The Effects of Weight Loss on Cholesterol Metabolism in Overweight and Obese Hyperlipidemic Women

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ABSTRACT

Obese individuals are at greater risk of various comorbidities including cancer, diabetes, and cardiovascular disease (CVD). Endocrine imbalances and dyslipidemia are likely contributors to the etiology of these diseases in obese individuals.

The objectives of this research project were: (1) to determine the effectiveness of a self-selected diet and exercise weight loss (WtL) protocol in overweight and obese women; (2) to investigate the effects of moderate WtL on hormones associated with the regulation of energy balance, blood lipid levels, and low density lipoprotein (LDL) particle size; (3) to characterize changes in cholesterol metabolism as a result of moderate WtL through an examination of factors that likely play a role in its modulation, specifically body composition and single nucleotide polymorphisms (SNP) in ATP binding cassette (ABC)G5 and ABCG8 transporter.

In carrying out these objectives, 35 women were included in a 24-week WtL trial. Hormone, lipids, and cholesterol metabolism were assessed at the end of two stabilization periods. During these periods, body composition was also measured via magnetic resonance imaging (MRI). WtL was achieved through a 20% decrease in energy intake using diet combined with a 10% increase in energy expenditure through physical activity.

Overall, participants lost an average of 11.7$\pm$2.5 kg. WtL resulted in improvements in blood lipid risk factors of CVD with minimal effect on LDL particle size. No associations were found between leptin, ghrelin, adiponectin, and insulin. Cholesterol synthesis decreased as a result of WtL, while cholesterol absorption and turnover did not change. Despite an absence of change in turnover, increases were predicted by decreases in visceral adipose tissue, and decreases in cholesterol absorption were associated with losses in total and upper body skeletal muscle. This study also showed that changes in cholesterol concentrations and metabolism after WtL are associated with SNPs in ABCG5 and ABCG8 genes.

These findings suggest that hormones important in the regulation of energy homeostasis may exert their effects independently. Moderate WtL results in cardioprotective changes in blood cholesterol levels primarily due to changes in cholesterol synthesis. These findings also indicate that the responsiveness of blood cholesterol levels and metabolism to weight loss is modulated by changes in body composition and SNPs in ABCG5 and ABCG8.

RÉSUMÉ

L'obésité est un facteur de risque pour des co-morbidités telles le cancer, le diabète et les maladies cardiovasculaires (MCV). Les déséquilibres hormonaux et la dyslipidémie contribuent au développement de ces co-morbidités associées à l'obésité.

Les objectifs de ce projet de recherche étaient de : 1) déterminer l'efficacité d'un protocole de perte de poids par la diète et l'exercice physique choisi par les sujets (PPP) chez les femmes ayant une surcharge pondérale ainsi que les femmes obèses, 2) investiguer les effets d'un PPP sur les hormones impliquées dans le maintien du bilan énergétique, le profile lipidique sanguin et la taille des particules de lipoprotéines à faible densité (LFD), 3) caractériser les altérations du métabolisme du cholestérol à la suite d'un PPP, en examinant les facteurs qui pourraient y jouer un rôle, spécifiquement la composition corporelle et les polymorphismes simples de nucléotides (PSN) des transporteurs ATP binding cassette (ABC)G5 et ABCG8.

Afin de réaliser ces objectifs, trente-cinq femmes ont été recrutées pour participer à une étude de perte de poids incluant deux périodes de stabilisation durant lesquelles les mesures de composition corporelle étaient prises, et une période de perte de poids pendant laquelle une diminution des apports énergétiques de 20% et une augmentation de dépenses de 10% par l'exercice physique étaient imposées.

Les participantes ont perdu une moyenne de 11.7$\pm$2.5 kg. Le PPP était à l'origine d'une amélioration du bilan lipidique, avec un effet minimal sur la taille des particules de LFD. Aucune association n'a été notée entre les hormones leptine, ghreline, insuline et adiponectine. La synthèse de cholestérol a diminué en réponse au PPP alors qu'aucun changement n'a été détecté quant à l'absorption et au renouvellement du cholestérol. Cependant, l'augmentation de ce dernier était inversement reliée à la réduction du tissu adipeux viscéral. De même, la diminution de l'absorption du cholestérol était inversement reliée à la perte de la masse musculaire totale et de la partie supérieure du corps. Cette étude démontre aussi que les changements du taux de cholestérol ainsi que de son

métabolisme en réponse au PPP sont associés aux PSN des transporteurs ATP binding cassette (ABC)G5 et ABCG8.

Nos résultats suggèrent que les hormones impliquées dans le maintien du bilan énergétique pourraient avoir des effets indépendants. Les effets protecteurs du PPP sont en grande partie dus aux altérations de la synthèse du cholestérol. Ces résultats démontrent aussi que les effets du PPP sur le profil lipidique et le métabolisme du cholestérol sont affectés par des changements de la composition corporelle et des PSN des transporteurs ATP binding cassette (ABC)G5 et ABCG8.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original contribution to knowledge

The following are the contributions of this thesis to knowledge in the field of obesity:

- Establishes successful motivation strategies, which when incorporated with a protocol of self-directed diet and exercise, resulted in the achievement of moderate weight loss in women.
- Confirms that moderate weight loss in women results in cardioprotective improvements in blood lipid profiles.
- Examines for the first time, the interaction of energy regulating hormones after moderate weight loss in women, and demonstrates that insulin, adiponectin, leptin, and ghrelin may affect weight loss independently.
- Shows that a decrease in weight is predictive of an increase in adiponectin and a reduction in leptin, but is not predictive of other lipid or hormone parameters.
- Demonstrates that initial lipid and insulin levels may be predictive of weight loss and therefore, indicates that knowledge of hyperlipidemia may be an important behavioural motivator for weight loss success.
- Shows that moderate weight loss though diet and physical activity minimally affects LDL particle size and distribution.
- Finds novel changes in cholesterol metabolism after moderate weight loss in women, where despite decreases in cholesterol synthesis, cholesterol absorption and turnover remain unchanged.
- Demonstrates for the first time, that decreases in VAT and VAT:SAT ratio predict increases in cholesterol turnover, and that gains in SM predict augmentations in cholesterol absorption.
- Provides original evidence that the SNPs in ABCG5 and ABCG8 are associated with changes in cholesterol concentrations and cholesterol metabolism.

2. Research publications in refereed scientific journals

- Santosa S, Demonty I, Lichtenstein AH, Cianflone K, Jones PJH. An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr*. (in press)
- Varady KA, Lamarche B, Santosa S, Demonty I, Charest, A, Jones PJH. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women. *Metabolism* 2006:55;1302-1307.

3. Research manuscripts submitted to refereed journals

- Santosa S, Varady KA, AbumWeis S, Jones PJH. Physiological and therapeutic factors affecting cholesterol metabolism: Does a reciprocal relationship between cholesterol absorption and synthesis really exist? (Accepted with revisions at *Life Sci*)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. An effective self-directed protocol for moderate weight loss through diet and exercise in women. (Accepted with revisions at *Can J Diet Pract Res*)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. Changes in cholesterol metabolism and body composition as a result of moderate weight loss through diet and exercise in women. (Accepted with revisions at *Int J Obes*)
- Santosa S, Demonty I, Lichtenstein AH, Ordovas JM, Jones PJH. ABCG5 and ABCG8 single nucleotide polymorphisms contribute to the responsiveness of cholesterol kinetics to weight loss. (Submitted to *J Lipid Res*)

CONTRIBUTION OF CO-AUTHORS TO MANUSCRIPTS

The candidate was responsible for writing the main body of the review article, which integrated the observed relationship between cholesterol absorption and synthesis. The candidate also integrated the sections written by the co-authors and coordinated revisions among the co-authors. The candidate conducted and coordinated the clinical weight loss trial in women with Dr. Isabelle Demonty, a postdoctoral research fellow. As a clinical co-coordinator, the candidate shared the responsibility for recruiting of the study participants. The candidate also shared the responsibility for developing, and executing the study protocol, as well as, collecting and analyzing the data. The candidate analyzed all cholesterol absorption, synthesis and turnover measurements. The candidate also analyzed all magnetic resonance images. With the exception of forward stepwise regression and best subset analyses in the paper entitled "An Investigation of Hormone and Lipid Associations after Weight Loss in Women", which was carried out by Katherine Cianflone, the candidate conducted all statistical analyses. With the exception of the LDL particle size manuscript that was put together with the guidance of the candidate, the candidate under the guidance of the co-authors wrote the original manuscripts.

Dr. Peter Jones, the candidate's supervisor, edited all the manuscripts included in this thesis. Dr. Jones was the principal investigator, developing the initial protocol of the study. Dr. Jones also conducted regular weekly meetings with the candidate to monitor progress and provide any necessary guidance.

