM by 2.7-fold compared to those with a stable weight. On the other hand, a weight loss of 9 kg reduced the risk of developing NIDDM by more than 50% [Colditz et al. 1995]. Weight loss improves or even reverses the abnormalities of glucose and insulin associated with NIDDM and impaired glucose tolerance, as well as reversing hyperinsulinemia in obese non-diabetic individuals. In NIDDM patients, a 9 kg weight loss reduces mortality by 30-40% in overweight women [Williamson et al. 1995], whereas in another study, a 5% weight loss was found to reduce fasting blood glucose by 15% and glycosylated hemoglobin by 7% [Wing et al. 1990]. A weight loss of 10-20% in the NIDDM patients can normalize metabolic control and possibly life expectancy [Wing et al. 1990].

Cardiovascular Risk Factors: It has been demonstrated that a weight loss of 11 kg produced a 20% decrease in both systolic and diastolic pressure in hypertensive patients even when sodium intake was kept constant. And it has been suggested that as a

general rule, blood pressure is reduced by 1 mm systolic and 2 mm diastolic for each 1% reduction in body weight [Reisen et al. 1978]. On the other hand, a meta-analysis by Dattilo and Kris-Etherton of 70 published studies has indicated that for every kg of weight lost, there is a corresponding reduction of about 1% in total cholesterol and LDL-C, a rise of 1% in HDL-C and a reduction of 3% of TG [Dattilo and Kris-Etherton 1992]. Such data on blood pressure, lipids and other haemostatic factors most likely account for the reduction in morbidity and mortality from cardiovascular disease with the modest weight loss.

Other Related Disease: A 10 kg weight loss decrease deaths from obesity related cancers by 40-50% [Williamson et al. 1995]. In addition, weight loss improves respiratory function decreasing sleep hypoxia and apnoeic episodes, and relieves pain in the lower back, ankles and feet in the obese. Moreover, improved mirror image and self esteem are probably the most direct psychological effect of weight loss leading to improved physiological and social quality of life [for review Jung 1997].

I. 1. 2. 2. Problems with Maintenance of Lost Weight

Obesity with its cost to the individual's health and that of the society, is not a simple burden but rather a rampant disease. Weight loss is sought for its associated physiological and psychological benefits. However, weight loss is usually maintained with great difficulty, most of obese individuals who lose significant amount of weight regain it [Prentice 1997]. Hypotheses regarding regaining lost weight and the reestablishment of obesity have been extensively studied for decades, but findings are far from conclusive. There are two distinct schools of thought regarding this issue.

On one hand, there are those who propose that genetically determined predictors of obesity inevitably result in weight regain in post-obese individuals. Some studies have reported that, compared to normal never-obese matched individuals, post-obese individuals have a lower 24-hr metabolic rate. This was demonstrated at fasting basal metabolic rates [Shah et al. 1988, Ranneries et al. 1998], postprandially, expressed as the thermic effect of food (TEF) [Shah et al. 1988] and at all levels of physical activities [Geissler et al. 1987], even after correcting for differences in lean body mass. Some have also demonstrated that in order for post-obese to maintain a reduced weight, they must restrict their food intake to 25% less than anticipated, based on their metabolic body size [Rudolph et al. 1984]. In addition, Astrup and colleagues found that post-obese women had decreased fasting fat/carbohydrate oxidation rate, and failed to increase fat/carbohydrate oxidation rate when dietary fat content increased from medium fat (30%) to high fat diets (50%), indicating that the preferential source of energy is carbohydrate thus allowing more fat to be stored [Astrup et al. 1993, Astrup et al. 1994].

In a study looking at the 5-hr postprandial response of post-obese women and closely matched never-obese controls after ingestion of a high fat meal (50% energy from fat), although TEF was similar in the two groups, fat oxidation was suppressed postprandially in the post-obese women. Of interest, the postprandial plasma triglyceride (TG) concentration was decreased in the post-obese group and only amounted to 43% of that in the controls [Raben et al 1994]. In addition, it was demonstrated that the decreased fat oxidation in post-obese individuals was not due to reduced fat mobilization from adipose tissue, as glycerol release was identical to that in matched controls at resting and upon aerobic exercise. Rather, it was a consequence of the reduced fat utilization as

energy substrate by different tissues [Astrup et al. 1993, Ranneries et al. 1998]. In addition, upon weight loss, post-obese subjects were found to have higher insulin sensitivity, as they required less insulin for the same level of glycemia as matched control individuals [Ranneries et al. 1998]. Consequently, the shift in substrate utilization from fat to carbohydrate in the post-obese was explained as one aspect of improved insulin sensitivity and glucose uptake in glycolytic tissues [Franssila-Kallunki et al. 1992].

On the other hand, there are those who do not support the hypothesis that post-obese have diminished energy expenditure and metabolic abnormalities. They believe that obesity and regaining lost weight result, quite simply, from overeating and decreased physical activity [Hervey and Tobin 1983, Weinsier et al. 1995]. In a prospective study on premenopausal obese women, although mean basal metabolic rate, thermic effect of food and fasting and postprandial substrate oxidation decreased after weight loss to normal BMI, they nevertheless were not significantly different from matched never obese subjects [Weinsier et al. 1995]. In other studies, neither the 24-hr energy expenditure, carbohydrate/ fat oxidation rate [Burstein et al. 1995], thermic effect of food nor energy expenditure during various controlled daily activities were different from those of matched never-obese subjects [de Peuter 1992]. In addition, findings regarding insulin sensitivity in post-obese are inconsistent. While Ranneries and colleagues showed an improved insulin sensitivity in post-obese women as compared to matched never-obese controls [Ranneries et al. 1998], the same group concluded in earlier studies that no significant difference existed [Astrup 1993, Toubro et al. 1994].

Discrepancies among different studies regarding fasting and postprandial energy expenditure, substrate oxidation and TEF were attributed to differences in body

composition, meal size and composition, and length of study [Raben et al. 1993, Reed and Hill 1996]. Of interest, in a study looking at 131 TEF tests from a wide range of subjects ingesting meals of varying sizes and compositions suggested that the shape of REE curve may detect differences between the obese and their matched controls when the trapezoid AUC of the REE curve alone cannot, and that ≥ 5 hr are required when measurement of TEF is attempted [Reed and Hill 1996]. Measurements that last for 4 hr only can miss up to 22.5% of actual TEF, and those that last for 3 hr only can miss 40%. Thus, when studying differences in TEF between obese individuals and their matched controls, the shorter the measurement of TEF, the more likely the differences would exist [Reed and Hill 1996], which may prove to be true when measurement of TEF is attempted to compare post-obese individuals, rather than obese, and their matched controls.

Clearly, there are opposing beliefs regarding the causes underlying the pronounced tendency of the post-obese to regain weight. We believe that there are indeed some metabolic differences associated with the post-obese state, which may be partially responsible for the facilitated weight regain in this group. However, we have investigated this question from a different perspective; that of an enhanced adipose tissue lipogenesis induced by the acylation stimulating protein pathway.

I. 1. 3. Acylation Stimulating Protein

I. 1. 3. 1. Characterization

Acylation Stimulating Protein (ASP) is a 76-amino acid basic protein, with a mass of 8,933 Dalton, which has been identified from human plasma. ASP markedly increases TG synthesis through fatty acids esterification in human adipocytes and skin fibroblasts [Cianflone et al. 1987, Cianflone et al. 1989, Baldo et al. 1993]. ASP is generated through the interaction of the complement factors B and D (or adipsin) with C3, which is then cleaved by the C3 convertase complex to generate C3a and C3b. ASP (C3a-desArg) is formed when the terminal arginine is removed from C3a by carboxypeptidase, which is present in excess in human plasma.

In vitro studies on mature adipocytes established that these cells possess mRNA for the three precursors of ASP, complement C3, adipsin and factor B, and at greater levels than in preadipocytes [Cianflone et al. 1994]. During adipocyte differentiation, increase in ASP production occurs after the increase in lipoprotein lipase (LPL) expression. However, TG synthetic capacity of these adipocytes significantly increases only following the increase in ASP production [Cianflone and Maslowska 1995].

On average, fasting plasma ASP is elevated with obesity, decreasing proportionally with weight loss [Sniderman et al. 1991, Cianflone et al. 1995, Maslowska et al. 1999]. In a study looking at fasting plasma ASP concentration in obese and non-obese individuals, it was found that fasting plasma ASP frequency distribution is skewed to the left in both groups [Maslowska et al. 1999]. In non-obese men (N = 82, age = 41 ± 11years, and BMI = 24.6 ± 3.2 kg/m^2, mean ± SD), median fasting plasma ASP concentration was 20.0 nM, with the 10th to 90th percentile between 10.9 to 38.8 nM. In

non-obese women (N = 101, average age = 39 ± 11, and BMI = 23.5 ± 3.5 kg/m^2, mean ± SD), median fasting plasma ASP concentration was 20.4 nM, with the 10th to 90th percentile between 14.5 to 40.8 nM). There is no statistically significant difference in median fasting plasma ASP concentration between non-obese men and women. On the other hand, in obese males (N = 12, average age = 41 ± 7 years, and BMI = 50.2 ± 15.0 kg/m^2, mean ± SD), median fasting plasma ASP concentration was 37.6 nM with the 10th to 90th percentile between 23.7 to 101.1 nM. In obese women (N = 42, average age = 41 ± 9 years, and BMI = 48.9 ± 8.5 kg/m^2, mean ± SD), median fasting plasma ASP concentration was 71.8 nM with the 10th to 90th percentile between 33.0 to 178.7 nM. In obese individuals, median fasting plasma ASP concentration was 1.9 fold higher in women than men [Maslowska et al. 1999].

I. 1. 3. 2. Regulation of Triglyceride Synthesis

In order for TG to be synthesized, a sequential coupling of three fatty acids to a glycerol-3-phosphate backbone is required. ASP affects both of these two components of the TG molecule, fatty acids as well as glycerol.

In vitro studies on isolated human adipocyte microsomes demonstrated that ASP had a significant effect on diacylglycerol acyltransferase, the last enzyme involved in TG synthesis, which adds a third fatty acid to the diacylglycerol, and which is believed to be the rate limiting step. ASP affected the maximal activity of diacylglycerol acyltransferase rather than affecting substrate delivery [Yasruel et al. 1991]. However, increased diacylglycerol acyltransferase activity will increase the rate at which fatty acids are incorporated in the cells to produce TG, and thus indirectly increase cellular fatty acid

uptake. In addition, both insulin and ASP were found to inhibit FFA release from fat cells, through stimulation of fractional FFA resterification, to the same extend by insulin and ASP, and by inhibition of FFA release during lipolysis, to a lesser extend by ASP than by insulin [Harmelen et al. 1999].

On the other hand, ASP has a direct effect on the transport of glucose, the precursor of glycerol, in cultured human skin fibroblasts [Germinario et al. 1993], human adipocytes [Maslowska et al. 1997], and rat muscle cells [Yuzhen et al. 1996]. This increase is both concentration and time dependent [Germinario et al. 1993, Cianflone et al. 1994]. ASP achieves this effect in the same manner as insulin, by causing translocation of glucose transporters (Glut 1, Glut 4, Glut 3) from intracellular vesicles to the cell surface, thereby increasing specific membrane glucose transport. The ASP effect on glucose transport in fibroblasts and muscle cells is independent of but additive to that of insulin, suggesting that they function *via* different mechanisms [Germinario et al. 1993, Yuzhen et al. 1996].

Insulin is less effective in stimulating glucose transport and TG synthesis when fat cell size increases with obesity [Ciaraldi et al. 1981, Kashiwagi et al. 1984]. However, unlike insulin, not only do adipocytes from morbidly obese subjects remain responsive to ASP [Walsh et al. 1989], but the extent to which adipocytes generate and are responsive to ASP is proportional to the degree to which they have accumulated TG mass during differentiation [Cianflone et al. 1994]. As it matures, the adipocyte increases expression of the elements of the ASP pathway, as well as the response to ASP through a positive feedback loop [Sniderman and Cianflone 1994]. Thus, as fat cells expand, as in the case

of obesity, ASP may favor the maintenance of the enlarged cell size, and thus the maintenance of obesity.

I. 1. 3. 3. Role in Postprandial Triglyceride Clearance

After ingestion of fat, postprandial plasma TG usually peaks to ≥ 2 times its fasting level at 3 to 4 hr, returning to fasting levels at 6 to 10 hr [Karpe et al. 1992, Bergeron and Havel 1997]. Postprandial hyperglycemia is dose dependent. In studies where 20-40 g of fat was ingested, plasma TG and triglyceride rich lipoptoteins TG (TRL-TG) returned to near postprandial values (15-25% of baseline) 6 hr after food ingestion, but remained significantly higher (40-100%) at the same time point when 80-120 g of fat was ingested [Murphy et al. 1995]. As many *in vitro* studies demonstrate an effect of ASP on increasing FFA reesterification and TG synthesis in adipose cells, it was suggested that ASP might play a significant role in enhancing postprandial TG clearance through its effects on enhanced adipose tissue TG synthesis.

To study postprandial ASP response after a fatty meal, blood samples were obtained from 12 subjects, after ingestion of a mixed meal with 60 g of fat, from an arterialized hand vein and an anterior abdominal wall vein that drains subcutaneous adipose tissue. Data from this study demonstrated that ASP is produced postprandially, as there was a positive postprandial ASP veno-arterial gradient across a subcutaneous adipose tissue bed, with maximal production at 3 to 5 hr. In addition, there was a correlation between the veno-arterial ASP production and calculated FFA uptake into adipose tissue [Saleh et al. 1998].

To identify the specific postprandial plasma component that stimulated ASP production, physiological concentrations of glucose, FFA, VLDL, LDL and HDL were added to cultured human adipocytes. None had any substantial effect on ASP production, whereas insulin resulted in a 2-fold increase in ASP production [Maslowska et al. 1997]. However, chylomicrons added at physiological concentrations (TG = 0-0.56 mM) to the cultured human adipocytes produced up to a 150-fold increase in generation of ASP and its precursor protein, C3, the effect being both time and concentration dependent. Insulin enhanced the effect of chylomicron on ASP generation, again demonstrating the interaction between insulin and ASP. Further studies demonstrated that the active chylomicron component responsible for the increase in ASP and C3 production was a protein loosely associated with the lipoprotein particle, transthyretin [Scantlebury et al. 1998]. Of interest, in a study looking at the response of plasma ASP to prolonged fasting, as plasma ASP level decreased with fasting and weight loss, plasma free fatty acid (FFA) and ketone bodies rose significantly. In addition, there was an inverse relationship between fasting plasma ASP and FFA [Cianflone et al. 1995].

To date, these *in vivo* and *in vitro* studies established the role of plasma ASP on enhancing adipose tissue FFA uptake and TG synthesis and suggest a role for plasma ASP in postprandial plasma TG clearance and thus, in the maintenance of obesity.

I. 2. Study Rationale

After consumption of a high fat meal, triglyceride (TG) circulate in plasma in triglyceride rich lipoproteins (TRL) namely, chylomicron (CHYLO) and very low density lipoprotein (VLDL) [Karpe et al. 1992, Bergeron and Havel 1997]. For the TRL to be cleared from plasma, two interconnected key processes are employed; hydrolysis of TG within the TRL core and uptake of the generated FFA into the peripheral tissues [Eckel et al. 1995]. Hydrolysis of TRL is regulated by lipoprotein lipase (LPL) [Karpe et al. 1992]. LPL is a secretory glycoprotein made in many tissues: adipose tissue, skeletal muscle, cardiac muscle, lactating mammary gland, and central nervous system [Eckel et al. 1995]. After its secretion, it is transported to the endothelial cells in the tissue of its origin where it is bound to the vascular endothelium through its affinity for heparin sulfate, and only low levels are present in plasma [Eckel 1989, Olivecrona and Bengtsson-Olivecrona 1990]. At the vascular endothelium, LPL acts on the core of the TRL hydrolyzing TG and releasing FFA and glycerol. Under normal metabolic condition, FFA released by the hydrolytic effect of LPL on TG are taken up to a larger extent by local tissue to meet the needs of that tissue, or circulate to other peripheral or hepatic tissues [Eckel et al. 1995].

Adipose tissue LPL activity is elevated in human obesity [Schwartz and Brunzell 1981]. One hypothesis suggests that elevated LPL is a primary defect in obesity that enhances the ability to "pull" TG into the cells. Although not conclusive, it has also been suggested that LPL activity rises further with weight loss [Schwartz and Brunzell 1981, Eckel and Yost 1987] and returns to lower, still elevated, values with weight regain [Schwartz and Brunzell 1981]. Further elevation of LPL with any weight drop tends to

enhance lipid clearance, increase TG storage, and restore the obesity state [Kern et al. 1990].