Dr. Alice H Lichtenstein was a co-investigator in the study and also developed the initial protocol of the study. Dr. Lichtenstein edited manuscripts 2, 3, 5, and 6, in which she was a co-author. Dr. Lichtenstein's lab analyzed all lipid parameters including cholesterol, triglyceride, apolipoprotein, and C-reactive protein.

Dr. Isabelle Demonty was a clinical co-cordinator in the study and also shared in the responsibility of recruiting study participants, developing, and executing the protocol, and collecting samples to be analyzed. Dr. Demonty analyzed all hormone parameters

including insulin, glucose, leptin, adiponectin, and ghrelin. Dr. Demonty also edited manuscripts 2-6, in which she was a co-author.

Dr. Jose M Ordovas provided all data on ATP-binding cassette protein G5 and G8 single nucleotide polymorphisms. Dr. Ordovas also edited manuscript 5, in which he was co-author.

Dr. Katherine Cianflone provided the facilities and guidance in measuring hormone parameters. Dr. Cianflone also edited manuscript 3, in which she was a co-author.

Krista Varady, who wrote the methods section in part 2 of the literature review, and helped in editing and revising the manuscript. She also wrote and revised the manuscript that examined LDL particle size under guidance of the candidate.

Suhad AbumWeis wrote the section on circadian rhythm and genetic influences section in part 2 of the literature review, and helped in editing and revising the manuscript.

Dr. Benoît Lamarche provided the facilities and guidance in measuring LDL particle size and distribution. Dr. Lamarche also edited manuscript 4, in which he was co-author.

Amélie Charest also provided guidance in measuring LDL particle size and distribution. She also edited manuscript 4, in which she was co-author.

Dr. Jose Morais, Dr. Linda Wykes, and Dr. Jean-Francois Yale, the candidate's committee members, edited the final draft of the thesis.

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INTRODUCTION

The worldwide prevalence of obesity has reached epidemic proportions affecting approximately 1 billion adults globally (World Health Organization, 2003). In Canada direct measurement of weight and height indicates that about 60% of Canadian are overweight or obese (Tjepkema, 2004). Thus, Canada is no exception to this global epidemic. It has been suggested that, if upwards trends in obesity continue, life expectancy of upcoming generations will be lower than that of their parents (Olshansky et al., 2005). Cancer, diabetes and cardiovascular disease (CVD) are among the well-established comorbidities associated with obesity. Obesity is not only associated with diminished quality of life, but is also associated with gross economic burden on the health care system (Daviglus et al., 2003; Katzmarzyk et al., 2004). Collectively, the cost and health risks associated with obesity have led to an emphasis on the importance of weight loss by organizations such as the Heart and Stroke Foundation of Canada, the American Heart Association, and the Canadian and American Diabetes Associations.

Weight loss through decreasing energy intake by diet and increasing energy expenditure by physical activity remains the number one recommended strategy to prevent or treat obesity related comorbidities (Donnelly et al., 2005). Additionally, structured programs where diet and physical activity are closely monitored or supervised achieve greater weight loss compared to programs where participants self-monitor (Andersen et al. 1999; Andersen et al. 2002; Evans et al. 1999; Janssen et al. 2002; Jeffery et al. 2003; Miller et al. 2002; Nicklas et al. 2003; Ross et al. 1995; Volek et al. 2002; Wing et al. 1998). The major limitation in using weight loss regimens with such stringent controls is the time and cost involved in their implementation. Accomplishing weight loss by a more self-directed protocol might include the use of motivational strategies in lieu of close supervision. Various approaches to designing motivational strategies have been suggested (Anderson, 2000). In the clinical setting, an optimal program would integrate these motivational strategies to produce comparable weight loss to more structured regimens, without the need for such rigorous controls.

The success of decreases in body weight cannot only be attributed to the method used as body weight may also be affected by endocrine imbalances of hormones that

regulate energy homeostasis (Diez et al., 2003; Maffei et al., 1995; Pischon et al., 2004; Spranger et al., 2003; Weyer et al., 2001; Wu et al., 2004). Not only do these hormones contribute to the etiology of obesity, they are also likely involved in the development of comorbidities associated with the disease. Adiponectin, found at reduced levels in obese individuals, is also lower in patients with insulin-resistance, type 2 diabetes, and CVD (Diez et al., 2003; Pischon et al., 2004; Spranger et al., 2003; Weyer et al., 2001). Thus, adiponectin may have some function in regulation of blood glucose and lipid homeostasis. Though the role of adiponectin in blood glucose regulation is not as clear, the function of insulin in energy regulation by signalling glucose storage has been well established. Ghrelin and leptin are also hormones that are thought to affect energy homeostasis through opposing effects. Leptin signals satiety, while ghrelin stimulates food intake and energy storage (Maffei et al., 1995; Wu et al., 2004). Studies show that each of the above mentioned energy regulatory hormones are likely affected by weight loss (Baratta et al., 2004; Hansen et al., 2002; Zahorska-Markiewicz et al., 2004). Despite this, no study has examined the combined changes in the hormones as a result of weight loss.

Weight loss also results in cardioprotective changes in blood lipids, yet the mechanism by which weight loss ameliorates blood lipid concentrations is not understood. In addition to low density lipoprotein (LDL) cholesterol concentrations, previous research indicates that LDL particle size and distribution may be an important consideration in CVD risk (Rizzo et al., 2006b). More specifically, small dense LDL particles have been shown to be atherogenic since they are prone to infiltrate arterial tissue and are associated with decreased receptor-mediated uptake (Austin et al., 1988; Bjornheden et al., 1996; Galeano et al., 1998; Stampfer et al., 1996). Weight loss through dietary means has been observed to beneficially affect LDL size and distribution (Archer et al., 2003; Katzel et al., 1995; Markovic et al., 1998; Purnell et al., 2000). The effects of physical activity on LDL size however, is not as clear as some studies have demonstrated a decrease in small LDL particles (Halle et al., 1999; Kang et al., 2002; Williams et al., 1990) and others have observed no change (Elosua et al., 2003) or slight increases (Varady et al., 2005). Therefore, whether moderate weight loss achieved through combined diet and physical activity affects LDL particle size remains to be seen.

Studies have shown that cholesterol synthesis decreases after weight loss (Di Buono et al., 1999a; Raeini-Sarjaz et al., 2001). The research suggests that a relationship exists between cholesterol absorption and synthesis, where such decreases in cholesterol synthesis would lead to increases in cholesterol absorption (Gylling et al., 2002a; Miettinen et al., 2003c). Whether cholesterol absorption and turnover respond to weight-related changes in cholesterol synthesis however, is unclear. Additionally, it has been suggested that changes in regional body composition, especially declines in visceral adipose tissue (VAT), result in cardioprotective changes in lipid levels (Janssen et al., 2002; Leenen et al., 1993; Nieman et al., 2002). However, how regional changes in body composition affect cholesterol metabolism is yet to be determined.

Genetics may also influence the effectiveness of weight loss in decreasing cholesterol levels. More specifically, ATP-binding cassette (ABC) G5 and ABCG8 transporters promote the efflux of cholesterol from hepatocytes and enterocytes (Klett et al., 2004). Single nucleotide polymorphisms (SNP) in these transporters may affect the efficacy with which ABCG5 and ABCG8 function, thereby affecting the response of cholesterol lowering therapies (Kajinami et al., 2004a; Kajinami et al., 2004b). The effect of weight loss on cholesterol levels and metabolism has not been examined in the context of SNPs on ABCG5 and ABCG8.

Study Objectives

Accordingly, the objectives of this project were to:

1. To determine the effectiveness of a self-selected diet and exercise weight loss protocol in overweight and obese women.
2. To investigate the effects of moderate weight loss in overweight and obese hyperlipidemic females on hormones associated with the regulation of energy balance, blood lipid levels, and LDL particle size.
3. To characterize changes in cholesterol metabolism as a result of moderate weight loss through an examination of factors that likely play a role in its modulation, specifically changes in body composition and SNP in ABCG5 and ABCG8 transporter.

CHAPTER 1.
LITERATURE REVIEW

1.1 Introduction

The purpose of this literature review is to delve into the current research surround blood lipid risk factors of cardiovascular disease (CVD) and their metabolism as it relates to weight and weight loss. As such, the literature review is divided into to two parts. The first part examines the influences of dietary cholesterol on cholesterol kinetics, the effects of weight loss and changes in body composition on lipids, the effects of weight loss on low density lipoprotein (LDL) particle size, the potential effects of energy regulatory hormones on weight and blood lipids, and the effects of weight loss and changes in body composition on cholesterol metabolism. The second part is presented in the form of a manuscript and reviews the connection between cholesterol absorption and synthesis and the therapeutic and physiological factors that may affect their relationship.

Part 1

The influence of dietary cholesterol on cholesterol kinetics in vivo

The effect of *in vivo* cholesterol levels on CVD risk and mortality has been well established in past research. A study by Wilson et al. (1998) showed that total cholesterol (TC) levels greater than 5.17 mmol/L account for 27% and 34% increases in CVD event risk in men and women, respectively. Cui et al. (2001) found that elevations in LDL cholesterol (LDL-C) of 0.78 mmol/L led to an augmentation in CVD risk of 15% in men and 8% in women. Contrary to the negative effects of TC and LDL-C on risk, higher levels of high-density lipoprotein cholesterol (HDL-C) have been shown to be cardioprotective (Cui et al., 2001; Kwiterovich, 2000).

Levels of cholesterol *in vivo* are dependent on absorption, synthesis and turnover. Various studies have indicated the influence of diet on cholesterol metabolism. McNamara et al. (1987a) showed a 6% decrease in absorption in American males consuming a diet containing 840 mg/d of cholesterol compared to one containing 240 mg/d. The decreases in percent of absorption occurring in the presence of high cholesterol diets may act in a compensatory fashion to prevent increases in plasma cholesterol. A Finnish study on men indicated a positive correlation of absorption on plasma LDL-C and TC (Kesaniemi et al., 1987b; Miettinen et al., 1989). On average,

cholesterol absorption may account for up to 60% of circulating cholesterol levels (Bosner et al., 1999a; Bosner et al., 1993; Gremaud et al., 2001b). However, a large range of absorption efficiency has been observed in humans (Bosner et al., 1999a; Bosner et al., 1993; Gremaud et al., 2001b). In regards to synthesis, human trials have produced a variety of outcomes, with some indicating no change (Kestin et al., 1989; Keys et al., 1956; Kummerow et al., 1977; McNamara et al., 1987a) and others associating increases in synthesis with the reduction of cholesterol in the diet (Connor et al., 1961; Connor et al., 1964; Hopkins, 1992; Jones et al., 1996; Keys, 1984; Roberts et al., 1981). Conversely, a human feeding trial by Jones et al. (1996) indicated a suppression of synthesis on increasing levels of dietary cholesterol.

1.3 Effects of weight loss and changes in body composition on lipid levels

Decreases in weight of 10% to 15% have been shown to produce cardioprotective changes in blood lipid profiles of overweight individuals (Wing et al., 1995). A meta-analysis of 70 studies by Dattilo and Kris-Etherton (1992b) found that every kilogram of weight loss through dietary means accounted for a 0.05 mmol/L decline in TC, a 0.02 mmol/L reduction in LDL-C and a 0.015 mmol/L decrease in triglycerides (TG). The same meta-analysis also found that increases in HDL-C of 0.009 mmol/L per kilogram of weight loss usually occurred at stabilized weights and increases in HDL-C of 0.007 mmol/L per kilogram weight loss were found in subjects activerly losing weight (Dattilo et al., 1992b). Many trials have been conducted that examine the effects of weight loss on lipids (Miller et al., 2005; Nordmann et al., 2006; Wood et al., 2006). However, most have focused on males with very few examining females. In these studies, weight loss was often achieved through diet only. Even less examined are the effects of changes in body composition on blood lipid levels.

Table 1-1 summarizes four trials conducted on women that examined the effects of weight loss and body composition on blood lipid levels. Overall, body weight was successfully reduced in programs that contained both diet and exercise (Janssen et al., 2002; Nieman et al., 2002; Stefanick et al., 1998). Trials by Stefanick et al. (1998) and Lofgren et al. (2005b) showed an average decrease of 3 – 4 kg and studies by Nieman et al. (2002), and Janssen et al. (2002), found reductions of 8 and 10 kg, respectively.

These studies found that groups which underwent a regiment of diet and exercise exhibited a significant decrease in TC and LDL-C levels (Janssen et al., 2002; Lofgren et al., 2005b; Nieman et al., 2002; Stefanick et al., 1998). The diet and exercise group in the trial by Stefanick et al. (1998) saw reductions in TC and LDL-C of 17.5 mg/dL (0.45 mmol/L) and 14.5 mg/dL (0.37 mmol/L), respectively. Similarly, the trial by Lofgren et al. (2005b) observed decreases in TC, LDL-C, and TG by 8%, 12.3%, and 19.5%, respectively, while no changes in HDL-C were found. The shorter term study by Janssen et al. (2002) which also examined individuals who underwent treatments of diet and exercise, exhibited TC and LDL-C declines of up to 0.60 mmol/L and 0.34 mmol/L respectively. Nieman et al. (2002) found that the diet and exercise groups had average decreases in TC (5.34 mmol/L at baseline vs. 4.70 mmol/L at week 12) and in LDL-C (3.49 mmol/L at baseline vs. 2.96 mmol/L at week 12). Although slight decreases of HDL-C in the diet and resistance group were indicated in the 16-week trial by Janssen et al. (2002), the results of the meta-analysis by Dattilo and Kris-Etherton (1992b) showed increases to occur after further treatment. Therefore, although HDL may decrease initially, increases may occur over the long term. The same study by Janssen et al. (2002) indicated no relation between the type of fat loss and lipid levels. However, declines in body fat percent were related to favourable changes in blood lipid profiles (Janssen et al., 2002; Nieman et al., 2002). Additionally, a review by Leenen et al. (1993) indicated that weight loss, resulting in decreases of visceral fat, were related to favourable changes in blood lipid profile. Other weight loss trials that examined the effects of diet on cholesterol levels also found significant decreases in TC and LDL-C after losses in adipose tissue in the trunk region (Farnsworth et al., 2003; Noakes et al., 2005).

Though these studies indicate an influence of changes in body composition after weight loss by diet and physical activity, the relationship between regional losses and cholesterol changes remains to be more closely examined.

1.4 Effects of weight loss on LDL particle size

Various studies have indicated that weight loss exerts postitive effects on low-density lipoprotein (LDL) size and distribution (Archer et al., 2003; Katzel et al., 1995; Lofgren et al., 2005a; Markovic et al., 1998; Purnell et al., 2000; Wood et al., 2006). More specifically, weight loss has been associated with reductions in smaller, more

atherogenic LDL particles (Archer et al., 2003; Katzel et al., 1995; Lofgren et al., 2005a; Markovic et al., 1998; Purnell et al., 2000; Wood et al., 2006). Despite this, studies in the current literature focus on the effects of diet-related weight changes on these parameters. A recent study by Wood et al. (2006) examined the effects of 7.5 kg of weight loss in men on LDL particle subfraction distribution and size. The weight loss in this study was produced from a diet that consisted of 10% carbohydrate, 65% fat, and 25% protein and resulted in 35% increase in large LDL particles and a 24.7% decrease in very small LDL particles (Wood et al., 2006). LDL particle size was also beneficially affected through 2% weight loss from a low fat/high carbohydrate diet in a study conducted by Archer et al. (2003). Another trial that produced 6.2 kg of weight loss through a low fat diet found a higher proportion of large LDL particles in the plasma (Markovic et al., 1998). Similarly, a study by Katzel et al. (1995) observed increases in LDL peak particle diameter after 10 kg of weight loss in men. One study that examined 70 premenopausal women found that weight loss of about 3.5 kg from diet and exercise resulted in increase in particle size and a decrease in smaller LDL subfractions (Lofgren et al., 2005a). Though the present literature shows that diet-induced weight loss positively alters LDL electrophoretic characteristics, the effect of moderate weight loss from combined low fat diet and exercise, another commonly implemented weight loss strategy, has yet to be tested.

1.5 Hormone interaction with weight and risk factors of cardiovascular disease

Insulin, ghrelin, adiponectin, and leptin are hormones implicated in exerting metabolic regulation of blood lipid risk factors of cardiovascular disease. Since these hormones are also play a role in maintaining energy homeostasis, they may influence or be modulated by weight loss.

The regulation of insulin in blood glucose control has been well established (James et al., 1994). More specifically, the release of insulin from the β-cell of the pancreas is key to glucose transport into myocytes and adipocytes (James et al., 1994). Resistance to insulin has been associated with an increase risk in cardiovascular events (DeFronzo et al., 1991). Indeed, insulin resistance has been linked to low HDL-C and high TG (DeFronzo et al., 1991; Howard et al., 1994; Riemens et al., 1999; Taskinen, 1990). Additionally, insulin may react with LDL receptors to stimulate clearance of LDL from the blood (Mazzone et al., 1984).

Though adiponectin is a cytokine released by adipose tissue (adipocytokine), its secretion has been shown to be reduced in obesity (Arita et al., 1999). Adiponectin has also been implicated in the regulation of blood glucose levels, since it has been shown to be attenuated in people with type 2 diabetes and patients with insulin resistance (Arita et al., 1999; Berg et al., 2002; Spranger et al., 2003; Weyer et al., 2001). Studies in mice indicate that adiponectin may decrease insulin resistance by stimulating fatty acid oxidation and decreasing the presence of TG in the muscle and liver (Yamauchi et al., 2001). Though the mechanism by which adiponectin modulates blood glucose homeostasis has not yet been elucidated, experiments in rats showed that adiponectin decreased hepatic glucose production through inhibiting enzymes of gluconeogenesis (Berg et al., 2001). Lower levels of adiponectin have also been shown to associated with CVD and has been implicated in the development of atherosclerosis (Diez et al., 2003; Ouchi et al., 1999; Ouchi et al., 2001; Pischon et al., 2004).