However, even with an enhanced LPL activity in the post-obese individuals, the rate of lipolysis cannot proceed beyond the limit of nearby cells to oxidize or store the FFA released. This should be expected given the potential toxicity of excess FFA concentrations on living cells. Excess plasma FFA impairs LPL activity causing both LPL and its activator apoliporptein-CII to detach from the endothelial wall, and lipolysis to diminish significantly [Peterson et al. 1990].

Therefore, we propose that there is a microenvironmental metabolic regulation of LPL activity on TG rich lipoprotein and FFA cellular uptake through the ASP pathway [Sniderman and Cianflone 1994]. ASP enhances the second step in TRL clearance by increasing normal FFA plasma clearance and intracellular esterification to form TG [Cianflone et al. 1987, Yasruel et al. 1991, Baldo et al. 1993]. As long as individuals have a normal ASP function, FFA released by the action of LPL on postprandial TRL will be cleared from the capillary space and LPL will continue to function. However, an exaggerated postprandial ASP response, manifested by increased plasma ASP concentration or increased cell sensitivity to ASP, will accelerate the rate at which FFA are taken up into the cells, preventing excess FFA from accumulating, and allowing LPL activity to proceed.

In North America, an average person consumes at least 3 meals per day, with fat contributing to 35- 40% of the total daily caloric intake [for review Golay and Bobbioni 1997]. This excess daily high fat intake along with an ASP enhanced postprandial cellular

FFA uptake and TG storage, could indeed sustain a constant push to store the exogenous fat, thus contributing to weight regain in the post-obese individuals.

I. 3. Study Objectives and Hypotheses

The primary objective of this study is to examine whether, compared to never-obese closely matched controls, post-obese women have metabolic differences in postprandial fat metabolism and plasma ASP concentration when challenged with an oral fat load. The secondary objective is to study postprandial plasma ASP response in relation to REE, TEF, carbohydrate/fat oxidation rate and insulin sensitivity, which are factors that have been implicated in the maintenance of the obesity state in the post-obese individuals.

In brief, this was done through following the fate of a ^{13}C-labeled high fat breakfast meal for 8 hr after food ingestion in 3 plasma pools and in expired breath CO_2, and indirectly assessing adipose tissue exogenous fat uptake. The 3 plasma pools were: TG fraction in TRL with density fraction corresponding to sedimentation factor $S_f > 400$ (referred to as CHYLO-TG), TG fraction in TRL with density fraction $S_f = 20 - 400$ (referred to as VLDL-TG), and in plasma FFA. In addition, measurements of various blood parameters (ASP, insulin, glucose, total TG, CHYLO-TG, VLDL-TG, and FFA), and metabolic parameters (REE, TEF and carbohydrate/fat oxidation rate) were conducted along the same time course of 8 hr.

We believe that, after ingestion of a high fat meal, the post-obese women would have an enhanced postprandial plasma ASP response, an enhanced plasma TRL clearance

rate, and an increased adipose tissue uptake of the exogenous fat, which would progressively favor their weight regain.

Thus, we hypothesize that as a response to the ingestion of the ^{13}C-labeled high fat meal:

1) Compared to matched never obese controls, the post-obese women would have;

A. greater % change in postprandial plasma ASP concentration from baseline

B. lower postprandial plasma total TG, CHYLO-TG, VLDL-TG and FFA concentrations, and an earlier return of the postprandial values of these plasma parameters to fasting baseline values. This would indicate faster clearance of postprandial plasma total TG and TRL-TG.

C. lower % of recovered ^{13}C in breath CO_2 per hour, lower ^{13}C concentration in CHYLO-TG, VLDL-TG and FFA pools, and an earlier return of postprandial ^{13}C concentration in plasma CHYLO-TG and VLDL-TG pools to fasting non-enriched baseline values. This would indirectly indicate greater adipose tissue uptake of the exogenous fat.

2) Area under the 8 hr time curve (AUC) of the % change in plasma ASP in the post-obese and control women would correlate:

A. negatively with TEF

B. positively with AUC of carbohydrate/fat oxidation rate

C. negatively with AUC of serum insulin x glucose concentrations

CHAPTER TWO
METHODS & PROCEDURES

II. 1. Study Population

II. 1. 1. Inclusion Criteria

Subjects for the control (never-obese) and post-obese groups were selected according to the following inclusion criteria;

- 30 - 50 years old women, with regular menstruation.
- Body mass index (BMI) of 20 - 27 kg/m^2.
- Normal fasting plasma concentration of total plasma TG (< 1.8 mM), plasma FFA (0.1-0.8 mM), total plasma cholesterol (< 6.4 mM), plasma HDL-C (0.8 - 2.3 mM) plasma LDL-C (1.3 - 4.9 mM), plasma, ApoB (45 – 120 mg/dl), serum insulin (36 - 179 pM), and serum glucose (2.6 - 5.2 mM) [Pagana and Pagana 1997].
- No reported history of cardiovascular disease, diabetes, hypertension, gastrointestinal, respiratory or any hormonal disorders.
- Not taking any medication that can affect lipid metabolism for 6 months prior to the study.
- Stable weight (Wt) for 6 months prior to the study (no loss or gain ≥ 4.5 kg).
- Not claustrophobic.

  Measurement of energy expenditure through indirect calorimetry requires the use of a transparent plastic canopy that covers the head, which is claustrophobic for some subjects and can affect the accuracy of the measurement due to the stress factor.
- No reported history of obesity or Wt problems (the never-obese group)

- No reported present post-operational gastrointestinal complications such as delayed or fast gastric emptying, also known as dumping syndrome (the post-obese group).

II. 1. 2. Matching Criteria

The post-obese women were recruited before the control women, thus, minimum to maximum ranges for some selected parameters before the study date were obtained, according to which, the control women were matched to the post-obese. The control women were matched to the post-obese women according to the following parameters:

- Age, as the post-obese women age ranged from 31- 48 years old.
- BMI, as the post-obese women BMI ranged from 20.1 - 26.8 kg/m^2.
- Body surface area (BSA), as the post-obese women BSA ranged from 1.55 – 1.84 m^2
- Percent body fat (% body fat), as the post-obese women % body fat ranged from 22.0 – 40.0 %.
- Waist/hip (W/H) ratio, as the post-obese women W/H ratio ranged from 0.66 – 0.84.
- Fasting total plasma TG and cholesterol, as the post-obese women total plasma TG ranged from 0.40 – 1.25 mM, and total plasma cholesterol ranged from 3.30 – 5.60 mM. Postprandial plasma TG responses increase with rising fasting basal TG level [Bergeron and Havel 1997]. Therefore, matching was done in order to eliminate the effect of inter-subject variation in fasting lipid levels on subsequent postprandial lipemia, and to eliminate significant difference among the two compared groups in previous dietary composition, if existing, particularly regarding fat quality and quantity.

- Habitual level of physical activity, as all subjects had light physical activity habits. Light physical activity was estimated as the level of activity for most housewives, office workers, doctors, teachers, lab technicians who do not maintain regular physical cardiovascular exercise every day.
- Physical activity and alcohol consumption for 3 days prior to the study. Measurement of % body fat is done by measuring electric conductivity through fat free mass (FFM), and is highly influenced by the hydration state of the body [Khaled et al. 1988]. Therefore subjects were instructed not to exercise, engage in heavy cardiovascular activity nor consume alcoholic beverages for the 3 days prior to the assessment of % body fat, so as not to create an artifact due to dehydration.
- Stage in menstrual cycle, as all subjects were between 2-7 days after menstruation (i.e. follicular stage). At this stage during menstruation, sex hormones such as estrogen and progesterone are at their lowest levels, and energy expenditure is at its minimum [Webb 1987, Bisdee et al. 1989]. Therefore this stage was selected to eliminate possible effects of inter-subject variation in sex hormones on plasma ASP, lipid metabolism and energy expenditure in both groups.
- Smoking, as 3 post-obese women were light smokers (less than half a package per day). Although tobacco smoking effect on plasma ASP has never been looked at, it is known to increase sympathoadrenal activity and may influence fat metabolism [Ranneries et al. 1998]. Thus, 3 never obese women were light smokers, and none of the women was allowed to smoke during the study.

II. 1. 3. Recruitment and Sample Size

The cardiovascular research unit at the Royal Victoria Hospital has been keeping records of patients who have undergone the gastric bypass procedure at the hospital between 1993 – 1995. Subjects who had this operation were morbidly obese with a BMI of ≥ 40 kg/m^2. In this procedure, the stomach is reduced to 30 % of its original volume by applying a multiple staple line horizontally along the stomach, with an opening of the new stomach pouch into the small intestine [Benotti and Forse 1995]. An empty stomach has a volume of 50 ml and can expand to 1500 ml with food consumption [Zeman 1991]. Thus, the new volume of gastric bypass stomach can expand to $\approx$ 500 ml with food consumption. Thus this type of bypass induces weight loss by obliging the obese subject to eat far less than normal, due to the reduced stomach size, and not by inducing controlled malabsorption as in some other procedures like jejuno-ileal bypass and biliopancreatic bypass [Benotti and Forse 1995].

Among the list of women who had the gastric bypass procedure at the Royal Victoria Hospital, those between 30 – 50 years old were contacted by phone, with permission of their physician, to screen for suitable subjects according to the inclusion criteria. Only eight post-obese women matched the inclusion criteria and agreed to participate, and thus were included in the study. The post-obese women were recruited before the controls, thus minimum to maximum ranges for the post-obese group regarding age, BMI, BSA, % body fat, W/H ratio, fasting plasma TG and cholesterol were defined according to which the control women were selected.

The control (never-obese) women were recruited through advertisements posted at the Royal Victoria Hospital, Montreal General Hospital, and McGill Campus. They

were selected by systemic sampling technique; the first 8 women who called in, who were qualified according to the inclusion criteria, and could be matched to the post-obese women were selected.

To determine qualified subjects from both groups, personal information regarding age, Wt history, menstruation, health problems, medications, alcohol, smoking and activity habits, and complications after the gastric bypass procedure (for the post-obese group) were obtained through a baseline questionnaire (Appendix A). The baseline questionnaire was completed by the study coordinator, during an interview with the subjects, in person or over the phone. In addition, baseline Wt, height (Ht), BMI, BSA, W/H ratio, % body fat, fasting plasma TG, total plasma cholesterol, plasma ApoB, plasma FFA, serum insulin and glucose were measured prior to the study date. These measurements were conducted to ensure normal values of the BMI and blood parameters and to match the control to the post-obese group according to the matching criteria. All subjects signed an informed written consent form prior to enrollment in the study. This study was approved by the Ethics Review Committee of the Royal Victoria Hospital.

II. 2. Study Design & Intervention

This study was cross-sectional in design (Appendix B). On the study day, subjects came in at 8 am after an overnight 12 hr fast (i.e. post-absorptive state). Subjects were instructed not to consume any alcoholic beverages or exercise for three days prior to the study. All subjects were on their usual diets before the study day. Fasting baseline Wt, Ht, BMI, BSA, % body fat, fasting REE, substrates oxidation rate were measured, and blood and breath samples were collected. A high fat mixed breakfast meal was given to

the subjects which was fully ingested over a period of 20 min, under the supervision of the study coordinator. All subjects were given the same meal considering the narrow range of BMI and BSA selected. The high fat breakfast meal consisted of:

60 g (2 slices)	white bread
1	large egg, omelet with cheese and whipped cream
2.5 tablespoon	whipping cream, 35% energy from fat
20 g	cheddar cheese, 33% energy from fat
105 g	bacon strips
70 g	banana, peeled
10 g	margarine, unsalted
16 g	peanut butter, smooth, unsweetened
16 g	raspberry jam, sugar free

(N.B. total meal weight was 384 g, and total homogenized volume ≈ 250 ml)

The same food brands were used for all the meals prepared so as to maintain consistent nutrient composition and ^{13}C background natural abundance. The nutrient analysis of this high fat meal was calculated based on the manufacturer's nutrient information available on the food labels combined with a nutritional software (The Food Processor for Windows, version 6.0, ESHA research, Salem, Or), with a Canadian database.

As such, the high fat meal consisted of 4446 joules (1062 kcal), which was, on average, equal to ≈ 60% of the daily caloric requirements for the selected range of subjects' age and BMI. The macronutrient distribution was; 12 % of energy from protein (31.9 g), 21% of energy from carbohydrate (55.8 g) and 67 % of energy from fat (80.0 g),

constituting of 25% saturated, 26% mono and 10% polyunsaturated fats in addition to 6% of other missing sources. Such a high fat and high energy meal was selected as to provide an equal acute challenge, above the normal fat and energy intake per meal of both groups, as average daily fat intake in North America = 35-40% energy from fat [for review Golay and Bobbioni 1997]. The high fat meal was labeled with 1.430 ml (1.280 g) of ^{13}C-oleate (99 % enrichment) (Cambridge Isotopes, Xenia, OH). ^{13}C-oleate mixes freely with other hydrolyzed dietary fat in the intestine, and gets incorporated along other long chain fatty acids into chylomicron [Wolfe 1992]. Oleate, as a label for the long chain dietary fat, was chosen because it is the most abundant fatty acid in dietary fat source [Willem and Vergroesen 1994], plasma TG (40.7%) and adipose tissue (46.2%) [Christie et al. 1971], and its metabolism is representative of other long chain unsaturated fatty acids [Beylot 1994]. The ^{13}C-oleate was spread over the 2 slices of toast. All subjects were instructed to eat the labeled toasts slowly throughout the meal.

After the initiation of food intake, postprandial blood samples, breath samples, energy expenditure and substrate oxidation rates measurements were collected at 2, 4, 6, and 8 hr, in addition to the previous fasting measurements. During the 8 hr study period, subjects were not allowed to smoke, or consume any food or beverage, except for water and 1 cup of decaffeinated tea or coffee (no sugar or milk added). They reclined on a sofa-bed and engaged in sedentary activities such as reading or watching TV, and were confined to the research unit. This was to eliminate the effect of activity on increasing measured REE, thus postprandial increase in energy expenditure could be solely attributed to the TEF.

There are some important issues to note when dealing with subjects who have a gastric bypass procedure. Studies have demonstrated that gastric bypass procedures can result in normal, delayed or rapid gastric emptying also called dumping syndrome. Delayed gastric emptying mostly occurs with solid food intake and usually normalizes with time, whereas rapid gastric emptying, the more frequent complication of gastric bypass procedures, usually occurs with ingestion of liquid or semi-liquid foods [Horowitz et al. 1982, Behrns and Sarr 1994]. Post-obese subjects who had dumping or delayed gastric emptying or any other gastrointestinal complication of the gastric bypass were automatically excluded at the recruitment stage. However, in order to avoid dumping in the post-obese group during the study, certain dietary conditions were followed. Rapid gastric emptying can be avoided by proper dietary therapy which controls this syndrome up to 95% [Behrns and Sarr 1994]. Therefore, the breakfast meal was free of simple carbohydrates and consisted of solid food only, with no beverage allowed during food intake or directly after. In addition, the post-obese subjects were instructed to eat slowly, over the 20 min period, and to lie down on a sofa bed after food consumption. All subjects from both groups were monitored during and after food consumption. The meal was well tolerated, and neither the post-obese women, nor the controls, reported any feelings of dizziness, flushing, sweating, or nausea which are symptoms associated with fast or delayed gastric emptying [Gustavsson et al. 1988, Behrns and Sarr 1994].

II. 3. Analysis and Measurements

II. 3. 1. Anthropometric Measurements

Subjects' height was measured with a meter to the closest 50 cm. Percent body fat was determined at fasting state with Bioelectric impedance analysis technique (Tanita Incorporation, Skokie, Illinois). In this technique, a light electric current is passed through the body and electric conductivity through FFM is measured [Khaled et al. 1988]. Fat mass is indirectly measured by subtracting the subject's FFM from total body Wt. To measure % body fat, subject's sex and height were entered into the Tanita memory, then the subject stepped barefoot on the scale, after rubbing the soles of their feet with alcohol, and a printout of % body fat, Wt, FFM, fat mass was obtained.

Waist circumference was measured as the horizontal circumference around the midway between the lowest rib margin and the iliac crest, where as the hip circumference was measured at the point yielding the maximum circumference over the buttocks [Jones et al. 1986]. Waist to hip (W/H) ratio was obtained by dividing waist circumference over hip circumference.