Although leptin is also an adipocytokine, its function is different than that of adiponectin. More specifically, leptin has been found to signal satiety, through a direct effect on the hypothalamus, and stimulate energy expenditure (Halaas et al., 1995; Lee et al., 1996; Maffei et al., 1995; Wu et al., 2004). Additionally, leptin has been shown to be elevated in people with higher body fat, as is found in obesity (Maffei et al., 1995; Ostlund et al., 1996). The importance of leptin to the contribution of obesity is made evident through the *ob/ob* mouse, that lacks a gene encoding leptin, and is significantly more obese than their normal counterpart (Pelleymounter et al., 1995). Not only has absence of leptin been shown to contribute to obesity, patients with aberrant leptin-secreting adipocytes have been shown to develop lipotoxicity and abnormal fat distribution (Garg, 2004; Garg et al., 2004). Indeed, leptin may act to maintain lipid homeostasis through limiting food intake and thus prevent the excess storage of lipids, and upregulate capacity of nonadipose tissues to oxidize excess lipids, which may have resulted from overnutrition (Unger, 2005).

In contrast to leptin, ghrelin, which is primarily secreted from the stomach, signals food intake and energy storage (Maffei et al., 1995; Wu et al., 2004). Indeed, studies have shown that ghrelin is negatively correlated to BMI and fat cell size (Purnell et

al., 2003; Ravussin et al., 2001; Tschop et al., 2001). Though the influence of ghrelin on cardiovascular disease risk in unclear, a recent study by Purnell et al. (2003) observed a positive correlation between ghrelin and HDL cholesterol. Ghrelin may also affect lipids indirectly through its action on insulin as experiments in mice and rats have found that ghrelin may inhibit insulin secretion from pancreatic islet cells (Qader et al., 2005; Salehi et al., 2004).

Despite the implications of insulin, adiponectin, leptin, and ghrelin in weight control and lipid risk factors of cardiovascular disease, these hormones have never been studied in combination in the context of weight changes produced through diet and exercise.

1.6 Effects of weight and changes in body composition on cholesterol metabolism

Very few studies have examined the effects of weight and more specifically, weight loss on cholesterol kinetics. A trial by Miettinen and Gylling (2000a) showed higher cholesterol synthesis in ten obese versus ten normal weight males. Additionally, the obese group was found to have lower dietary absorption (Miettinen et al., 2000a).

Weight loss has been indicated to affect cholesterol synthesis in experiments conducted on men as well as men and women together (Di Buono et al., 1999a; Griffin et al., 1998; Raeini-Sarjaz et al., 2001). Di Buono et al. (1999a) looked at weight loss in six males with average baseline BMI of 30.3 ± 3.8 kg/m^2. A six month period of weight loss using a caloric deficit of 1000 kJ/d following the AHA StepI diet led to reductions of 3 kg – 8 kg (Di Buono et al., 1999a). This was accompanied with decreases in fractional cholesterol synthesis (FSR) from $8.42\% \pm 3.90\%$/d to $3.04\% \pm 1.90\%$/d ($P<0.05$), as well as reduced absolute cholesterol synthesis rate from 1.66 ± 0.84 g/kg/d to 0.59 ± 0.38 g/kg/d (Di Buono et al., 1999a). Similar results were found in a randomized cross-over trial by Raeini-Sarjaz et al. (2001). This study fed participants (n=11) normal and low energy diets that either contained typical amounts of fat or low amounts of fat for 4 weeks with 6 weeks between phases (Raeini-Sarjaz et al., 2001). The low energy diets resulted in weight loss of approximately 3 kg and were accompanied by FSR decreases of 27.7% and 25.5% (in the low energy and low energy-low fat diet, respectively).

Contrary to this, the trial by Griffin et al. (1998) found no differences in fasting synthesis measured by C14-acetate. In their trial, obese reduced insulin resistant (RI; n=7) and insulin resistant (IR; n=6) participants with diabetes were given a diet designed to decrease daily caloric intake by 25% and fat intake to less than 30% or a diet reducing caloric intake by 800 kcal, whichever was less (Griffin et al., 1998). This diet resulted in weight changes in the RI and IR groups from 92.5±6.4 kg to 85.3±5.5 kg and 107.9±5.5 kg to 99.8±5.2 kg, respectively (Griffin et al., 1998).

Another study in overweight, people with type 2 diabetes by Simonen et al. (2000) also observed no significant changes in cholesterol synthesis after a 3-month weight loss program, followed by a one and a half year maintenance period (n=19). The same experiment attributed an average rise in cholesterol absorption from 29.5% to 37.6% with a weight loss of 6 kg (Simonen et al., 2000). However, no significant changes were observed in cholesterol levels despite these variances in absorption (Simonen et al., 2000). It should be noted that, despite this loss in weight, the lack of significant results in these studies may have been due to the limited number of participants.

Absorption of dietary cholesterol occurs in the intestine, whereas endogenous production of cholesterol occurs in many cells including adipocytes and hepatocytes. In obesity, both adipocytes and hepatocytes increase in number. The meta-analysis of Datillo and Kris-Etherton (1992b) estimated the cholesterol synthetic rate of fat as 20 mg/kg/d. However, they indicate that in vitro studies of cholesterol synthesis demonstrate limited ability for human adipose tissue to convert labeled precursors into sterols (Dattilo et al., 1992b). Since the liver is known to be the primary source of cholesterol synthesis, its higher rate of occurrence in obesity is likely due to hepatic rather than adipose tissue increases (Dattilo et al., 1992b). Indeed, cholesterol synthesis per gram of hepatic tissue has been found to be equivalent in obese and non-obese individuals (Miettinen et al., 2000a). In weight loss, the mobilization of adipose tissue stores of cholesterol during caloric restriction may result in the inhibition of hepatic cholesterol synthesis (Dattilo et al., 1992b).

Studies examining the effects of weight loss on cholesterol metabolism have traditionally used diet as the only method of achieving weight loss. Current guidelines

for a healthy lifestyle also emphasize the importance of physical activity. To our knowledge, the effect of weight loss through diet and exercise on cholesterol kinetics has yet to be studied. Additionally, none of these studies examine the effects of changes in body composition on cholesterol metabolism. A decrease in cholesterol synthesis through weight loss may have effects on cholesterol absorption. However, there are no known trials in overweight healthy individuals that examine this relationship.

LITERATURE REVIEW PART 2.

Manuscript 1.

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Physiological and therapeutic factors affecting cholesterol metabolism: Does a reciprocal relationship between cholesterol absorption and synthesis really exist?

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1.7 Abstract

Cholesterol absorption and synthesis contribute to maintaining cholesterol homeostasis. Several physiological and therapeutic factors affect cholesterol homeostasis, including: genetics, circadian rhythm, body weight, plant sterols, ezetimibe, and statin therapy. The present objective is to determine the main vector, i.e. cholesterol absorption or synthesis, affected by each of these factors, and to examine whether an alteration in one vector is linked to a reciprocal change in the other. Current techniques used to assess cholesterol absorption and synthesis are also reviewed. Review of physiological factors affecting cholesterol metabolism suggest a reciprocal relationship between these two vectors. Carriers of the E2 isoform of apolipoprotein E and ATP binding cassette (ABC) G8 19H (exon 1 mutation) show a decrease in cholesterol absorption accompanied by a corresponding increase in synthesis. Circadian rhythm affects cholesterol synthesis, however, its effect on absorption has yet to be established. Obese subjects show an increase in cholesterol synthesis with a subsequent decrease in cholesterol absorption. Weight loss down regulates cholesterol synthesis, but has little or no effect on absorption. In the case of therapeutic factors, plant sterols and stanols inhibit cholesterol absorption, which results in a compensatory increase in synthesis. Ezetimibe also decreases intestinal absorption, while reciprocally increasing synthesis. Statin therapy down regulates synthesis, which is accompanied by a rise in absorption. These findings suggest that a change in one vector, fairly consistently, results in a compensatory and opposing change in the other. An understanding of this reciprocal relationship between cholesterol absorption and synthesis may allow for the development of more effective interventions for dyslipidemic disorders.