Subject's BSA was calculated based on Wt and Ht using DuBois approximation [Wang et al. 1992];

$$BSA = Wt^{0.425} \times Ht^{0.725} \times C \quad \text{......} \quad 1$$

Where BSA = Body surface area in cm^2

Wt = Weight in kg

Ht = Height in cm

C = Constant = 71.84

II. 3. 2. Resting energy Expenditure, Thermic Effect of Food and Carbohydrate/Fat Oxidation Rate Measurements

Resting energy expenditure was assessed through open circuit indirect calorimetry using the Deltatrac instrument (SensorMedics Corporation, Anaheim, CA) which was calibrated for every subject using a reference gas with 96% O_2 and 4% CO_2. As first demonstrated by Atwater and Benedict, indirect calorimetry is a technique that measures energy expenditure, or heat, through quantitation of O_2 consumption and CO_2 production [Donald and Ralph 1990].

Measurement of REE was taken at fasting state at 0 hr, and postprandially at 2, 4, 6 and 8 hr. After 15 min bed rest to equilibrate, subject's O_2 consumption and CO_2 production were measured using a plastic transparent canopy that covered the head completely while the subject was lying down. Measurements were carried out for 20 min, of which the first 5 min were automatically discarded. During the measurements, the subject remained awake and at comfortable room temperature. The Deltatrac employs the Weir equation for the calculation of REE [Cunningham 1990]:

$$REE = 5.68\ VO_2 + 1.59\ VCO_2 - 2.17\ N_u \quad \text{.....} \quad 2$$

Where REE = Resting energy expenditure in Kcal/ 24 hr

VO_2 = O_2 consumption in ml/min

VCO_2 = CO_2 production in ml/min

N_u = Uninary nitrogen excretion in g/24 hr

(N.B. Urinary nitrogen excretion was assumed to be an average of 12 g/day [Schutz et al. 1991]. Actual differences in nitrogen excretion due to previous 24 hr protein intake results in a negligible difference in calculated energy and will not influence the relative

partitioning between carbohydrate and fat oxidation determined during indirect calorimetry [Schutz et al. 1991].

To measure TEF, the trapezoid AUC of the increment increase in energy expenditure above fasting level after ingestion of the fat load was calculated as:

$$TEF = AUC \text{ of } REE - (REE_{fast} \times 8) \quad \ldots\ldots 3$$

Where TEF = Thermic effect of food in Kcal/8 hr

AUC = Trapezoid Area under the 8 hr time curve of resting energy expenditure in Kcal/8 hr

REE_{fast} = Fasting resting energy expenditure in Kcal/hr

Substrate oxidation of carbohydrate, fat and protein were automatically calculated by Deltatrac using the following equations:

$$CHO = 5.926\ VCO_2 - 4.189\ VO_2 - 2.539\ N_u \quad \ldots\ldots 4$$

$$Fat = 2.432\ VO_2 - 2.432\ VCO_2 - 1.943\ N_u \quad \ldots\ldots 5$$

$$Protein = 6.250\ N_u \quad \ldots\ldots 6$$

Where CHO = Carbohydrates oxidized in g/24 hr

Fat = Fat oxidized in g/24 hr

Protein = Protein oxidized in g/24 hr

VO_2 = O_2 consumption in ml/min

VCO_2 = CO_2 production in ml/min

The carbohydrate oxidation rate in grams was divided by the fat oxidation rate in grams to obtain the ratio of carbohydrate to fat oxidation rate (carbohydrate/fat oxidation rate). Coefficient of variation of the 15 min measurements of REE and substrate oxidation was < 6%.

II. 3. 3. Blood Parameter Measurements

Venous blood samples (40 ml) were collected in 4 (10 ml) vacutainer tubes with (1 tube) and without (3 tubes) anticoagulant (EDTA), at fasting and postprandial states at 0, 2, 4, 6 and 8 hr. Total volume of blood collected during the study was 200 ml. Blood was immediately centrifuged at 1500 rpm, at 4° C for 15 min (Beckman J6-MC Centrifuge, Palo Alto, CA). Plasma (EDTA tubes) and serum aliquots were frozen away at −80° C for later measurements of plasma ASP, plasma lipids, serum insulin and glucose in duplicates. The remaining plasma was kept at 4° C for the isolation of CHYLO, VLDL and FFA fractions, which was immediately performed.

II. 3. 3. 1. Plasma ASP Concentration

Plasma ASP was assayed by in-house enzyme linked immunosorbant assay (ELIZA) [Saleh et al. 1998]. ASP precursor (C3) was precipitated in plasma samples, in in-house plasma controls samples, and in standard ASP samples at 4° C for 90 min with 20% solution of polyethylene glycol (PEG) 8000 (Fisher, Nepean, ON) in phosphate buffered saline (PBS) for a final PEG concentration of 10%. Samples were centrifuged at 2300 rpm, at 4° C for 20 min and the supernatant was used to assay for ASP. In-house monoclonal anti-ASP antibody 4H3 (7ug/ml in PBS, 100 µL/well) was coated, overnight at 4° C, on Nunc-Immuno Module MaxiSorp F8 plates (Gibco, Missisaga, On). The plates were blocked for 2 hr, at room temperature with 250 µL/well of bovine serum albumin (1.5% in PBS) (Sigma, St. Louis, MO). PEG precipitated plasma samples and in-house plasma controls were diluted in PBS (1:40) and added to the plate at 100 µL/well. ASP standards (100 µL/well) were also added to each plate for a concentration curve ranging from 0.08 nM to 3.06 nM. Plates were incubated for 90 min at 37° C in a shaking bath.

The plates were then washed with wash solution, 0.05% Tween 20 (Sigma, St. Louis, MO) in 0.9% NaCl and incubated for 90° C min at 37° C with 100 μL of rabbit polyclonal anti-ASP antibody (1:2000 dilution in PBS). Goat anti-rabbit IgG conjugated to horseradish peroxidase (100μL , 1:3000 dilution in 4% PEG in PBS, 0.05% Tween 20) was added for 30 min at 37° C. Finally the plates were rinsed and the color reaction was initiated by the addition of 100 μL of phenylenediamine dihydrochloride (0.5 mg/ml in 100 mM NaCitrate, pH 5.0, containing 0.005% Tween 20). The reaction was stopped using 50 μL/well of 4 N H_2SO_4 solution and absorbance was read with a spectrophotometer (LKB Biochrom Ltd, Cambridge, England) at a wavelength of 490 nm. A log [wavelength] versus log [concentration] curve was used to calculate the sample concentration. The linear range of the concentration curve was from 0.14 nM to 1.83 nM with a coefficient of correlation > 0.98, and a coefficient of variation between the measured duplicates of < 5%.

II. 3. 3. 2. Serum Insulin and Glucose Concentrations

Serum insulin and glucose were measured at fasting and postprandially at 2, 4, 6, and 8 hr. Although serum insulin and glucose are expected to peak postprandially earlier than 2 hr, between 30 min to 1.5 hr [Astrup et al. 1994], it was not practicable to withdraw blood at more time points because of the large amount of blood volume already needed (200 ml). Fasting and postprandial blood insulin was assayed in serum samples by commercial radio-immunosorbant assay (RIA) for human insulin (Medicorp Incorporation, Montreal, QC). Samples were counted for one minute in a gamma counter (LKB-Wallac Ria Gamma 1274, Wallac Oy, Finland). The standard curve range was

0-2000 pM. A [radioactivity] versus log [concentration] curve was used to calculate glucose concentration with a coefficient of correlation > 0.99, and coefficient of variation between the measured duplicates of < 5%. Fasting serum insulin was considered normal if it was between 36 – 179 pM.

Fasting as well as postprandial blood glucose was assayed in serum samples by a commercial enzymatic colorimetric kit (Sigma, St. Louis, MO). The standard concentration curve range was 0 – 11.0 mM and absorbance was read with a spectrophotometer at a wavelength of 490 nm. An [absorbance] versus [concentration] curve was used to calculate glucose concentration with a coefficient of correlation > 0.99, and a coefficient of variation of the measured duplicates of < 5%. Fasting serum glucose was considered normal if it was between 2.6 – 5.2 mM.

II. 3. 3. 3. Plasma Lipid and Apolipoprotein B Concentrations

Total plasma TG concentration, as well as CHYLO-TG and VLDL-TG concentrations were measured at fasting state and postprandially at 2, 4, 6, and 8 hr after food consumption. Isolation of the density fractions corresponding to CHYLO and VLDL was performed by ultracentrifugation (Beckman L8-80 Ultracentifuge, Palo Alyo, CA). Plasma was centrifuged with 1.006 g/ml NaCl density solution at 30,000 rpm at 4° C for 30 min in Ti50 rotor. The floating milky top layer containing the CHYLO fraction (density fraction with $S_f > 400$) was removed and CHYLO-TG concentration and volume were measured, after correction for dilution with the density solution. The remaining solution was centrifuged at 40,000 rpm at 4° C for 18 hr in Ti50 rotor [Havel et al. 1955]. The floating top layer containing the VLDL fraction (density fraction with $S_f = 20$-400)

was removed and the VLDL-TG concentration and volume were measured, again after correction for the dilution factor. The remaining solution, the CHYLO solution and VLDL solution were used for isolation of FFA, TG fraction in CHYLO and in VLDL respectively by thin layer chromatography (see below).

Plasma total TG, CHYLO-TG and VLDL-TG were assayed by a commercial enzymatic colorimetric kit (Boehringer Mannheim Biochemica, Laval, QC). The standard curve range was 0 - 2.25 mM. Absorbance was read with a spectrophotometer at a wavelength of 490 nm. An [absorbance] versus [concentration] curve was used to calculate TG concentration with a coefficient of correlation of > 0.99, and a coefficient of variation between the measured duplicates of < 5%. Fasting plasma total TG was considered normal if it was < 1.8 mM.

Total plasma FFA was measured at fasting and postprandial states by a commercial enzymatic colorimetric kit (Boehringer Mannheim Biochemica, Laval, QC). The standard curve range was 0 - 1.0 mM and absorbance was read with a spectrophotometer at a wavelength of 490 nm. An [absorbance] versus [concentration] curve was used to calculate FFA concentration with a coefficient of correlation > 0.99, and a coefficient of variation between the measured duplicates of < 5%. Fasting plasma FFA was considered normal if it was within a range of 0.1 – 0.8 mM.

Total cholesterol and HDL-C concentrations were measured in fasting samples only as cholesterol concentration does not change significantly through out the fat load. (previously tested in a pilot study on 6 subjects). Cholesterol was assayed by a commercial enzymatic colorimetric kit (Boehringer Mannheim Biochemical, Laval, QC). Plasma HDL-C was separated according to Gidez by heparin/manganese chloride

precipitation (Sigma, St. Louis, MO and Fisher, Nepean, ON) (1:1, v/v) [Gidez et al. 1982]. The standard curve range was 0 - 10.4 mM and absorbance was read with a spectrophotometer at a wavelength of 490 nm. An [absorbance] versus [concentration] curve was used to calculate total cholesterol and HDL-C concentrations with a coefficient of correlation > 0.99, and a coefficient of variation between the measured duplicates of total cholesterol and HDL-C of < 5%. Fasting plasma cholesterol was considered normal if it total plasma cholesterol was < 6.4 mM, and HDL-C was within a range of 0.8 – 2.3 mM. Plasma LDL-C was calculated according to Friedewald [Schectman et al. 1996] as:

$$\text{LDL-C} = \text{Total Chol} - (\text{HDL-C}) - \text{TG}/2.3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 7$$

Where LDL-C = Low density lipoprotein cholesterol in mM

Total Chol = Total cholesterol in mM

HDL-C = High density lipoprotein cholesterol in mM

TG = Total triglyceride concentration in mM

Fasting LDL-C was considered normal if it was within a range of 1.3 – 4.9 mM.

Fasting plasma ApoB concentration was measured by nephlometric technique in clinical biochemistry laboratory at the Royal Victoria Hospital. Plasma ApoB was considered within the normal range if it was between 45 – 120 mg/dl.

II. 3. 4. Stable Isotope Measurements

II. 3. 4. 1. Breath Samples

Breath samples were collected at fasting and postprandially at 0, 2, 4, 6 and 8 hr after ingestion of the fat load. Immediately after terminating the indirect calorimetry measurements, breath samples at each time point were collected by blowing into two 2-liter urine bags. To trap CO_2 in the breath samples, samples were slowly bubbled into a 100 cm spiral glass trap containing 10 ml of 1 N NaOH solution [Jones et al.1985]. NaOH solution was frozen in sealed and labeled polypropylene tubes at –80° C for later determination of ^{13}C enrichment in breath CO_2 within 2 months.

Frozen NaOH samples were thawed on the day of the isotope enrichment analysis. CO_2 trapped in NaOH solution was released by addition of 2 ml of O-phosphoric acid 85% (Fisher, Fair Lawn, NJ) to 2 ml of NaOH solution in a vacutainer [Jones et al.1985]. Vacutainers containing CO_2 gas samples, in duplicates, were directly transferred to a Dual Inlet Stable Isotope Ratio Mass Spectrometry (IR/MS) (Vacuum Generators, Cheshire, UK)] for ^{13}C enrichment analysis. In the dual inlet IR/MS system, each sample is compared with a reference standard under identical instrumental conditions. The reference limestone standard, Pee Dee Belemnite (PDB) is defined to be 0 ppm [Wolfe 1992]. The instrument was calibrated using CO_2 gas of known isotopic enrichment of $^{13}C/^{12}C = 0.0107403$ (Delta ^{13}C (‰) = -44.221 ± 0.296 ppm).

^{13}C enrichment was calculated using the following formula [Binnert et al. 1995]:

$$\delta\,^{13}C\,(‰)_{t=i} = [(R_{S\,t=i} - R_{PDB}) / R_{PDB}] \times 10^3 \quad \text{.....} \quad \mathbf{8}$$

Where $\delta^{13}C\ (‰)_{t=i}$ = Delta at time = i hr in ppm (part per million)

$R_{S\ t=i}$ = $^{13}C/^{12}C$ of the sample at time = i hr

R_{PDB} = $^{13}C/^{12}C$ of PDB = 0.0112372

^{13}C enrichment in breath samples was expressed as % of the administered ^{13}C dose recovered in breath CO_2 per hour, and was calculated according to (Schoeller 1980):

$$\%\ ^{13}C_{rec/hr} = \frac{\text{mM excess } ^{13}C/\text{mM } CO_{2\ rec}}{\text{mM } ^{13}C_{administered}} \times (\text{mM } CO_{2\ excreted/hr}) \times 1.25 \times 100 \quad \ldots\ldots\ 9$$

Where $\%\ ^{13}C_{rec/hr}$ = % administered ^{13}C recovered in breath CO_2/hr

mM excess ^{13}C/mM $CO_{2\ rec}$ = $(\delta^{13}C(‰)_{t=i} - \delta^{13}C(‰)_{t=0})\ R_{PDB} \times 10^{-3}$ ... **10**

mM ^{13}C administered = $\frac{\text{mg } ^{13}C\text{-oleate}}{M} \times \frac{(P) \times (n)}{100}$ **11**

mg ^{13}C-oleate = Weight of administered ^{13}C-oleate = 1280 mg

M = Molecular weight of ^{13}C-oleate = 283.45

P = ^{13}C isotope purity = 99

n = Number of labeled carbon position = 1

mM $CO_{2\ excreted/hr}$ = (BSA) x 300 $mM/m^2.hr$ **12**

mM $CO_{2excreted/hr}$ = Excreted breath CO_2 in mM/hr

BSA = Subjects' body surface area in m^2

1.25 = Correction factor to adjust for uptake of label into the HCO_3 pool [Irving et al. 1983]

Coefficient of variation between the measured duplicates of $^{13}CO_2$ enrichment in breath CO_2 samples was < 2%.

II. 3. 4. 2. Blood Samples

^{13}C enrichment was measured in plasma FFA and the TG fraction of CHYLO and VLDL at fasting and postprandial states at 0, 2, 4, 6, and 8 hr. The lipid layer was extracted from CHYLO, VLDL and FFA samples according to Bligh & Dyer with 5 times chloroform/ methanol (2:1, v/v) (Fisher, Nepean, ON). The organic extract was then evaporated under nitrogen gas. Dried extracts were re-dissolved in chloroform, and FFA (Retardation factor: R_f = 0.22) and TG (R_f = 0.5) were separated by thin layer chromatography on LK5 silica gel plates 150A° (Whatman, Clifton, NJ) using hexane/ ether/ acetic acid (Fisher, Nepean, ON) (75:25:1, v/v/v) as mobile phase. TG and FFA were stained with iodine vapor and scraped off the plates then extracted from silica gel powder with hexane/ chloroform/ diethyl ether (5:2:1, v/v/v) (Fisher, Nepean, ON).