1.8 Introduction

Circulating cholesterol levels are a primary target of therapy for the prevention and treatment of coronary heart disease (NCEP Adult Treatment Panel III, 2001). Cholesterol absorption and synthesis contribute to maintaining cholesterol homeostasis, and thus, cholesterol levels in the blood. Exogenous cholesterol is absorbed within the small intestine, whereas endogenous cholesterol is produced in both hepatic and peripheral tissues (Dietschy et al., 1967; Spady et al., 1983). Cholesterol homeostasis has been shown to be affected by several key physiological factors, such as: an individual's genetics, the body's circadian rhythm, and body weight. In addition to these internal modulators of cholesterol metabolism, certain external therapeutic factors may also affect cholesterol balance. Examples of commonly implemented therapeutic options include: plant sterol or stanol supplementation, ezetimibe treatment, statin therapy, and body weight loss. Each of these physiological or therapeutic factors *directly* affects one of the vectors involved in cholesterol homeostasis, i.e. cholesterol absorption or synthesis. For instance, in the case of ezetimibe therapy, these agents decrease circulating cholesterol levels by directly inhibiting cholesterol absorption, while having no direct effect on cholesterol synthesis. In view of the selective effect of these factors either on cholesterol absorption or synthesis, certain questions that arise include: Does an alteration in one vector result in a reciprocal effect in the other? What is the magnitude of the reciprocal effect? What are current methodologies implemented in measuring cholesterol absorption and synthesis? Moreover, what are the implications of these reciprocal effects with regard to cholesterol lowering therapy? Thus, in order to address these questions, the objective of this paper is to determine the main vector affected by each of the physiological and therapeutic factors mentioned above, and to examine whether an alteration in one vector is linked to a reciprocal change in the other. In assessing the relationship between cholesterol absorption and synthesis, the current methods used to examine these kinetic parameters will also be discussed.

1.9 Methods of measuring cholesterol absorption and synthesis

A variety of techniques exist to measure cholesterol absorption and synthesis. The following section briefly describes the most common methods currently implemented, and also touches upon the advantages and limitations of each.

1.9.1 Cholesterol absorption assessment methods

Sterol balance approach

The gold standard approach to measuring cholesterol absorption is the sterol balance method (Grundy et al., 1969a; Wilson et al., 1965). This technique measures the mass absorption of cholesterol as the difference between dietary intake of exogenous cholesterol and fecal excretion of exogenous cholesterol. So as to not underestimate the amount of exogenous cholesterol absorbed, the quantity of endogenous cholesterol excreted in the feces is accounted for by the administration of intravenous radio labeled cholesterol. Thus, the mass absorption (mg/d) of cholesterol is calculated as the balance between dietary cholesterol intake and fecal cholesterol excretion. Although this method is limited in that it is time consuming, this technique is advantageous in that it allows for the absolute mass absorption of cholesterol to be calculated.

Plasma isotope ratio technique

The plasma isotope ratio technique differs from the balance method in that, instead of quantifying cholesterol absorption, it estimates the fraction of cholesterol absorbed over a defined period of time (Bosner et al., 1999b; Gremaud et al., 2001a; Jones et al., 2000a). Cholesterol absorption is calculated from the ratio of oral cholesterol tracer enrichment, taken to represent the amount of exogenous cholesterol absorbed, to intravenous tracer enrichment, taken to represent the total cholesterol pool in the blood. This method requires that volunteers receive a stable or radioactive isotope labeled cholesterol tracer orally (e.g. $^{2}H/^{3}H$-cholesterol) while simultaneously receiving a second tracer intravenously (e.g. $^{13}C/^{14}C$-cholesterol). Following isotope administration, blood samples are collected over several days. Free cholesterol is isolated from plasma or red cells, and is subsequently combusted to $^{2}H/^{3}H$ -labeled-hydrogen gas and $^{13}C/^{14}C$ -labeled-CO_2 gas (Jones et al., 2000a). Isotopic enrichments are then analyzed and expressed against international standards (Jones et al., 2000a). Alternatively, on-line GC/ combustion and GC/ pyrolysis/ IRMS has been employed. Once the steady state ratio of the $^{2}H/^{3}H$ - to $^{13}C/^{14}C$ -cholesterol enrichments are reached, generally within 2 to 3 days of isotope administration, the fraction cholesterol absorbed can be determined. The plasma isotope ratio technique has been shown to be beneficial in clinical settings in that it allows for the direct

quantification of the percent of cholesterol absorbed. This method, however, may be seen as invasive due to intravenous isotope administration.

Plant sterol-to-cholesterol ratio determination

Evidence suggests that plant sterol metabolism is related to cholesterol absorption. Results of recent human studies indicate that circulating plant sterol levels are correlated with cholesterol absorption rates. More specifically, in a study by (Tilvis et al., 1986), it was shown that the ratio of serum campesterol-to-cholesterol was strongly correlated to percent cholesterol absorption. In support of these findings, in a trial performed in 63 randomly selected Finish subjects, campesterol-to-cholesterol ratios were shown to vary directly with percent cholesterol absorption (Miettinen et al., 1990). These findings indicate that circulating levels of non-cholesterol sterols are proportional to the amount of cholesterol absorbed. This method of absorption assessment is advantageous as it is quick and relatively easy to perform by way of gas-liquid chromatography. Thus, it is feasible for application in large sample populations. This technique, however, is limited in that it only indicates whether absorption has increased or decreased, and does not provide any indication of the absolute amount of cholesterol absorbed. Also, identification of plant sterol peaks is sometimes difficult, confounded by the presence of other substances co-eluting off the GC column at the same retention time.

1.9.2 Cholesterol synthesis assessment methods

Deuterium incorporation approach

The deuterium incorporation approach determines cholesterol synthesis as the rate of incorporation of deuterium from body water into red cell membrane free cholesterol over a 24 h period (Wang et al., 2004b; Woollett et al., 2003). Employing this technique requires that subjects consume a small amount of deuterated water following the study intervention. Shortly after ingestion, the deuterated water equilibrates with intra- and extra-cellular body water pools. In this way, any newly formed cholesterol synthesized within 24 h will have a certain number of H atoms replaced by deuterium. The fractional cholesterol synthesis rate (FSR) (pools/d) over a 24 h period is then calculated as the amount of enriched free cholesterol relative to the total cholesterol pool. Although this method allows for the direct measurement of

the cholesterol pool synthesized over a 24 h period, the laboratory techniques employed to quantify isotope enrichments are quite time-intensive and laborious.

Cholesterol precursor quantification

Previous studies suggest that circulating cholesterol precursors, i.e. lathosterol, lanosterol, squalene, and desmosterol levels, may reflect whole body cholesterol synthesis (Kempen et al., 1988; Pfohl et al., 1999). The rationale for using precursor levels as an indicator of cholesterol synthesis lies in the assumption that these compounds leak into plasma lipoproteins at a rate proportional to that of their formation in the cholesterol synthetic pathway (Ordovas et al., 2002). Levels of cholesterol precursors are determined by gas-liquid chromatography from the non-saponifiable material of serum or plasma lipids. This cholesterol synthesis assessment method benefits from being efficient and inexpensive. On the other hand, the method is limited in that it does not allow for the direct quantification of the amount of cholesterol synthesized. Instead, this method simply indicates whether or not increases or decreases in cholesterol precursors occur as a result of specific interventions.

In view of the above discussion of methodologies, the advantages and limitations of these various methods should be taken into consideration when reviewing the studies employing these techniques.

1.10 Physiological factors affecting cholesterol absorption and synthesis

Certain physiological factors, such as genetics, circadian rhythm, and body weight, have been shown to affect cholesterol homeostasis by directly modulating either cholesterol absorption or synthesis. The following section aims to discuss the primary effect of each of these factors on either one of these vectors, and attempts to delineate whether a corresponding effect between the two vectors exists. A summary of the effects of these physiological factors on cholesterol absorption and synthesis is displayed in **Table 1-2**.

Genetic factors

Cholesterol metabolism is regulated by a host of different proteins and transporters, many of which have been shown to possess genetic polymorphisms. The involvement

of hereditary factors in cholesterol absorption and synthesis is supported by human studies showing inter-individual variability in response to cholesterol lowering by statins (Miettinen et al., 2002, 2003a; O'Neill et al., 2001), heritability of indicators of cholesterol synthesis and absorption (Boomsma et al., 2003; Gylling et al., 2002b), and differences in cholesterol absorption between different mouse strains (Carter et al., 1997; Jolley et al., 1999; Schwarz et al., 2001; Wang et al., 2001a).

Apolipoprotein E (apoE) has been shown to play a major role in lipid transport and metabolism. Three common isoforms of the apoE gene exist, these being E2, E3, and E4. Carriers of the E2 isoform may have lower rates of cholesterol absorption and higher rates of cholesterol synthesis compared to carriers of the E3 or E4 isoform (Gylling et al., 1995; Kesaniemi et al., 1987a; Miettinen et al., 1992). In contrast, carriers of the E4 isoform exhibit increased cholesterol absorption rates, accompanied by a reciprocal decrease in bile acid synthesis (Kesaniemi et al., 1987a). As a result, carriers of the E4 isoform may be better candidates for cholesterol-lowering drugs that inhibit absorption, i.e. plant sterols or ezetimibe. These effects, however, have not been consistently observed. For instance, Miettinen et al. (1992) showed that the effect of apo E polymorphism is poorly related to cholesterol metabolism at low cholesterol intakes. In addition, Jones et al. (1993b) found no relationship between apo E genotype and cholesterol synthesis rate in subjects on self-selected low cholesterol and high cholesterol diets. Therefore, the effect of the apo E isoforms on cholesterol absorption, and their possible reciprocal effect on cholesterol synthesis, may only be apparent at high intakes of dietary cholesterol.

Sitosterolemia, a rare autosomal recessive disorder characterized by high absorption and retention of sterols, is caused by mutations in the ATP binding cassette (ABC) transporters, ABCG5 and ABCG8 (Berge et al., 2000; Lee et al., 2001). ABCG5 and ABCG8 genes express transporters at the intestine and the liver, which play a key role in regulating cholesterol absorption (Ordovas et al., 2002). Although their specific functions are unclear, it has been postulated ABCG transporters may be responsible for the selective reverse transport of cholesterol from the enterocyte into the intestinal lumen. Studies examining non-sitosterolemic individuals were carried out to investigate the effect of sequence variants in ABCG5 or ABCG8 on cholesterol metabolism. Berge et al. (2002) reported that two variants in ABCG8, D19H and

T400K, are linked to lower plasma plant sterols concentrations. Further research found that the 19 H allele (exon 1 mutation) of the ABCG8 gene was associated with low cholesterol absorption markers and high cholesterol synthesis markers (Gylling et al., 2004a). An intervention study that examined genetic associations in 337 subjects after 52 weeks of treatment with 10 mg atorvastatin also observed lower total cholesterol (TC) levels in carriers of at least one mutant 19H allele (Kajinami et al., 2004b). The same study did not find any differences according to the Q604E on ABCG5 and T400K polymorphisms (Kajinami et al., 2004b). Additionally, a novel ABCG8 exon 2 mutation was identified on the Island of Kosrae (Sehayek et al., 2004). The carriers of the mutant allele had increased plasma plant sterols levels and decreased lathosterol levels, indicating enhanced absorption, and a reciprocal decrease in synthesis. These findings indicate that the existence of a relationship between absorption and synthesis may be mediated by mutations in ABCG8.

Niemann Pick Cl Like 1 (NPC1L1), a protein with a sterol-sensing domain, was recently discovered as the intestinal cholesterol transporter (Altmann et al., 2004). A recent investigation on the Dallas Heart Study participants by Cohen et al. (2006) resulted in the identification of various mutations in NPC1L1 that were common in individuals with low cholesterol absorption. Additionally, variant alleles were associated with a 10% lower low-density lipoprotein (LDL) cholesterol (Cohen et al., 2006). The variations were found to be common in 6% of 1832 African-American men and women, however, the investigators were unable to determine which alleles may lead to functional impairment of NPC1L1 (Cohen et al., 2006). Though it is unclear whether individual variations in NPC1L1 affect its function, a haplotype common to 1 in 8 subjects was found to be associated with inter-individual variation in the response of plasma LDL-C to 12 week treatment with ezetimibe, a drug that inhibits cholesterol absorption (Hegele et al., 2005). Further studies have indicated that feeding a cholesterol/cholate diet to wild type mice results in down regulation of intestinal mRNA expression of NPC1Ll (Davis et al., 2004). Moreover, mice that were deficient in NPC1L1 had upregulated intestinal 3-hydroxy-3-methylglutaryl (HMG) CoA synthase mRNA and increased cholesterol synthesis (Davis et al., 2004). Thus, NPC1L1 mutations may play a major role in the indirect control of cholesterol synthesis through regulation of cholesterol absorption.

Studies using knockout mouse models have identified additional genes that express proteins that may affect cholesterol absorption and synthesis including sterol regulatory element binding protein-1 (SREBP-1), acyl CoA:cholesterol acyltransferase 2 (ACAT2) , farnesoid X-receptor (FXR), and mucin gene 1 (Muc1) (**Table 1-2**) (Buhman et al., 2000; Lambert et al., 2003; Shimano et al., 1997). Sterol regulatory element binding protein-1 is a membrane-bound transcription factor that regulates cholesterol synthesis and uptake from circulating LDL. Mice lacking the SREBP-1 gene had elevated levels of levels of mRNAs for 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase and squalene synthase, consequently cholesterol synthesis was elevated threefold in livers of these mice and hepatic cholesterol content was increased by 50% (Shimano et al., 1997). Buhman et al. (2000) showed that ACAT2 is the enzyme responsible for cholesterol esterification in mouse small intestine as well as liver. In addition, it was demonstrated that ACAT2-deficient mice had reduced capacity to absorb dietary cholesterol when fed high cholesterol diet but not when fed a chow diet (Buhman et al. 2000). Thus, deficiency of ACAT2 has a protective effect against hypercholesterolemic diet in mice. Farnesoid X-receptor is a nuclear hormone receptor that play regulate many aspects in cholesterol metabolism including reverse cholesterol transport, intestinal cholesterol absorption and enterohepatic circulation of cholesterol and thus FXR-deficient mice had increased intestinal cholesterol absorption (Lambert et al. 2003). Recently, it was shown that disruption of Muc1, one of the genes that regulate mucin secretion in the small intestine, resulted in 50% reduction in intestinal cholesterol absorption in mice (Wang et al., 2004a). Another regulatory protein, scavenger receptor class B type 1 (SR-BI) which may facilitate cholesterol uptake by enterocytes, has also been identified (Altmann et al., 2002; Mardones et al., 2001). However, existing evidence indicates that SR-BI may not be necessary for cholesterol absorption (Altmann et al., 2002; Mardones et al., 2001). Therefore, studies in human are needed to investigate whether genetic variations in genes mentioned above may play a role in explaining absorption and synthesis responsiveness to various therapeutic factors. Candidate genes that play a role in cholesterol absorption and synthesis are potential targets for the development of cholesterol lowering drugs or functional ingredients.

Circadian rhythm

A circadian rhythm of cholesterol biosynthesis has been identified in animals and humans. In studies involving rats and hamsters, maximum cholesterol synthesis was shown to occur at midnight while minimum synthesis occurs at noon (Edwards et al., 1972; Hamprecht et al., 1969; Ho, 1975, 1979; Jurevics et al., 2000). The peak in synthesis likely corresponds to the nocturnal feeding cycle of rats and hamsters (Edwards et al., 1972; Ho, 1979). When rats are fed during the light period, the maximum rate of cholesterol synthesis occurs 9 h after feeding (Edwards et al., 1972). Another study in hamsters further indicates that regardless of the lighting period, the peak in cholesterol synthesis occurs 4 to 6 h after feeding and the nadir occurred at the end of the fasting period (Ho, 1979). In humans, circadian rhythm in cholesterol synthesis has been measured directly by deuterium incorporation and indirectly by blood concentration of cholesterol precursors (Cella et al., 1995; Jones et al., 1990; Miettinen, 1982; Pappu et al., 1994; Parker et al., 1982). Deuterium incorporation from body water into plasma cholesterol indicates that free cholesterol FSR values peak at 06:00 h and attain a nadir at 14:00 to 18:00 h (Jones et al., 1990). Another study showed maximal cholesterol FSR to occur at 22:00 h while minimal FSR occurred at 11:30 h (Cella et al., 1995). Plasma concentrations of cholesterol precursors also demonstrate circadian variation with highest blood concentrations of mevalonic acid observed at midnight (Pappu et al., 1994; Parker et al., 1982). Furthermore, increased blood concentrations of squalene and lipid soluble methyl sterols occur at midnight and 04:00 h (Miettinen, 1982). In humans, delaying meal time 6.5 h, resulted in 8.6 h and 6.5 h delay in maximum and minimum cholesterol synthesis rates, respectively (Cella et al., 1995). Thus, akin to what was seen to occur in animals, the circadian rhythm of cholesterol synthesis in humans also seems to be affected by food intake.

Many animals and humans studies have examined circadian rhythm in cholesterol synthesis but not circadian rhythm in cholesterol absorption. Nevertheless, the possibility of diurnal variation of cholesterol absorption is supported by data obtained in rats (Edwards et al., 1972; Ishida et al., 2000). Here, hepatic cholesterol synthesis appears low at noon, and also indicated that maximal expression and activity of sterol 12α-hydroxylase (CYP8B) occurred at mid-afternoon in animals subjected to light/dark cycling (Edwards et al., 1972; Ishida et al., 2000). The CYP8B enzyme is

responsible for the synthesis of cholic acid and the determination of the cholic acid-to-chenodeoxycholic acid ratio (Vlahcevic et al., 2000). In mice lacking the gene encodes CYP8B enzyme, cholesterol absorption was reduced and cholesterol synthesis was unregulated (Murphy et al. 2005). Seeing as cholic acid facilitates cholesterol absorption in the intestine, and since cholic acid supplementation enhanced cholesterol absorption in humans, it can be hypothesized that maximal rates of CYP8B results in maximal rates of cholesterol absorption (Chiang, 2004; Woollett et al., 2004). The mechanism by which circadian rhythm affects cholesterol synthesis and absorption could be explained at the molecular level. The expression of many genes encoding enzymes involved in cholesterol metabolism have demonstrated circadian rhythm, such as the expression of HMG-CoA (Jurevics et al. 2000) and CYP8B enzymes (Ishida et al. 2000). Future research in this area should examine the level or the expression of the newly discovered proteins that have a role in cholesterol absorption such as NPC1Ll, as well as ABCG5 and ABCG8 genes.