TG and FFA samples were dried under N_2 and transferred into 18 cm combustion tubes (Vycor, Corning GlassnWorks, Corning, NY). CuO (0.6 g) and 2 cm silver wire were added, and tubes were flame sealed under vacuum at less than 20 mtorr pressure. TG and FFA samples were fully combusted at 520° C for 4 hr. The generated CO_2 and H_2O were separated by trapping H_2O with a –95° C methanol slurry, and CO_2 in a vacutainer submerged in a –198° C liquid N_2 bath [Jones et al. 1985]. Vacutainers containing the CO_2 samples were analyzed in duplicates for ^{13}C enrichment within 12 hr.

Analysis of ^{13}C enrichment in the plasma FFA samples and the CHYLO-TG and VLDL-TG samples in duplicates were conducted in the IR/MS. TG or FFA samples before combustion weighed between 0.25 - 0.9 mg/ sample in order to generate enough CO_2 after combustion to be measured on the IR/MS. Enrichment was calculated using the following formula [Binnert et al. 1995]:

$$\delta\,^{13}C\ (‰)_{t=i} = [(R_S - R_{PDB}) / R_{PDB}] \times 10^3 \quad \ldots\ldots\ldots\ldots\ 8$$

Where $\delta\,^{13}C\ (‰)_{t=i}$ = Delta at time = i hr in ppm (part per million)

$R_{S\,t=i}$ = $^{13}C/^{12}C$ of the sample at time = i hr

R_{PDB} = $^{13}C/^{12}C$ of PDB = 0.0112372

^{13}C enrichment in plasma CHYLO-TG, VLDL-TG and FFA pools were expressed as ^{13}C concentration in each of the 3 plasma pools and calculated as follows:

$$[^{13}C]\ CHYLO\text{-}TG_{t=i} =$$

$$(mM\ excess\ ^{13}C/mM\ CO_{2\,t=i})_{CHYLO\text{-}TG} \times mM\ CHYLO\text{-}TG_{t=i} \quad \ldots\ 13$$

$$[^{13}C]\ VLDL\text{-}TG_{t=i} =$$

$$(mM\ excess\ ^{13}C/mM\ CO_{2\,t=i})_{VLDL\text{-}TG} \times mM\ VLDL\text{-}TG_{t=i} \quad \ldots\ 14$$

$$[^{13}C]\ FFA_{t=i} =$$

$$(mM\ excess\ ^{13}C/mM\ CO_{2\,t=i})_{FFA} \times mM\ FFA_{t=i} \quad \ldots\ 15$$

Where $[^{13}C]_{t=i}$ = ^{13}C concentration at time = i hr in CHYLO-TG, VLDL-TG and FFA pools respectively

$mM\ excess\ ^{13}C/mM\ CO_{2\,t=i} = (\delta\,^{13}C_{PDB\,t=i} - \delta\,^{13}C_{PDB\,t=0})\ R_{PDB} \times 10^{-3}$ 10

in CHYLO-TG, VLDL-TG and FFA pools

mM CHYLO-TG = TG concentration in CHYLO pool in mM

mM VLDL-TG = TG concentration in VLDL pool in mM

mM FFA = FFA concentration in FFA pool in mM

II. 4. Statistical Analysis

Fasting baseline measures comparing the post-obese and control groups for anthropometric measurements, body composition, blood parameters concentration, REE/FFM/hr, carbohydrate/fat oxidation rate and for TEF were analyzed by unpaired *t*-test. The concentration curves of blood parameters, blood and breath ^{13}C enrichments, REE/FFM were analyzed by a repeated measures 2 way analysis of variance (RM 2-way ANOVA), conducting all pairwise multiple comparisons using Bonferroni *t*-tests.

There were 3 null hypotheses tested by the RM 2-way ANOVA:

- mean group difference: comparing the post-obese group mean of the 5 time points (0, 2, 4, 6, and 8 hrs) versus the control group mean of the 5 time points.
- mean time difference: comparing the 5 time points to each other, the 0 hr mean of the post-obese and control groups versus the 2 hr mean of the post-obese and control groups versus the 4 hr mean of the post-obese group and the control groups and so on.
- mean group x time interaction, comparing the post-obese vs the control group within each time point, called group difference within time, and comparing the 5 time points within each group, called time difference within group. Interaction is present, as calculated by 2-way ANOVA, when the main effects (mean group difference or mean time difference) were not sufficient to describe the data, and further analysis of group x time interaction was required. When an interaction was present, the group difference within time and/or time difference within group was described in the results. It is by the group x time interaction analysis that the difference in clearance rate of various blood parameters between the post-obese and control groups was

determined. The group that had an earlier return of postprandial blood sample to fasting baseline levels was determined to have a faster clearance

Statistical analysis was performed using SigmaStat (Jandel scientific software for windows '95, San Rafael, CA). Prior to running ANOVA and *t*-test analysis, SigmaStat automatically tests for samples' normality and equal variance assumptions required for these tests. When normality or equal variance test failed, data to be measured by RM 2-way ANOVA (carbohydrate/fat oxidation rate and ^{13}C concentration in VLDL-TG) were transformed to the trapezoid AUC, and mean difference was tested by an unpaired *t*-test. Correlation between the AUC of selected parameters was conducted by Pearson product moment correlation. Significance was set at $p < 0.05$ for all parametric, non-parametric and correlation tests, except for % change in ASP, as this is a 2-tailed test and significance at $p < 0.025$ was required.

CHAPTER THREE
RESULTS

III. 1. Subjects' Baseline Characteristics

Tables 1 and 2 show values for age, fasting baseline anthropometric characteristics; Wt, Ht, BMI, BSA, W/H ratio, % body fat, FFM, and fat mass, as well as blood parameters; total TG, cholesterol, HDL-C, calculated LDL-C, ApoB, insulin and glucose of the post-obese and control women on the study date.

There was no statistically significant group difference between the post-obese and control women in the mean value of age, BMI, BSA, W/H ratio, % body fat, total plasma TG and cholesterol as a result of matching. There was also no significant difference between the two groups in any of the other parameters shown, which taken together with the matched parameters, results in a more homogeneous background between the post-obese and the control groups.

Table 1. Baseline Characteristics of the Post-Obese Women on the Day of the Study

	Age (yrs)	Wt (kg)	Ht (cm)	BMI (kg/m^2)	BSA (m^2)	W/H ratio	% B. fat	FFM (kg)	Fat mass (kg)	TG (mM)	FFA (mM)	Chol (mM)	HDL-C (mM)	LDL-C (mM)	ApoB (mg/dl)	Insulin (pM)	Glucose (mM)
1	45	69.4	162.5	26.3	1.73	0.83	39.5	42.0	27.4	0.54	0.23	4.14	1.33	2.58	64.0	131.2	3.02
2	37	57.7	166.0	20.9	1.64	0.74	22.6	44.7	13.0	0.50	0.44	3.31	1.18	1.91	49.7	124.5	3.89
3	31	52.7	162.0	20.1	1.55	0.66	22.4	40.9	11.8	0.83	0.36	3.90	1.74	1.80	48.5	112.4	4.27
4	35	73.1	170.5	25.1	1.84	0.81	29.3	51.7	21.4	0.70	0.40	3.61	1.00	2.31	56.5	108.1	4.05
5	38	64.0	163.0	24.1	1.69	0.82	36.0	41.0	23.0	1.04	0.42	5.60	1.51	3.64	82.3	129.2	4.77
6	44	74.2	166.5	26.8	1.82	0.84	41.7	43.2	30.9	1.04	0.62	5.47	1.64	3.38	80.6	121.6	3.76
7	48	58.8	163.0	22.1	1.63	0.76	27.2	42.8	16.0	0.64	0.38	4.28	1.76	2.24	78.6	125.2	4.57
8	44	58.5	156.0	24.0	1.57	0.79	28.4	41.9	16.6	0.92	0.19	3.78	0.95	2.43	91.7	163.2	5.20
Avg	**40**	**63.6**	**163.7**	**23.7**	**1.68**	**0.78**	**30.9**	**43.5**	**20.0**	**0.78**	**0.38**	**4.26**	**1.39**	**2.54**	**69.0**	**126.9**	**4.19**
SD	6	7.9	4.2	2.4	0.11	0.06	7.3	3.5	6.8	0.21	0.13	0.84	0.32	0.66	16.4	16.6	0.67

Normal range of fasting plasma concentration is: total plasma TG (<1.8 mM), FFA (0.1-0.8 mM), total cholesterol (<6.4 mM), HDL-C (0.8-2.3 mM), LDL-C (1.3-4.9 mM), ApoB (45-120 mg/dl), serum insulin (36-179 pM), and serum glucose (2.6-5.2 mM)

Table 2. Baseline Characteristics of the Control Women on the Day of the Study

	Age (yrs)	Wt (kg)	Ht (cm)	BMI (kg/m^2)	BSA (m^2)	W/H ratio	% B. fat	FFM (kg)	Fat mass (kg)	TG (mM)	FFA (mM)	Chol (mM)	HDL-C (mM)	LDL-C (mM)	ApoB (mg/dl)	Insulin (pM)	Glucose (mM)
1	40	53.4	161.0	20.6	1.54	0.68	23.3	41.0	12.4	1.29	0.68	3.78	1.53	1.69	53.4	98.6	3.55
2	32	66.5	159.5	26.1	1.70	0.77	38.8	40.7	25.8	1.21	0.56	3.76	0.54	2.69	77.9	147.1	4.30
3	31	69.0	168.5	24.3	1.79	0.75	30.2	48.2	20.8	0.34	0.14	3.30	1.48	1.67	52.7	105.6	4.23
4	38	63.5	169.0	22.2	1.73	0.74	30.8	44.0	19.6	0.68	0.13	3.80	1.07	2.43	65.6	141.9	4.03
5	48	61.8	166.5	22.3	1.69	0.69	36.2	39.4	22.4	0.59	0.68	4.03	2.20	1.58	66.2	124.4	4.98
6	35	65.8	169.0	23.0	1.63	0.78	35.3	42.6	23.3	0.87	0.59	4.90	1.44	3.08	93.9	88.6	4.66
7	40	55.2	165.0	20.3	1.60	0.80	30.7	38.3	16.9	0.70	0.42	4.67	1.66	2.71	77.1	139.8	4.47
8	36	71.0	174.0	23.5	1.84	0.74	35.2	46.1	24.9	0.72	0.19	4.25	1.56	2.38	84.6	93.9	4.41
Avg	**38**	**63.3**	**166.6**	**22.8**	**1.69**	**0.74**	**32.6**	**42.5**	**20.8**	**0.80**	**0.42**	**4.06**	**1.44**	**2.28**	**71.4**	**117.5**	**4.33**
SD	5	6.3	4.7	1.9	0.10	0.04	4.8	3.4	4.4	0.31	0.24	0.52	0.48	0.56	14.6	23.6	0.42

Normal range of fasting plasma concentration is: total plasma TG (<1.8 mM), FFA (0.1-0.8 mM), total cholesterol (<6.4 mM), HDL-C (0.8-2.3 mM), LDL-C (1.3-4.9 mM), ApoB (45-120 mg/dl), serum insulin (36-179 pM), and serum glucose (2.6-5.2 mM)

III. 2. Metabolic Parameters

III. 2. 1. Resting Energy Expenditure per Hour

Fasting and postprandial REE per hour (REE/hr) for the post-obese and control groups following ingestion of the high fat meal are shown in figure 1. There was no statistically significant difference in mean REE/hr between the two groups. There was however a significant mean time difference ($p < 0.001$), as REE is expected to increase postprandially as a result of the TEF, and there was a significant group x time interaction ($p < 0.001$).

Fasting REE/hr was similar between the post-obese and control group. In both groups, REE/hr increased significantly at 2 and 4 hr after ingestion of the high fat meal, with the post-obese being significantly higher than the controls at 2 hr ($p < 0.008$). However, whereas the REE/hr remained significantly elevated at 6 hr ($p < 0.001$) and at the end of the study at 8 hr ($p < 0.02$) in the control group, it returned to its fasting baseline levels at 6 hr in the post-obese group.

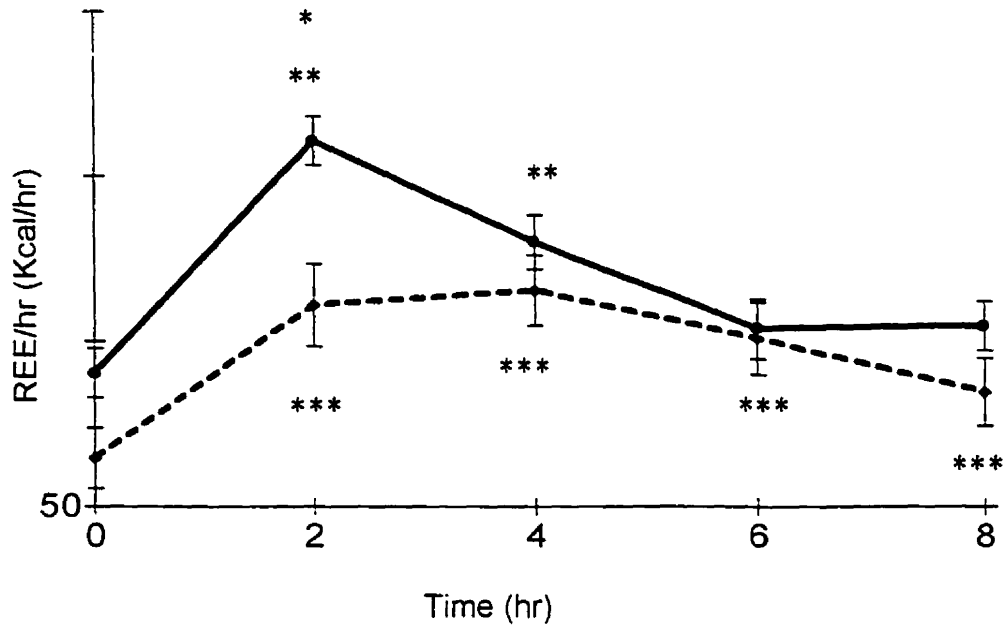

Figure 1. Resting energy expenditure per hour (REE/hr) in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time points, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 2. 2. Thermic Effect of Food

Mean thermic effect of the high fat meal over the 8 hr of the post-obese women was 517 ± 11 Kcal/8 hr, and that of the control women was 481 ± 16 Kcal/8 hr (Mean ± SEM). Although REE/hr returned to its fasting level earlier in the post-obese group than the controls, there was no statistically significant difference in mean TEF between the two groups.

This can be explained by the shape of the curve of REE/hr. In the post-obese group, there was a sharper increase in REE/hr at 2 hr, above its level in the controls at the same time point, whereas the increase in REE/hr in the controls was less acute at 2 hr, maintaining a plateau afterwards at 2, 4, and 6 hr. The result being that mean AUC of the REE, or TEF, in the two groups was similar. Some studies (see introduction) suggested that the shape of the REE curve is important, and that if TEF was measured for a sufficient period of time, more than 5 hr, then no difference would exist between the compared groups (obese vs their matched controls in that study). The same could be said in this study, regarding the importance of the shape of the curve of the REE and length of measurement in comparing TEF of the post-obese and their matched controls, and may explain the discrepancies about TEF differences in post-obese subjects and their matched controls found in other studies.

III. 2. 3. Carbohydrate/Fat Oxidation Rate

Figure 2 shows values for fasting and postprandial carbohydrate to fat oxidation rates (g/g) for the post-obese and control groups, after ingestion of the labeled high fat mixed meal over the 8 hr study period. Data were transformed to the trapezoid AUC of carbohydrate/fat oxidation rate as normality test failed (p = 0.003). There was no statistically significant difference in the mean AUC of carbohydrate/fat oxidation rate between the post-obese 18.54 ± 5.34, and that of the control groups 20.22 ± 2.99 (Mean ± SEM). Thus, there is no difference in utilization of carbohydrate and fat as the source of energy between the post-obese and control groups.

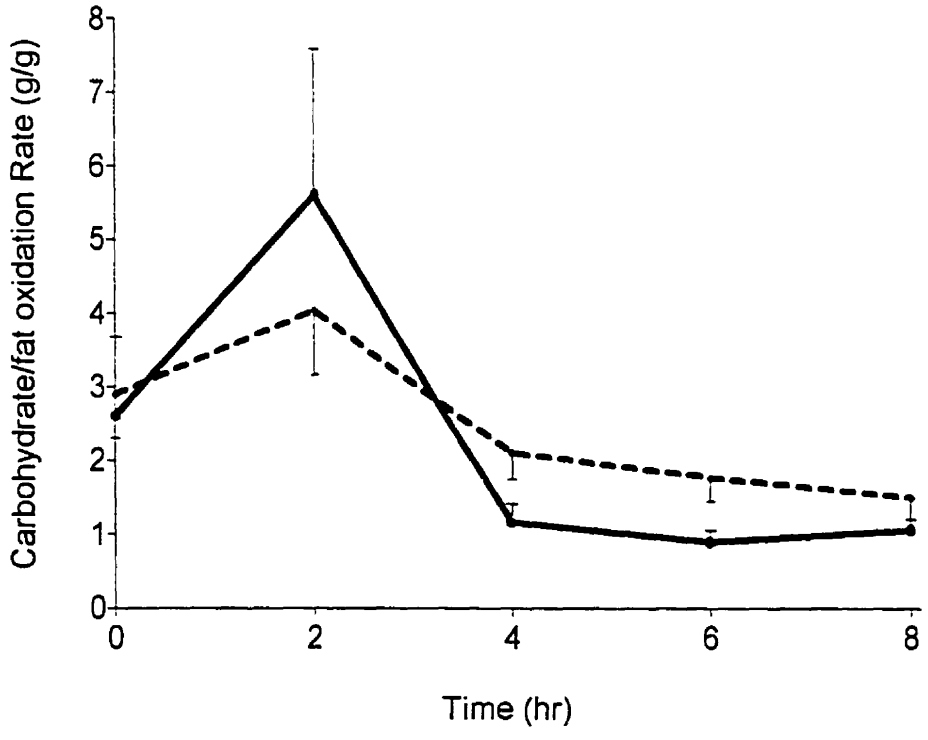

Figure 2. Carbohydrate/fat oxidation rate in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM.

III. 3. Blood Parameters

III. 3. 1. Plasma Acylation Stimulating Protein Concentration

Figure 3 show values for fasting and postprandial plasma ASP concentrations, and Figure 4 show values for the % change in postprandial plasma ASP concentration from baseline for the post-obese and control women after ingestion of the high fat meal. In both groups, plasma ASP concentration was between the 10^{th} (14.5 nM) and 90^{th} (40.8 nM) percentile of the normal non-obese distribution (see introduction), and there was no difference in mean fasting plasma ASP concentration in both groups. Although there was a trend for lower fasting and postprandial plasma ASP concentration, and a greater % change from fasting baseline in the post-obese group, there was no significant difference in mean plasma ASP concentration, mean % change from baseline, and there was no group x time interaction (ASP concentration or % change).

However, there was a statistically significant mean time difference where, compared to its fasting level, mean plasma ASP concentration, of both groups averaged together, was significantly decreased at 6 hr (-18.5 ± 7.5%) and 8 hr (-25.4 ± 7.1%) (mean ± SEM) after food ingestion ($p < 0.001$). It cannot be determined however if the decrease (% change from baseline) in plasma ASP concentration was greater in the post-obese at 6 and 8 hr because there was no group x time interaction.

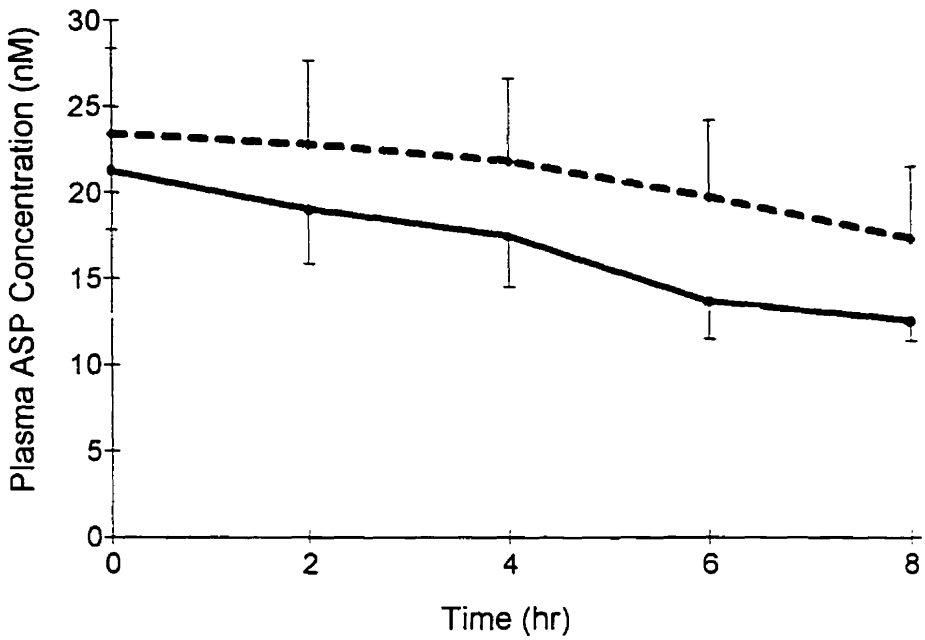

Figure 3. Plasma Acylation Stimulating Protein (ASP) concentration in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM.

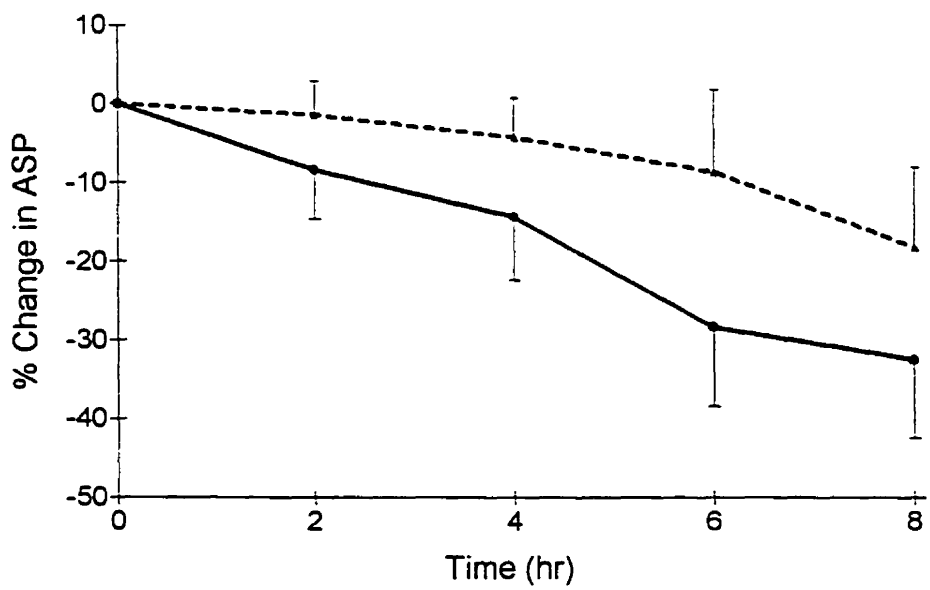

Figure 4. Percent change in plasma Acylation Stimulating Protein (ASP) concentration from baseline in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM.

III. 3. 2. Serum Insulin and Glucose Concentrations

III. 3. 2. 1. Serum Insulin Concentration

Figure 5 shows values for fasting and postprandial serum insulin concentrations in the post-obese and the control women after ingestion of the high fat mixed meal. There was no group difference between the post-obese and control groups in serum insulin concentration, but there was a significant mean time difference ($p < 0.001$), and a group x time interaction with $p = 0.05$. In both groups, mean serum insulin rose significantly 2 hr after food ingestion ($p < 0.01$). However, in the post-obese group serum insulin concentrations at 4, 6, and 8 hr were similar to that at fasting where as in the control group, serum insulin remained significantly elevated at 4 hr ($p < 0.05$), returning to fasting levels only at 6 hr. In addition, serum insulin in the control group was significantly higher than that in the post-obese at 4 hr (p =0.003).

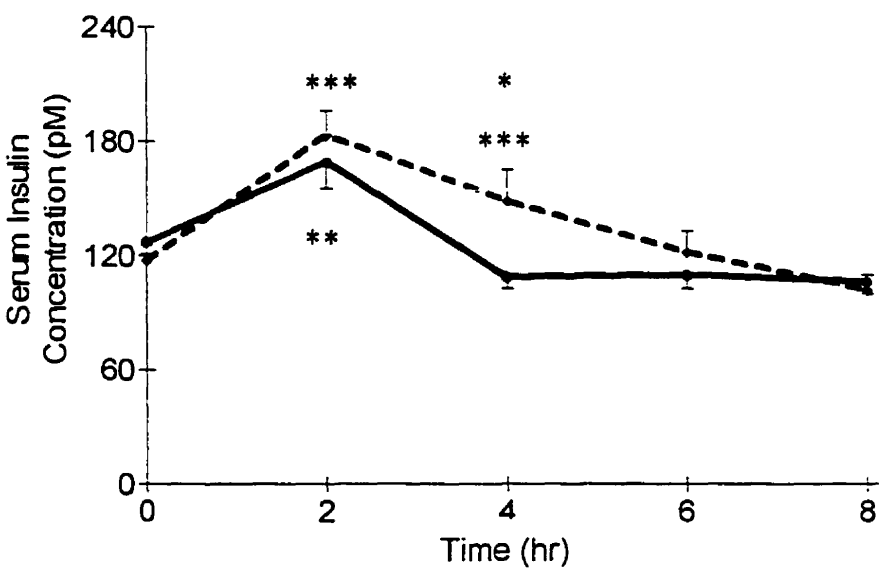

Figure 5. Serum insulin concentration in the post-obese (▬▬) and control (▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time points, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 3. 2. 2. Serum Glucose Concentration

Figure 6 shows values for fasting and postprandial serum glucose concentrations in the post-obese and the control women after ingestion of the high fat mixed meal. There was a group difference in mean serum glucose concentration between the post-obese and control women, where the post-obese group were significantly lower than the controls (p =0.006). In addition, there was a significant mean time difference ($p < 0.05$), and a mean group x time interaction with $p < 0.05$. Fasting serum glucose concentration was similar in both groups. However, while in the post-obese group postprandial serum glucose concentrations were similar to fasting values at all time points measured, serum glucose in the control group was significantly higher than fasting values at 2 and 4 hr, and higher that that of the post-obese at the same time points ($p < 0.05$).

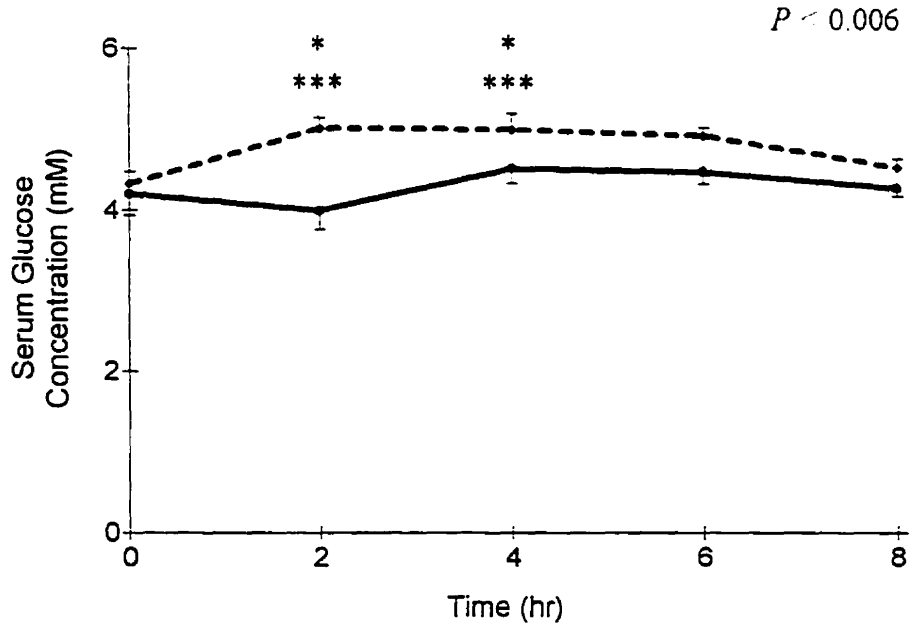

Figure 6. Serum glucose concentration in the post-obese (▬▬) and control (▪▪▪▪) women after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, $p < 0.006$ indicates significance for mean group difference, * $p < 0.05$ post-obese vs control women at indicated time points, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 3. 2. 3. Serum Insulin and Glucose Concentration Product

To examine whether the post-obese individuals have higher insulin sensitivity, as was previously suggested in some studies (see introduction), insulin sensitivity in the post-obese group compared to the matched controls was examined. A novel index of insulin sensitivity was used in this study which was the product of serum insulin and glucose concentration (insulin x glucose). This index reflects actual absolute values rather than ratios. Lower serum glucose concentration maintained by even lower levels of serum insulin concentration would thus indicate higher cellular sensitivity to circulating insulin. Mean fasting and postprandial serum insulin x glucose values in the post-obese and control groups are shown in Figure 7. Data shown suggest that the post-obese have, on average, higher insulin sensitivity than the control group as they have significantly lower mean serum insulin x glucose concentration values (p =0.02).

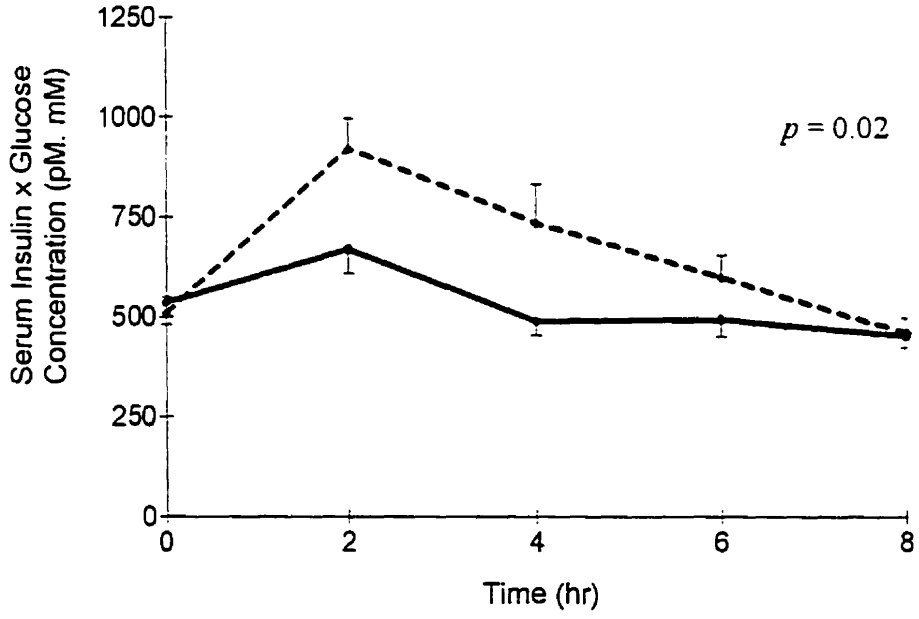

Figure 7. Serum insulin x glucose concentration in the post-obese (▬▬) and control (▪▪▪▪▪) women in response to the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, p = 0.02 represents significance for mean group difference.

III. 3. 3. Plasma Lipid Concentration

III. 3. 3. 1. Total Plasma Triglyceride Concentration

Fasting and postprandial total plasma TG concentrations for the post-obese and control groups after ingestion of the labeled high fat meal are shown in Figure 8. There was no statistically significant difference between the post-obese and the control groups in mean total plasma TG. There was, however, a significant mean time difference ($p < 0.001$), and a group x time interaction with $p = 0.01$. In both groups, total plasma TG was significantly higher than fasting levels at 2 and 4 hr after food ingestion ($p < 0.05$) with both groups returning to baseline values by 6 hr. However, many studies (as earlier mentioned in introduction) have demonstrated the close correlation between fasting total plasma TG and postprandial plasma TG response. The two studied groups have been matched for the baseline fasting total plasma TG, which can explain the finding of no significant difference in total plasma TG clearance curves between the two groups.