Body weight

Dyslipidemia is common in individuals who are overweight and obese (Dattilo et al., 1992a). These abnormal fasting lipid profiles may be due to altered cholesterol metabolism commonly found in overweight populations (Di Buono et al., 1999b; Miettinen et al., 2000a). Compared to normal weight individuals, obese subjects demonstrate elevated cholesterol synthesis (Miettinen et al., 2000a). Liver biopsies taken from normal and overweight individuals reveal that the expression of HMG CoA reductase, the enzyme catalyzing the rate-limiting step in hepatic cholesterol synthesis, is augmented in those who are overweight (Stahlberg et al., 1997). Additionally, the activity of other enzymes participating in the cholesterol synthesis pathway are elevated in obese individuals (Stahlberg et al., 1997). Furthermore, it was observed that the livers of obese participants are enlarged (Stahlberg et al., 1997). Since synthesis of cholesterol per g of hepatic tissue is similar in lean and obese individuals, the larger livers of obese compared to lean individuals results in an overall augmentation in synthesis (Angel et al., 1979; Angelin et al., 1982). This increase in cholesterol synthesis in obese subjects has been associated with a decrease in cholesterol absorption (Miettinen et al., 2000a). Thus, it can be speculated that elevations in cholesterol synthesis in the overweight may reciprocally down regulate cholesterol absorption.

1.11 Therapeutic factors affecting cholesterol absorption and synthesis

In addition to the physiological mediators of cholesterol metabolism mentioned above, certain therapeutic factors have also been shown to modulate cholesterol homeostasis. These factors include plant sterol and stanol supplementation, ezetimibe treatment, statin therapy, and weight loss. **Table 1-3** summarizes the effect of these various therapeutic interventions on cholesterol absorption and synthesis.

Plant sterol and stanol supplementation

The chemical structures of plant sterols and stanols are similar to that of cholesterol (Katan et al., 2003; Mel'nikov et al., 2004; Vanstone et al., 2002a). This similarity results in the displacement of micellular cholesterol at the level of the small intestine (Mel'nikov et al., 2004; Vanstone et al., 2002a). Since brush border cells do not readily take up plant sterols and stanols, attenuation in cholesterol absorption occurs (Mel'nikov et al., 2004; Vanstone et al., 2002a). Even though ingestion of plant sterols and stanols effectively lowers cholesterol absorption, studies indicate a compensatory rise in cholesterol synthesis. The effect of sitostanol ester supplementation (3 g/d) on cholesterol metabolism was examined in women with angiographically documented coronary artery disease (Gylling et al., 1997). After 7 weeks of treatment, cholesterol absorption was reduced by 45%, while cholesterol synthesis reciprocally increased by 39%. A similar reciprocal relationship between cholesterol absorption and synthesis was identified by Jones et al. (2000a). This study (Jones et al., 2000a) tested the effect of 1.84 g/d of plant sterol or stanol margarine on cholesterol metabolism in hypercholesterolemic patients. Cholesterol absorption and synthesis were measured following each intervention period by employing the plasma isotope ratio technique. Following sterol and stanol interventions, intestinal absorption of cholesterol decreased while synthesis increased. Comparable results were noted by Vanstone et al. (2002a). Results of this trial (Vanstone et al., 2002a) revealed that absorption diminished while the fractional rate of synthesis rose accordingly in response to 4 weeks of either plant sterol or stanol supplementation (1.84 g/d). Increased synthesis was also found in a study by Jakulj et al. (2005) after participants underwent treatment of 2 g/d of plant sterols. Collectively, these studies indicate a reciprocal response of cholesterol synthesis to changes in cholesterol absorption.

The mechanism by which a compensatory elevation in cholesterol synthesis occurs upon abatement of absorption may be explained through closer examination of the pathway of plasma cholesterol production. In this pathway, the rate-limiting enzyme, HMG CoA reductase, is regulated by intracellular cholesterol through negative feedback. More specifically, intracellular cholesterol regulates HMG CoA reductase concentrations through its ability to influence transcription, mRNA translation, and degradation of the enzyme (Liscum, 2002). Suppression in cholesterol absorption by plant sterols results in lower circulating cholesterol concentrations. As a result, negative feedback of cholesterol on HMG CoA reductase is inhibited, leading to increases in cholesterol synthesis. The reciprocal effect of decreasing cholesterol absorption on cholesterol synthesis is therefore, biologically plausible.

Ezetimibe treatment

Ezetimibe, also known as SCH 58235, is the first of a series of new pharmaceutical agents developed to reduce cholesterol absorption (Jeu et al., 2003). The inhibition of cholesterol absorption by ezetimibe is thought to occur through its interference with the function of the NPC1L1 transporter (Altmann et al., 2004; Davies et al., 2005). Ten mg/d of ezetimibe may lower total cholesterol up to 15% and LDL- cholesterol up to 20% (Jeu et al., 2003). Sudhop et al. (2002) recently examined the effect of ezetimibe on cholesterol absorption and synthesis in humans. The study was a randomized, double-blind, placebo-controlled crossover trial in individuals with mild to moderate hypercholesterolemia. In comparing ezetimibe with placebo, researchers found a 54% decrease in cholesterol absorption. Not surprisingly, the decline in absorption was accompanied by a corresponding rise in cholesterol synthesis. Thus, as with plant sterols, inhibiting cholesterol absorption through ezetimibe results in compensatory increases in cholesterol synthesis.

Statin therapy

Statins lower circulating levels of cholesterol by inhibiting the action of HMG CoA reductase (Smith et al., 2000). The reduction of HMG CoA reductase at the hepatocytes leads to increased expression of LDL receptors, which bind circulating LDL in turn effecting plasma LDL levels (Lennernas et al., 1997; Ray et al., 2004). As a result of this inhibition of hepatic cholesterol synthesis, statin therapy has been shown to reduce cholesterol concentrations by 20-30% (Miettinen et al., 2003d).

Since statin therapy aims to lower cholesterol synthesis, the effect of these pharmaceutical agents on overall cholesterol metabolism has been investigated. Smith et al. (2000) conducted a study where 31 gallstone patients participated in a randomized, parallel-arm, placebo-controlled trial examining the effect of statin therapy on cholesterol kinetic parameters. Seventeen of these patients received 20 mg/d of simvastatin and the other 14 served as controls. Cholesterol precursor-to-cholesterol ratios were used as indicators of hepatic cholesterol synthesis. After 3 weeks of treatment, the plasma lathosterol-to-cholesterol ratio decreased, suggesting a decrease in cholesterol synthesis. Despite these reductions, no changes in cholesterol absorption occurred. More recently, the absorption markers, campesterol, sitosterol and cholestanol, were used to determine the effects of statin therapy in subjects with high initial cholesterol absorption versus those with low absorption (Gylling et al., 2002a). After one year of statin treatment, no differences in circulating total cholesterol levels were observed between the two groups. Conversely, a greater suppression in synthesis in those with low baseline cholesterol absorption levels was noted following treatment. Furthermore, cholesterol absorption was shown to increase more in those who had greater declines in synthesis. Thus, although the study by Smith et al. (2000) did not observe an association, this study (Gylling et al., 2002a) indicates that cholesterol absorption responds reciprocally to alterations in cholesterol synthesis.

The long-term effect of simvastatin treatment on cholesterol metabolism was studied in a subset of the Scandinavian Simvastatin Survival Study (4S) Group (Miettinen et al., 2000b). Eight hundred and sixty seven patients were first divided into four quartiles based on cholestanol-to-cholesterol ratios, and were then randomized to receive either placebo or simvastatin (20 to 40 mg/d) for five years. The cholestanol-to-cholesterol ratio served as an indicator of baseline cholesterol absorption. Results revealed that lower baseline cholesterol absorption was associated with higher baseline synthesis precursor concentrations. After five years, the magnitude by which simvastatin decreased cholesterol synthesis was positively related with initial levels. In turn, cholesterol absorption parameters rose accordingly. Another study observed that 6 months of atorvastatin therapy in men with type 2 diabetes led to decreases in serum markers of cholesterol synthesis and an increase in markers of cholesterol absorption (Miettinen et al., 2003b). A trial which also studied the 4S group found a

similar relationship between synthesis and absorption in a double-blinded trial comparing supplementation of atorvastatin and simvastatin (Miettinen et al., 2003d). After one year of treatment, those who were taking atorvastatin displayed significantly lower cholesterol synthesis rates than those who were taking simvastatin. As a result of this decrease in synthesis, a reciprocal increase in cholesterol absorption was observed as was seen in other studies. Interestingly, cholesterol absorption was shown to be higher in those taking atorvastatin compared to those taking simvastatin. This study therefore supports the hypothesis that cholesterol absorption responds reciprocally to changes in cholesterol synthesis. It should be noted, however, that the compensatory elevation in cholesterol absorption was not equivalent to the decrease in cholesterol synthesis caused by the drugs. Thus, a beneficial effect on circulating cholesterol levels was still observed.