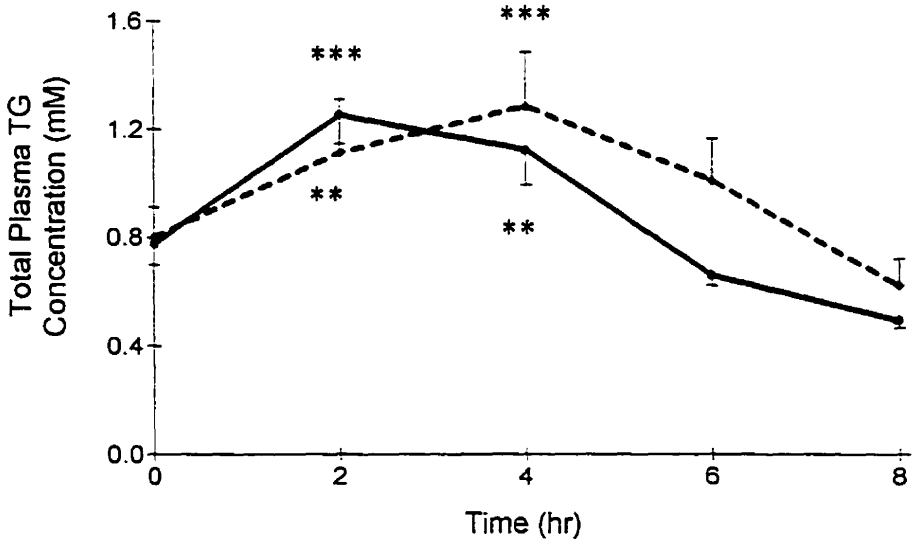

Figure 8. Total plasma triglyceride (TG) concentration in the post-obese (▬▬) and control (▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 3. 3. 2. Plasma Triglyceride Concentration in Chylomicron

Fasting and postprandial plasma TG concentrations in plasma density fraction with $S_f > 400$ (CHYLO-TG) in the post-obese and control groups are shown in Figure 9. Mean CHYLO-TG concentration was similar in the post-obese and control groups, but there was a significant mean time difference ($p < 0.001$), and a group x time interaction ($p = 0.02$). In both groups, plasma CHYLO-TG was significantly higher at 2 and 4 hr after food ingestion ($p < 0.001$). However, as hypothesized, data suggest a faster clearance of plasma CHYLO-TG in the post-obese group as plasma CHYLO-TG concentration returned to fasting levels by 6 hr, and only at 8 hr in the control group. In addition, plasma CHYLO-TG concentration at 6 hr was significantly lower in the post-obese than in the control group ($p = 0.02$).

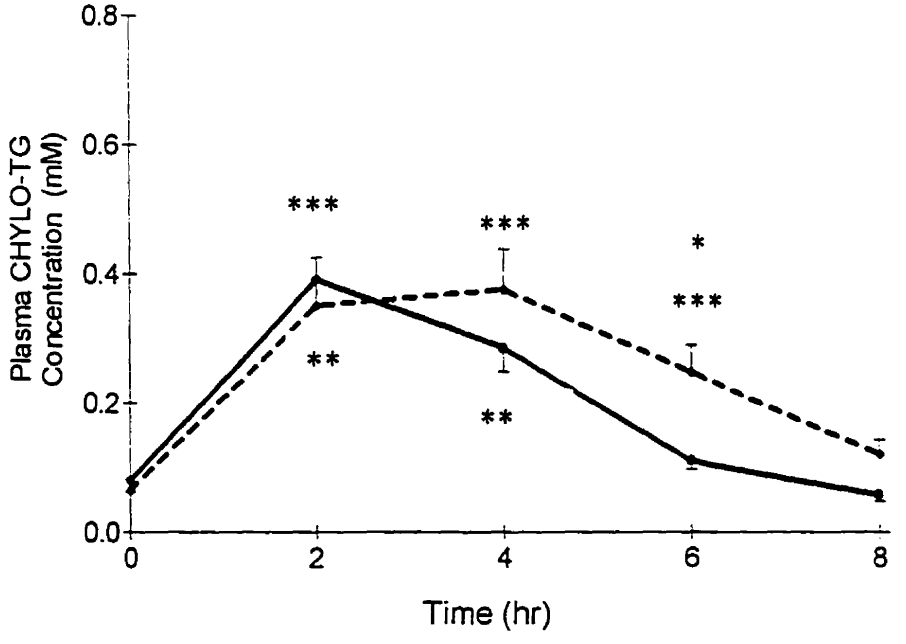

Figure 9. Plasma triglyceride concentration in chylomicron (CHYLO-TG) in the post-obese (———) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time point, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 3. 3. 3. Plasma Triglyceride Concentration in Very Low Density Lipoprotein

Figure 10 shows values for fasting and postprandial plasma TG concentration in plasma density fraction with S_f = 20-400 (VLDL-TG) in the post-obese and control groups after ingestion of the high fat meal. There was no statistically significant group difference in VLDL-TG concentration between the two groups, nor was there a group x time interaction. However, there was a significant time difference ($p < 0.001$) where, compared to its fasting levels, mean VLDL-TG concentrations of both groups significantly increased at 2 and 4 hr after food ingestion returning to its fasting level by 6 hr.

Of interest is the shape of the curve of total plasma TG which parallels that of plasma CHYLO-TG ($S_f > 400$) and VLDL-TG ($S_f = 20$-400). This should be expected given that postprandial plasma TG mainly circulate in TRL of plasma density fraction with $S_f > 20$.

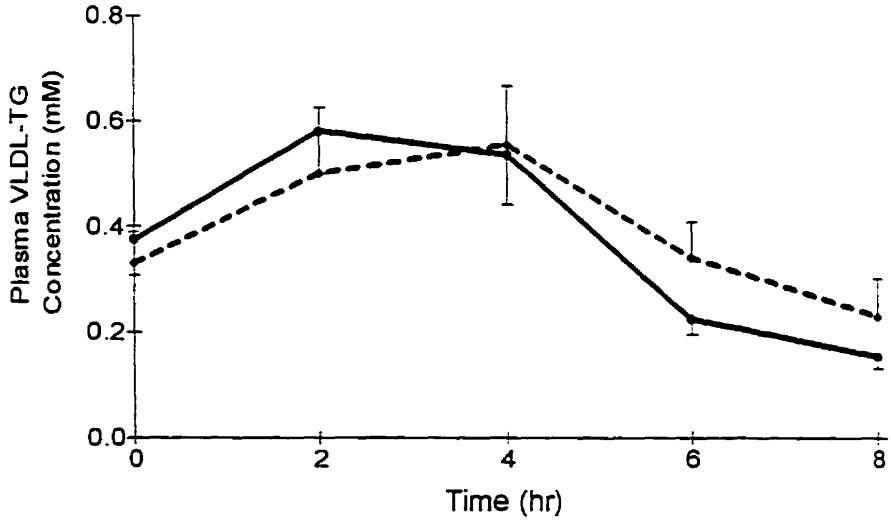

Figure 10. Plasma triglyceride concentration in very low density lipoprotein (VLDL-TG) in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM.

III. 3. 3. 4. Plasma Free Fatty Acid Concentration

Fasting and postprandial plasma FFA concentration in the post-obese and the control women after ingestion of the high fat meal are shown in Figure 11. There was no statistically significant group difference in mean plasma FFA concentration between the post-obese and the control women, however, there was a significant mean time difference ($p < 0.001$), and a strong group x time interaction ($p = 0.008$).

In both groups, postprandial plasma FFA concentration decreased significantly 2 hr after food ingestion ($p < 0.05$), as would be expected due to postprandial, carbohydrate-induced insulin secretion as apparent from Figure 5, and inhibition of adipose tissue hormone sensitive lipase and lipolysis. However, although plasma FFA concentration was significantly higher in the post-obese group compared to the controls 4 hr after food ingestion ($p = 0.005$), its value at that time point merely returned to fasting level, and remained at such concentration throughout the rest of the study. On the other hand, in the control group, plasma FFA concentration increased steadily after 2 hr reaching a significantly higher concentration than the fasting value 8 hr after food ingestion ($p = 0.02$). The sharp increase in plasma FFA in the post-obese group and not in the control at 4 hr, may be in part due to the sharp drop in serum insulin concentration in the post-obese where it remained elevated in the controls at same time point.

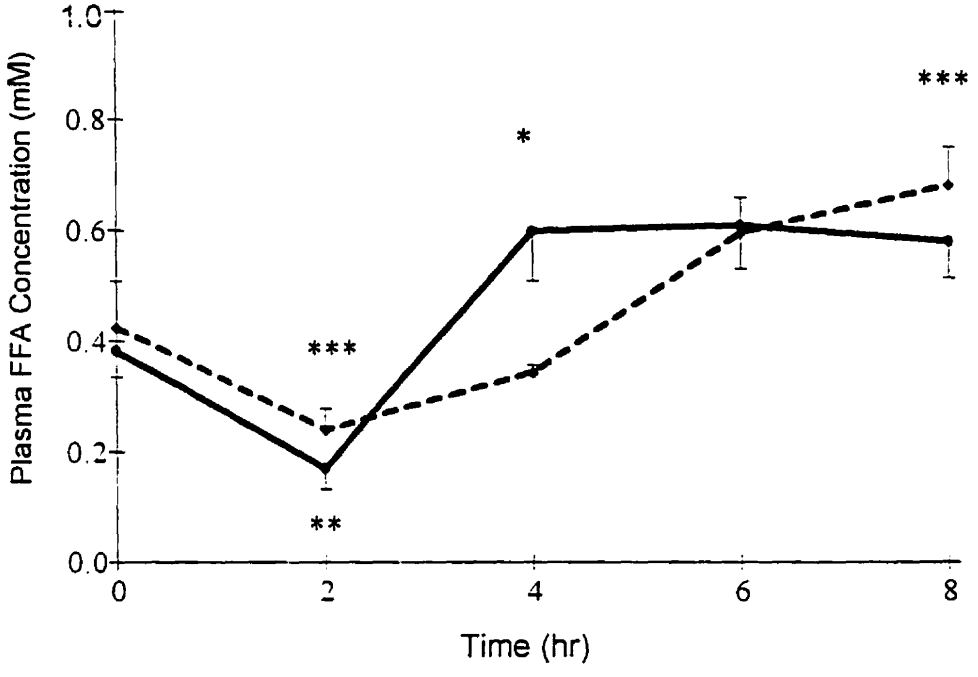

Figure 11. Plasma free fatty acid concentration (FFA) in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time point, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 4. ^{13}C Enrichment

III. 4. 1. Breath Samples

Figure 12 show values for mean postprandial ^{13}C enrichment in breath CO_2 pool for the post-obese and control groups following ingestion of the ^{13}C-labeled high fat meal, and expressed as the % of administered ^{13}C dose recovered in breath CO_2 per Hr. There was a significant mean time difference ($p < 0.001$) as enrichment in breath CO_2 increased in postprandial samples in both groups. However, contrary to our hypothesis, there was no statistically significant group difference in mean % recovered dose/hr in breath CO_2 between the post-obese and the control groups, but there was a strong group x time interaction ($p = 0.003$).

In the post-obese group, breath $^{13}CO_2$ enrichment increased significantly 2 and 4 hr after food ingestion ($p < 0.01$) and reached a plateau there after, where 13C enrichment were similar at 4, 6, and 8 hr. In the control group, however, breath $^{13}CO_2$ enrichment increased significantly at 4 and 6 hr ($p < 0.001$), and reached a plateau between 6 and 8 hr. In addition, at 8 hr, breath $^{13}CO_2$ enrichment was significantly lower in the post-obese group then in the controls ($p = 0.002$).

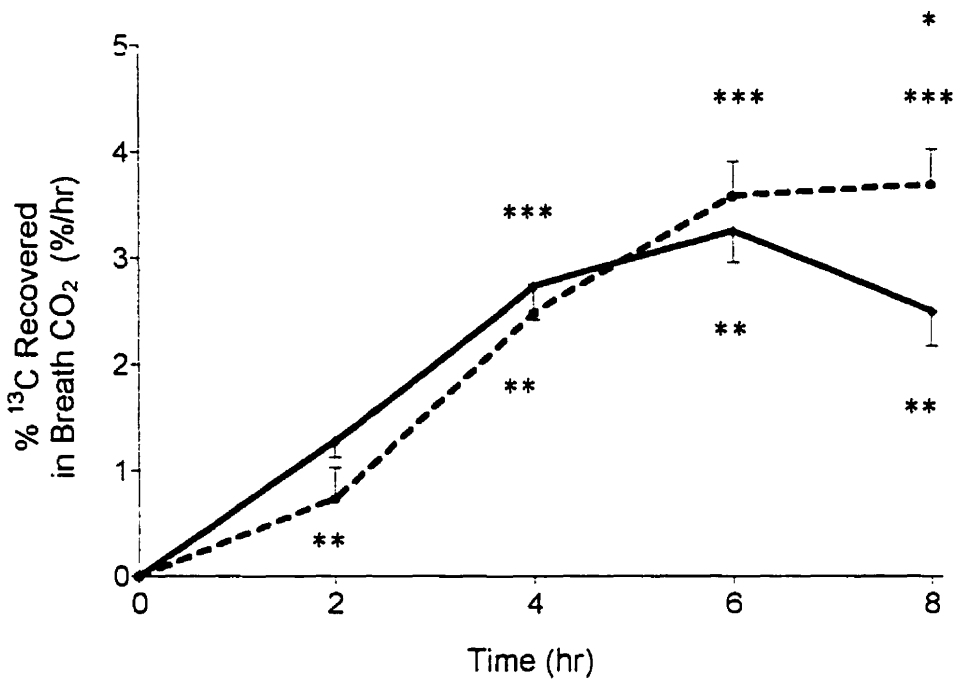

Figure 12. Percent recovered ^{13}C in breath CO_2 per hour in the post-obese (——) and control (•••••) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time points, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 4. 2. Blood Samples

III. 4. 2. 1. Triglyceride Fraction of Plasma Chylomicron Pool

Figure 13 show values for mean ^{13}C enrichment above baseline in CHYLO-TG in the post-obese and the control women after ingestion of the ^{13}C labeled high fat meal expressed as ^{13}C concentration in plasma CHYLO-TG.

There was no significant difference in mean plasma CHYLO-TG ^{13}C concentration between the post-obese and the control groups, but there was a significant mean time difference ($p < 0.001$), and a strong group x time interaction with $p = 0.001$. In both groups, plasma CHYLO-TG pool was significantly enriched 2 hr after food ingestion ($p < 0.005$). However, as hypothesized, ^{13}C concentration in CHYLO-TG pool was cleared earlier in the post-obese than the controls, as it returned to baseline non-enriched values 4 hr after food ingestion in the post-obese, whereas it required 8 hr in the control group. In addition, compared to the controls, CHYLO-TG ^{13}C concentration was significantly lower at 4 and 6 hr after food ingestion in the post-obese group ($p < 0.05$).

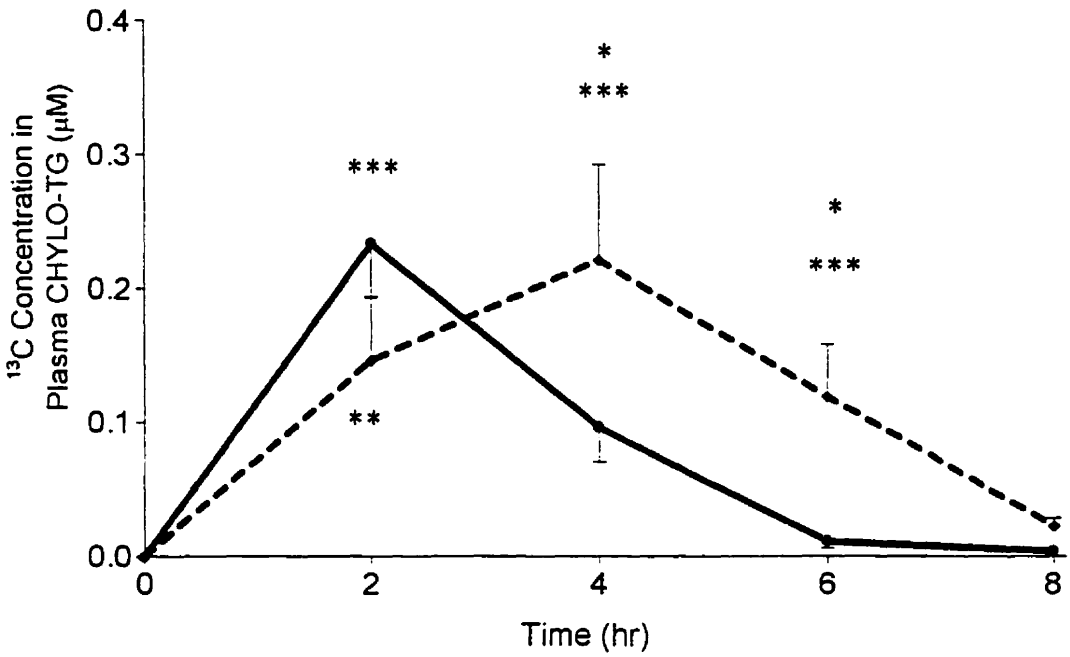

Figure 13. ^{13}C concentration in the triglyceride fraction of plasma chylomicron pool (CHYLO-TG) in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time points, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$ postprandial vs fasting in the control group.

III. 4. 2. 2. Triglyceride Fraction of Plasma Very Low Density Lipoprotein Pool

Figure 14 show values for mean ^{13}C enrichment above baseline levels in plasma VLDL-TG in the post-obese and the control women after ingestion of the ^{13}C labeled high fat meal, and expressed as plasma VLDL-TG ^{13}C concentration. Data were transformed to AUC of plasma VLDL-TG ^{13}C concentration as normality test failed (p = 0.002). Thus, although there is a clear trend for a faster clearance in the post-obese group of ^{13}C concentration in VLDL-TG pool as hypothesized, it cannot be determined which group attained an earlier return to fasting value and at what time point, as group x time interaction was not calculated (2-way ANOVA test was not conducted). Mean AUC of ^{13}C enrichment in plasma VLDL-TG pool, however, shows no significant difference between the post-obese and the control groups (mean ± SEM, 0.279 ± 0.040 ^{13}C μM/ 8 hr vs 0.379 ± 0.138 ^{13}C μM/ 8 hr respectively).

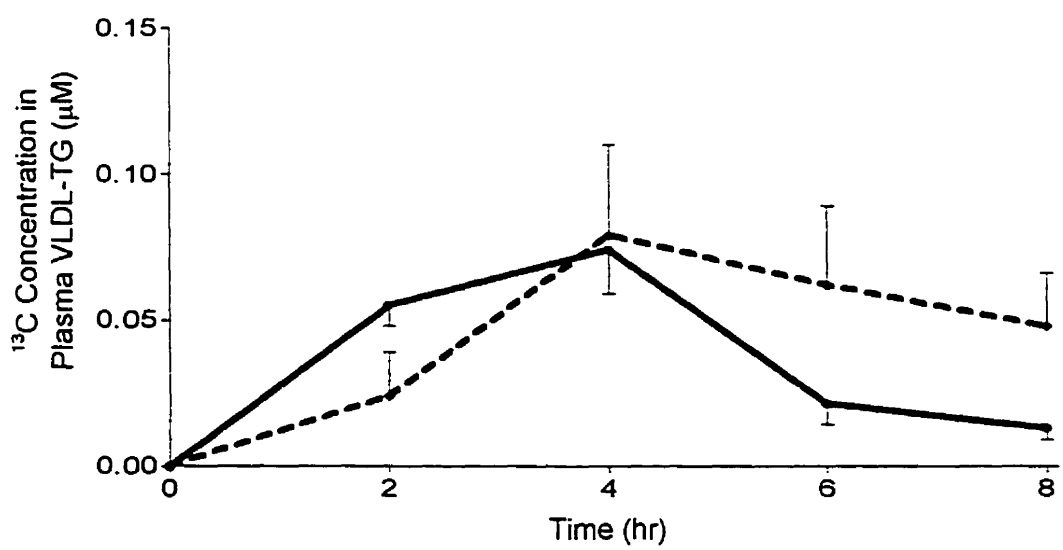

Figure 14. ^{13}C concentration in the triglyceride fraction of plasma very low density lipoprotein pool (VLDL-TG) in the post-obese (———) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM.

III. 4. 2. 3. Plasma Free Fatty Acid Pool

Figure 15 shows values for mean ^{13}C enrichment above baseline levels in plasma FFA in the post-obese and the control women after ingestion of the ^{13}C labeled high fat meal, and expressed as plasma FFA ^{13}C concentration.

By far, the greatest difference between the post-obese and the control groups was in the fractionation of the labeled FFA between the plasma and tissue compartments. As hypothesized, mean plasma FFA ^{13}C concentration was significantly higher in the control group than in the post-obese ($p = 0.009$). In addition, there was a significant time difference ($p < 0.001$) as well as a strong group x time interaction ($p < 0.001$).

The appearance of the ingested ^{13}C-label in the postprandial plasma FFA pool is a result of hydrolysis of plasma TG in TRL core (S_f >20 CHYLO-TG and VLDL-TG). Although, in the post-obese group, the ^{13}C was cleared from plasma CHYLO-TG pool only 4 hr after food ingestion, with a trend for the same rate of clearance in plasma VLDL-TG pool, postprandial plasma FFA pool ^{13}C concentration did not increase significantly above baseline at any of the time points measured. In the control group, however, as ^{13}C concentration decreased after 4 hr in plasma CHYLO-TG pool, with the same trend in plasma VLDL-TG pool, plasma FFA ^{13}C concentration increased dramatically 6 and 8 hr after food ingestion, and thus, was higher than that of the post-obese at the same time points ($p < 0.001$). Thus, in the post-obese group, whatever label was released from TRL by TG hydrolysis was preferentially assimilated into the surrounding tissue and prevented from accumulating in plasma FFA pool.

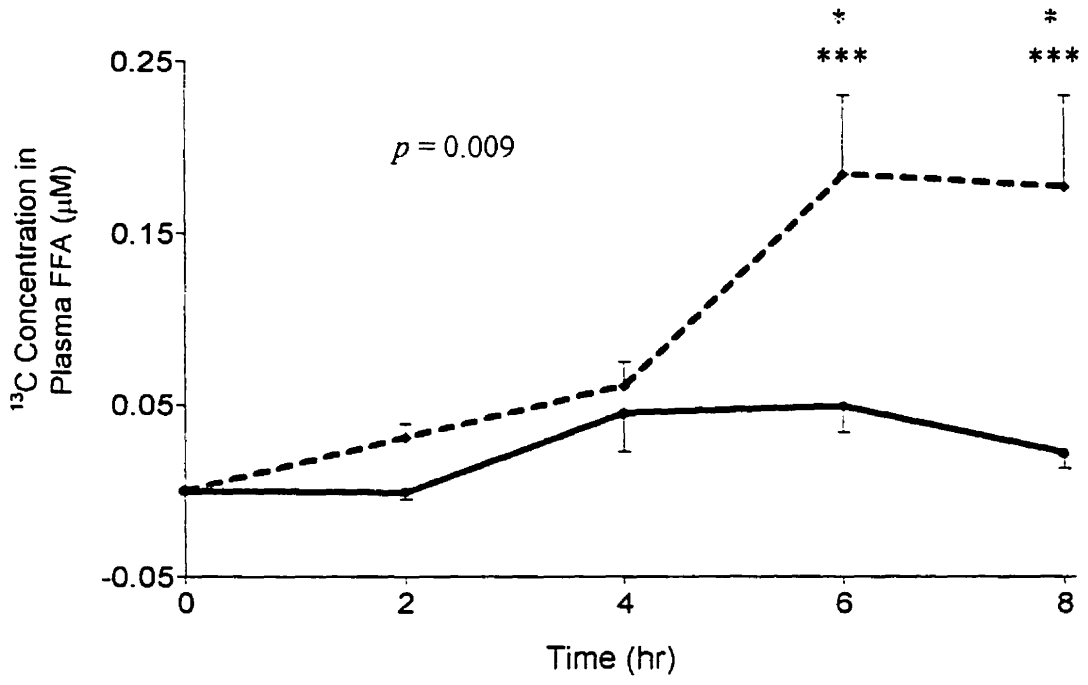

Figure 15. ^{13}C concentration in plasma free fatty acid pool (FFA) in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, p = 0.009 indicates significance for mean group difference, * $p < 0.05$ post-obese vs control women at indicated time points, *** $p <$ 0.05 postprandial vs fasting in the control group.

III. 4. 2. 3. Total Plasma Compartment

To estimate total enrichment of the plasma compartment with the ingested label, ^{13}C concentrations in the 3 measured plasma pools, plasma CHYLO-TG, VLDL-TG, and FFA, were summed. Figure 16 shows values for mean ^{13}C enrichment above baseline levels in total plasma compartment in the post-obese and the control women after ingestion of the ^{13}C labeled high fat meal, and expressed as total plasma ^{13}C concentration.

There was no mean group difference between the post-obese and the controls, but there was a significant mean time difference ($p < 0.001$), and a strong group x time interaction ($p < 0.001$). Compared to the controls, there was an earlier clearance of ^{13}C from total plasma compartment in the post-obese group. In both groups, ^{13}C concentration in total plasma compartment increased significantly 2 and 4 hr after food ingestion ($p < 0.01$). However, although in the control group total plasma compartment was still significantly enriched at the end of the study, 8 hr after food ingestion, plasma ^{13}C concentration returned to baseline non-enriched values at 6 hr in the post-obese group. In fact, it is unclear at which time the label would be cleared from the plasma in the control group, as enrichment has reached a plateau after 4 hr where ^{13}C concentrations in plasma at 4, 6, and 8 hr after food ingestion were similar. Thus, compared to matched controls, this data suggest a far greater exogenous fat clearance efficiency in the post-obese women examined in this study.

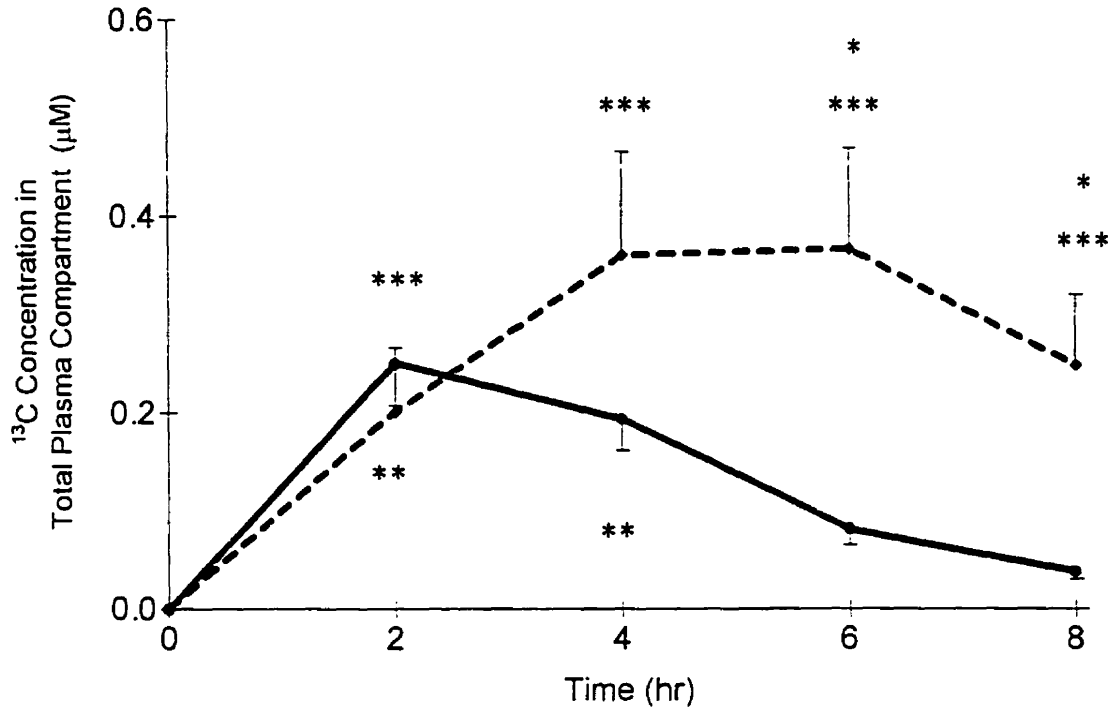

Figure 16. ^{13}C concentration in total plasma compartment in the post-obese (▬▬) and control (▪▪▪▪▪) groups after ingestion of the ^{13}C-labeled high fat meal. Data are shown as mean ± SEM, * $p < 0.05$ post-obese vs control women at indicated time points, ** $p < 0.05$ postprandial vs fasting in the post-obese group, *** $p < 0.05$, postprandial vs fasting in the control group.

III. 5. Correlation

AUC of selected parameters in the post-obese and control groups, taken together, were tested for correlation as shown in Table 3. There was no significant correlation between the AUC of the % change in plasma ASP from fasting level and TEF, carbohydrate/fat oxidation rate or insulin x glucose concentrations. In addition, insulin sensitivity, which was suggested to influence carbohydrate/fat oxidation in other studies by favoring tissue glucose uptake, did not correlate either with carbohydrate/fat oxidation or TEF.

Of interest, AUC of plasma ASP concentration correlated positively with AUC of total plasma TG and VLDL-TG, and AUC of % change in postprandial plasma ASP from baseline correlated negatively with AUC of CHYLO-TG. In addition, AUC of insulin x glucose concentration, the index of insulin sensitivity, correlated positively with AUC total plasma TG and CHYLO-TG. Thus, women in both groups with lower mean plasma ASP, greater % change of postprandial plasma ASP or higher insulin sensitivity had also lower postprandial total plasma TG concentration and faster clearance of circulating postprandial TRL. Other parameters were also tested for correlation, as shown in table 3, and are discussed in chapter 4.

Table 3. Correlation Between Area Under the Curve of Selected Parameters of the Post-Obese and Control Groups

AUC	TEF	Carbohydrate / Fat oxidation	[Insulin xGlucose]	[Total TG]	[CHYLO -TG]	[VLDL -TG]	[^{13}C] Breath CO_2	[13C] CHYLO-TG	[^{13}C] VLDL-TG	[^{13}C] FFA
[ASP]	*NS*	*NS*	*NS*	0.58 *0.02*	*NS*	0.59 *0.02*	*NS*	*NS*	*NS*	*NS*
ASP % Change	*NS*	*NS*	*NS*	*NS*	-0.56 *0.02*	*NS*	*NS*	*NS*	*NS*	*NS*
[Insulin]	*NS*	*NS*	-	0.69 *<0.01*	0.72 *<0.01*	0.60 *0.01*	*NS*	0.57 *0.02*	*NS*	0.57 *0.02*
[Insulin X Glucose].	*NS*	*NS*	-	0.65 *<0.01*	0.73 *<0.01*	*NS*	*NS*	0.58 *0.02*	*NS*	0.68 *<0.01*
[^{13}C] CHYLO-TG	*NS*	*NS*	0.58 *0.02*	*NS*	0.49 *0.05*	0.52 *0.04*	*NS*	-	0.84 *<0.0001*	0.77 *<0.001*
[^{13}C] VLDL-TG	*NS*	*NS*	*NS*	*NS*	0.52 *0.04*	0.56 *0.02*	*NS*	0.84 *<0.0001*	-	0.57 *0.02*
[^{13}C] FFA	*NS*	*NS*	0.68 *<0.01*	*NS*	*NS*	*NS*	*NS*	0.77 *<0.001*	0.57 *0.02*	-

Data in table represent r-value, and *p*-value (*italic number* underneath the r-value), n =16 for each category.
AUC = Area under the 8 hours time curve, [ ] = concentration of enclosed parameters, *NS* = not significant

CHAPTER FOUR
DISCUSSION

The results in this study suggest that the metabolic differences between the post-obese and the control women exist in the clearance efficiency of the exogenous fat source ingested. Data obtained from the post-obese women provide clear evidence for a faster removal of the ingested label from the plasma compartment.

Most of the studies conducted in the past decade on post-obese individuals compared to never-obese matched individuals have investigated metabolic differences in fasting and postprandial REE, TEF, insulin-glucose responses and substrate oxidation between the 2 groups. To date, few have examined differences in the oxidation versus storage of dietary fat. The use of a ^{13}C-labeled high fat meal is a specific, validated, non-invasive technique that have been used to trace the fate of dietary fat through breath CO_2 and plasma lipoproteins in obese individuals [Binnert et al. 1996, Binnert et al. 1998]. We have employed this technique as a novel method to investigate fat metabolism in post-obese women.

The post-obese group had a gastric bypass procedure that resulted in massive weight loss 2 - 3 years prior to this study. Studies have demonstrated that gastric bypass procedures can result in normal, delayed or rapid gastric that usually normalize with time specially with intake of solid foods [Behrns and Sarr 1994, Horowitz et al. 1982]. The possibility of rapid gastric emptying or dumping was avoided by tailoring the meal composition and consistency to fit the dietary therapy guidelines for this syndrome, which have been demonstrated to adequately control this condition [Behrns and Sarr 1994]. On the other hand, delayed gastric emptying symptoms, had it occurred, would

have been easily detected, as food is being retained in the stomach, and postprandial abdominal pain, distention, nausea and vomiting directly follow food ingestion [Gustavsson et al. 1988]. All subjects were monitored during and after food ingestion, particularly for the first 2 hours, when symptoms of dumping or delayed emptying would occur. None was noticed by the study coordinator nor reported by the post-obese or control women. Thus, it is unlikely that the differences in clearance rate of the label from the blood compartment between the 2 groups studied was due to difference in rate of delivery of the label into that compartment.

A second issue to note regarding the post-obese gastric bypass procedure is that, in order to avoid dumping, patients who undergo gastric bypass procedures are advised to consume small frequent meals, 4 - 6 meals a day [Behrns and Sarr 1994]. In terms of meal size, the meal volume, 250 ml, equals approximately half the gastric bypass stomach expansion capacity which is approximately 500 ml [Zeman 1991, Benotti and Force 1995]. However, we would not expect that meal frequency pattern prior to the study day would have affected lipogenesis, as recent studies demonstrated no significant difference in plasma lipid, glucose and lipogenesis with subjects placed on various meal frequency patterns, ranging from 1 to 12 meals per day [Jones et al. 1995, Murphy et al. 1996, Bellisle et al. 1997].

In addition, it is unlikely that dramatic dietary differences in fat quality or quantity prior to the study day existed between the 2 groups, as they had similar baseline fasting plasma lipid profile of TG, FFA, total cholesterol, HDL-C, and LDL-C. Fasting plasma total TG has a great influence on postprandial lipemia [Bergeron and Havel 1997], and the 2 groups were matched at baseline to exclude this possible confounding

variable. Therefore, a "spill over effect" of previous dietary pattern and meal frequency and size on the outcome of this study is unlikely to have occurred.

Postprandially, as TRL enter the plasma compartment, two interconnected processes are essential for their clearance. The first process requires hydrolysis of the TG core of the TRL releasing FFA and glycerol, and the second requires assimilation of the generated FFA into surrounding tissues [Eckel et al. 1995]. Evidence from this study points out that both processes are enhanced in the post-obese group.

Lipolysis of circulating TRL is controlled by the lipolytic activity of LPL localized to the endothelium, which in turn is regulated by insulin. Fasting adipose tissue LPL activity has been demonstrated to increase with weight loss and stabilization in obese humans [Schwartz and Brunzell 1981, Kern et al. 1990]. Importantly, an intervening period of isocaloric weight stabilization must occur before LPL is measured to avoid the effects of hypocaloric periods on LPL [Eckel and Yost 1987, Eckel et al. 1995], and subjects in this study were weight stable for 6 months prior to the study. Insulin is an important regulator of LPL activity. In healthy normal weight individuals following intake of a mixed meal, insulin secretion stimulates LPL activity [Farese et al. 1991]. In the post-obese state, increased insulin sensitivity, indicated by the lower ratio of plasma insulin to glucose, was demonstrated in several studies [Eckel 1992, Ranneries et al. 1998]. LPL activity was not measured in our study, but insulin sensitivity was, and the product of insulin and glucose was used as an index of sensitivity. Studies have demonstrated that, as insulin sensitivity increased following weight reduction and maintenance, insulin stimulation of fasting and postprandial LPL activity increased as well [Schwart and Brunzell 1981, Kern et al. 1990]. In the present study, evidence of

enhanced insulin sensitivity in the post-obese group was demonstrated by a lower serum glucose concentration maintained by an even lower serum insulin concentration. In addition, AUC of total TG, CHYLO-TG, and CHYLO-TG ^{13}C concentrations correlated positively with AUC of insulin x glucose concentrations, and more so than with AUC of insulin concentration alone. This is compatible with an enhanced insulin sensitivity and LPL activity.

Once FFA are generated by TRL lipolysis, the second process in TRL clearance is assimilation of FFA into the surrounding tissue. This process is regulated by the additive effects of ASP and insulin. It has generally been assumed that the rate-limiting step in TRL clearance is a function of LPL mass and activity available at the endothelium. This has been supported by animal studies that have shown that the amount of cellular uptake of FFA generated from CHYLO is directly related to the activity of LPL in that tissue [Peterson et al. 1990]. However, essential to that assumption is the supposition that the FFA released by LPL activity can be assimilated by the local tissue at the same rate that LPL can generate them and the possibility that the efficiency of cellular assimilation of FFA could be rate limiting should be raised.

An enhanced LPL lipolytic activity must be accompanied by an equal rate of tissue assimilation of the generated FFA for lipolysis to continue, as buildup of FFA would inhibit the LPL activity [Saxena et al. 1989, Peterson et al. 1990, Karpe et al. 1992]. In fact, *in vitro* studies have demonstrated that LPL has a built-in product-control through a negative feedback inhibition mechanism. These studies have demonstrated that FFA can bind to LPL which decreases its affinity for lipoprotein particles, its affinity for heparin-like polysaccharides on the endothelium, and abolishes LPL activation by

apolipoprotein C-II [Bengtsson and Olivecrona 1980, Saxena et al. 1989, Peterson et al. 1990]. Thus, accumulation of FFA beyond the capacity of nearby cells to assimilate would be rate limiting for TRL clearance. In the present study, an enhanced TRL lipolytic rate in the post-obese is also accompanied by an enhanced FFA uptake. This was demonstrated by the finding that postprandial ^{13}C concentration in plasma FFA pool was similar at all time points to the fasting non-enriched basal levels, and thus there was far less accumulation of ^{13}C in the FFA pool in the post-obese than the controls.

Postprandially, insulin also increases fatty acid uptake into adipose tissue, and with weight loss, higher insulin sensitivity may enhance cellular uptake of FFA generated from TRL hydrolysis [Eckel et al. 1995]. In fact, in the present study, AUC of ^{13}C concentration in the plasma FFA pool correlated negatively with higher insulin sensitivity, as indicated by lower plasma insulin x glucose concentration, and more so than with AUC of plasma insulin alone.

The activity of the ASP pathway on cellular uptake and esterification of FFA is determined by the level of ASP present and the cellular responsiveness or sensitivity to ASP. There was a trend for lower fasting and postprandial plasma ASP concentration in the post-obese women. As with insulin, this could reflect a higher cellular sensitivity to plasma ASP in the post-obese. Of interest, as AUC of total TG correlated positively to AUC of plasma insulin x glucose, the index of insulin sensitivity, so did it correlate to AUC of plasma ASP concentration in both groups, which suggests that subjects with higher insulin sensitivity or lower plasma ASP concentration had a faster clearance of plasma total TG.

Plasma ASP did not increase in the circulating plasma samples measured in this study, and the % change in postprandial plasma ASP concentration from baseline was similar between the post-obese and the control women. In a recent *in vivo* study, looking at the effect of 2 meals with different fat quality and quantity on postprandial ASP production in healthy normal weight individuals, there was also no change in postprandial plasma ASP with either meal [Charlesworth et al. 1998]. However, recent studies have demonstrated that ASP is produced postprandially, locally in adipose tissue, as there was a positive increase in veno-arterial ASP gradient across a subcutaneous adipose bed [Saleh et al. 1998]. Although again, no change in circulating plasma ASP concentration, the increase in adipose tissue ASP gradient was associated with an increased calculated FFA uptake into the adipose tissue. In addition, it was suggested that the postprandial components that stimulate ASP production were chylomicron and insulin (to a lesser extent). In fact, *in vitro* studies demonstrated that chylomicrons added at physiological concentrations to cultured human adipocytes resulted in 150-fold in ASP production whereas insulin resulted in a 2-fold increase [Scantlebury et al. 1998].

Therefore, the postprandial increase in veno-arterial ASP gradient observed in the previous *in vivo* study may well be limited to the microenvironment around the adipocytes where ASP induces its stimulatory effect on TG synthesis. Nonetheless, higher plasma ASP levels were demonstrated in obese subjects and a positive correlation between plasma ASP and BMI was demonstrated [Cianflone et al. 1995, Maslowska et al. 1999]. Thus, acute changes in ASP production in response to high fat feeding would be better detected in the proximal vicinity of the adipocytes, whereas total plasma circulation might better reflect long term regulation of adipose tissue mass. In fact,

compared to normal weight individuals, postprandial veno-arterial ASP gradient was higher in obese subjects, as was their plasma ASP concentration [personal communication, with Dr. K. Cianflone].

A final point to mention is the target tissue of the LPL generated FFA. Postabsorptive dietary fat has 3 potential destinations: liver, muscle and adipose tissue. Hepatic uptake of ^{13}C labeled FFA would eventually be assimilated into TG and re-secreted into plasma circulation as VLDL particles, to prevent lipid accumulation within the liver [Sniderman and Cianflone 1993]. We would expect, then, an increase in the VLDL (density fraction $S_f = 20 - 400$) enrichment following the increase in plasma FFA pool enrichment [Binnert et al. 1996]. In the present study, ^{13}C concentration in the plasma density fraction $S_f = 20 - 400$ increased prior to increases in plasma FFA pool. Moreover, there was a strong positive correlation between ^{13}C concentration in plasma density fraction $S_f = 20 - 400$ and that in plasma density fraction $S_f > 400$. Thus, the early increase in ^{13}C concentration observed in plasma density fraction $S_f = 20 - 400$ pool suggests that this pool contains CHYLO remnants [Bjorkegren et al. 1997]. In addition, it is unlikely that the post-obese women had enhanced hepatic TRL clearance as they have normal fasting plasma total cholesterol, HDL-C, LDL-C and ApoB, similar to that of the controls. In any case, there was no difference in the 2 groups studied in the ^{13}C concentration in plasma density fraction with $S_f = 20 - 400$.

The second destination of dietary fat could be muscle tissue. Two major sites of LPL-activity exist, skeletal muscles and adipose tissue, both of which are under tissue-specific regulation by insulin [Eckel et al. 1995]. Postprandially, in normal weight healthy individuals, insulin enhances adipose tissue LPL activity and inhibits skeletal

muscle LPL activity [Farese et al. 1991]. In the post-obese state, as insulin sensitivity increases so does its tissue specific regulation of LPL, adipose tissue LPL is further increased and skeletal muscle LPL is further inhibited [Eckel et al. 1995]. This suggests that the post-obese are less likely to take up more FFA through direct muscle LPL activity. Although ^{13}C enrichment increased in breath CO_2, we cannot distinguish whether the ^{13}C fatty acids oxidized were derived from skeletal muscle LPL activity or adipose tissue LPL activity. In addition, energy expenditure, carbohydrate/fat oxidation and ^{13}C concentration in breath CO_2 were similar in both groups, which would suggest equal substrate utilization in the muscle tissue between the two groups.

The third potential destination of dietary fat is adipose tissue. Adipose tissue is by far the largest fat depot in the body, and its capacity to store fat is unlimited. ASP is a product of adipose tissue, and its greatest effect on TG synthesis is in adipocytes. Thus, in the post-obese state, an enhanced adipose tissue LPL activity closely coordinated with an enhanced ASP and insulin sensitivity would inevitably channel the dietary fat into the adipose tissue.

The data in this study support the findings in other studies where no difference, between the post-obese subjects and their matched controls, in fasting or postprandial REE, TEF or carbohydrate/fat oxidation rate were detected [Hervey and Tobin 1983, de Peuter 1992, Weinsier et al. 1995, Burnstein et al. 1995]. These metabolic factors have been implicated in the tendency of post-obese to regain weight and thus reestablish the obesity state. However, energy imbalance, between intake and expenditure, only needs to shifted by a small fraction for the cumulative effect to result in obesity. The fattest man in the world died recently in his mid-forties weighing 465 kg. Even this enormous body fat

accumulation requires only an excess intake of as little as a small bar of chocolate every day [Prentice 1997]. Thus, the small differences in energy balance that may exist in the post-obese state could be well beyond the limits of detection of most metabolic measurements limited to the study's length of time. The average intake of North Americans is 3 to 4 meals per day with 35-40% of energy furnished by fat [for review Golay and Bobbioni 1997]. Thus, a synergic effect of consecutive meals, particularly high in energy or fat content, along with an enhanced adipose tissue lipogenesis induced by an enhanced adipose tissue LPL, ASP and insulin activity, could sustain a state of constant push on the adipose depot to store the ingested fat. This prolonged increased fat intake and enhanced adipose tissue fat storage, not counterbalanced by an increased energy expenditure, would progressively and inevitably induce weight regain in the post-obese individuals.

In conclusion, data in this study do not support conclusions of previous studies that the differences between the post-obese women and their matched controls are represented by differences in their energy expenditure, thermic effect of food or substrate utilization [Shah et al. 1988, Astrup et al. 1993, Astrup et al. 1994, Ranneries et al. 1998]. This study casts light on a novel finding of metabolic differences that exist in the post-obese women. These differences are represented by an enhanced plasma clearance of the exogenous fat source, which is indicated by a faster hydrolysis of the circulating TRL accompanied by a faster tissue uptake of the generated labeled FFA, findings which are compatible with an increased sensitivity to insulin and the ASP pathway.

CHAPTER FIVE
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CHAPTER SIX
APPENDICES

Appendix A. Baseline Questionnaire

Royal Victoria Hospital
Cardiovascular Research Unit

STUDY OF POSTPRANDIAL PLASMA ACYLATION STIMULATING PROTEIN RESPONSE AND FAT METABOLISM FOLLOWING THE INGESTION OF A ^{13}C-LABELED HIGH FAT MEAL

Baseline Measurements Questionnaire

Date: _____ Family name: _____ First name: _____

Date of birth: _____ Tel #: _____ Hospital # : _____

Address: _____

Age: _____ Ht: _____ Wt: _____ BMI: _____ BSA: _____

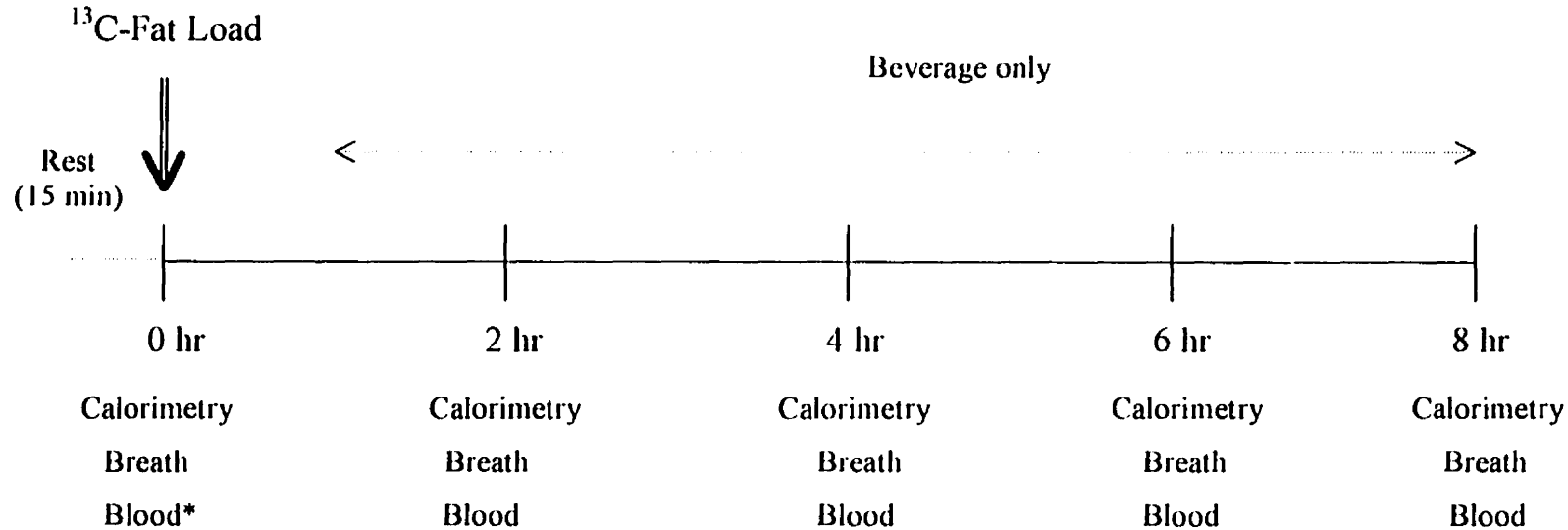
% B. Fat: _____ Fat mass: _____ FFM: _____ W/H ratio: _____

	Yes	No	Comments
Smoker?			# / day?
Alcohol?			Servings/ day?
Regular menstrual cycle?			When did the last cycle end?
Weight stable (4.5 kg/ 6 months)?			
Level of Physical activity?*	Sedentary	Light activity	Moderate activity Very active
Lactose intolerance?			
Gastrointestinal symptoms after food consumption? (Sweating, diarrhea, faint, nausea, vomiting, palpitations...)			
Have any of the following disorders: CVD, HTN, hyperlipidemia, DM, hypoglycemia, GI, pulmonary, liver, thyroid or other endocrine disorders?			
Medications? Pill?			
Hormones?			

*Sedentary: usually under the care of others, minimal activity like watching TV, reading...
Light activity: Most house wives, office workers, lawyers, doctors, teachers, drivers, lab technicians...
Moderate: Light industry, electrical, carpeting...If light activity, must exercise 1.5-2 hr/d to have moderate activity
Very active: Full time athletes, heavy laborers,...

Appendix B. Study Design

Study Design



Calorimetry : From indirect calorimetry, REE, TEF and carbohydrate/ fat oxidation rate were measured

Breath : From breath samples, ^{13}C enrichment in breath CO_2 was measured.

Blood : From blood samples, plasma ASP, total TG, CHYLO-TG, VLDL-TG, FFA, serum glucose and insulin concentrations, in addition to ^{13}C concentration in CHYLO-TG, VLDL-TG and FFA pools were measured.

* for measurement of fasting total plasma cholesterol, HDL-C, LDL-C and ApoB.

(N.B. Anthropometric measurements of Wt, Ht, % body fat, W/H ratio were conducted before the 15 min rest)