Weight loss

The assertion that weight loss decreases cholesterol synthesis is supported by the work of Di Buono et al. (1999b) who showed that a weight loss of 3 to 8 kg by diet in six overweight and obese men, resulted in a decline in whole body cholesterol synthesis. The reciprocal effect of weight loss on cholesterol absorption, however, has yet to be clarified. Though the action of insulin and glucose on cholesterol metabolism may confound the results (Pihlajamaki et al. 2004), weight loss of approximately 15 kg in 10 overweight people with type 2 diabetes decrease TC by 21% (Simonen et al., 2002b). The decrease in cholesterol was accompanied by a suppression in cholesterol synthesis and an elevation cholesterol absorption (Simonen et al., 2002b). Although a cause and effect relationship has yet to be established, it can be hypothesized that a synergy exists whereby changes in cholesterol synthesis result in an opposite response of cholesterol absorption to maintain cholesterol homeostasis. Further studies that examine the effects of weight loss on cholesterol metabolism in normoglycemic individuals are needed to clarify whether this relationship does indeed exist. Additionally, the question still remains as to how a decrease in hepatic synthesis signals an upregulation in intestinal cholesterol absorption. Future investigation should aim to establish a clear mechanistic cause and effect relationship between synthesis at the liver, and absorption at the intestine.

1.12 Summary and conclusion

In sum, cholesterol absorption and synthesis are affected by an array of physiological and therapeutic factors. The purpose of this paper was to identify the primary vector affected by each of these factors, and to determine whether a reciprocal effect in the other vector occurs in response to this initial change. Studies examining the effect of physiological factors on cholesterol metabolism suggest that a reciprocal relationship does indeed exist (**Table 1-2**). More specifically, in trials examining human genetic polymorphisms, a decrease in cholesterol absorption was consistently accompanied by an increase in synthesis. Such a relationship was shown in carriers of the E2 isoform of apoE, and also in those who carry a mutation on exon 1 of the ABCG8 19H allele. In the case of circadian rhythm, though this factor likely affects cholesterol synthesis, its effect on absorption has yet to be established. With regards to body weight, evidence suggests that the obese state results in an increase in cholesterol synthesis, which is accompanied by a reciprocal decrease in absorption. On the other hand, a loss of body weight down regulates cholesterol synthesis, while having little or no effect on absorption. Similarly, the majority of studies examining the effect of therapeutic interventions on cholesterol metabolism also support a reciprocal relationship between the two vectors (**Table 1-3**). Plant sterols and stanols inhibit cholesterol absorption, which often results in a compensatory increase in synthesis. Ezetimbe also decreases intestinal absorption, while reciprocally increasing synthesis. Conversely, statin therapy down regulates synthesis, which is frequently accompanied by an increase in cholesterol absorption. In view of these findings, it can be concluded that a change in one vector appears to, fairly consistently, result in a reciprocal and opposing change in the other.

Since the majority of these studies support a reciprocal relationship between these two vectors, an important question that arises is the magnitude of this effect. Such a question is imperative as the reciprocal response of the second vector may completely account for the initial change in the first. If such an effect occurred in response to therapy, the effect of the intervention would be obsolete. The magnitude of the reciprocal response was only established in trials examining the effect of plant sterols and statins on cholesterol metabolism. In the case of plant sterols, the treatment-induced decrease in absorption was consistently more pronounced than that of the reciprocal rise in synthesis. As for statins, the drug-induced decrease in synthesis was

generally larger than of the complementary increase in absorption. In this way, the reciprocal change in the second vector did not fully compensate for the change in the first, thus allowing for a reduction circulating cholesterol levels to occur. Establishing the magnitude of response for each factor is not possible due to the techniques employed in certain studies. The magnitude of the reciprocal effect can only be established when certain direct methods are used, such as: sterol balance approach, plasma isotope ratio technique, and the deuterium incorporation method. Since these direct methods were not always implemented, the magnitude of the reciprocal effect could not be established for certain factors.

In conclusion, findings from the current review suggest that a change in one vector is generally accompanied by a reciprocal and opposing change in the second. This relationship however, is not fully compensatory, allowing for the net reduction in body pools of cholesterol by therapeutic treatments. Physiological factors that affect cholesterol homeostasis may be implemented as future targets in the development of effective cholesterol lowering agents. Furthermore, the presence of such a relationship suggests that combination therapies which simultaneously affect both vectors would be more advantageous than therapies that effect one.

Table 1-1 Summary of trials examining the effects of weight loss and changes in body composition through diet and exercise on blood lipid levels in overweight women.

Author	Subjects	Methods	Type of Diet/Exercise	Weight & Body Composition Changes	Changes in Lipids
Stefanick et al. (1998)	180 overweight, postmenopausal women ↑ LDL-C, ↑ HDL-C Age: 45-64 yrs	1 year 4 Groups Diet, Exercise, Diet & Exercise, Control	NCEP Step II 3 x 60 min/wk, aerobic	↓ ~3 kg and ~3.5 kg, no change in waist:hip in Diet and Diet & Exercise	↓ TC ↓ LDL-C in Diet & Exercise
Lofgren et al. (2005b)	70 women, premenopausal Age: 20-45 yrs BMI: 25-37 kg/m^2	10 weeks DEXA Diet & Exercise	↓ energy intake 15%/d ↑ increase 4500 steps/d	↓ 3.6 kg ↓ 2.4 kg fat mass ↓ 0.5 kg fat free mass ↓ 1.3 kg trunk fat ↓ 0.3 kg trunk lean	↓ TC ↓ LDL-C ↓ TG
Nieman et al. (2002)	91 women slightly ↑ TC, ↑ LDL-C Age: 45.6 ± 1.1 yr BMI: 33.1 ± 0.6 kg/m^2 30 control normal C Age: 43.2 ± 2.3 yr BMI: 21.4 ± 0.34 kg/m^2	12 weeks Underwater weighing 4 Groups Diet (D), Exercise (DE), Diet & Exercise (DE), Control	1,200 – 1,300 kcal/d, NCEP Step I 5 x 45 min/wk, aerobic	↓ ~8 kg , ↓ BMI and ↓ body fat percent in D and DE vs. control	↓ TC, ↓TG in D and DE vs. controls ↓ LDL-C in DE vs. controls
Janssen et al. (2002)	38 premenopausal women normolipidemic Age: ~ 37 yrs BMI: ~ 33 kg/m^2 WHR: ~ 0.8	16 weeks MRI 3 Groups Diet alone (DO), Diet & Aerobic (DA), Diet & Resistance (DR)	↓ 1000 kcal/d Aerobic: 5 x 60 min/wk Resistance: 3 x seven ex, one set 8-12 rep	↓ ~10 kg to 11 kg in all ↓ total, ab, subcutaneous, visceral and intermuscular fat in all	↓ TC all ↓ LDL-C in DA and DR ↓ HDL-C in DR no relation to type of fat loss and lipid levels

Table 1-2. Physiological factors that play a role in modulating cholesterol absorption and synthesis.

Physiological factors	Cholesterol absorption	Cholesterol synthesis	Reference
Genetic effects Human model			
Apolipoprotein E			
Isoform E2	↓	↑	(Gylling et al., 1995)
Isoform E4	↑	↓ Bile acid synthesis	(Kesaniemi et al., 1987a; Miettinen et al., 1992)
ABCG8 (allele)			
19H (exon 1 mutation)	↓	↑	(Berge et al., 2002; Gylling et al., 2004a)
19H (exon 2 mutation)	↑	↓	(Sehayek et al., 2004)
Genetic effects Knockout mouse model			
NPC1L1	↓	?	(Altmann et al., 2002)
SREBP-1	?	↑	(Shimano et al., 1997)
ACAT-2	↓	↔ mRNA HMG CoA reductase w/high chol diet	(Buhman et al., 2000)
Farnesoid x-receptor	↑	?	(Lambert et al., 2003)
Mucin gene 1	↓	?	(Wang et al., 2004a)
Circadian rhythm effects Human model			
06:00 and 22:00 hr	↑	?	(Cella et al., 1995; Jones et al., 1990)
14:00 – 18:00 hr	↓	?	(Jones et al., 1990)
Body weight effects			
Obese subjects (BMI >30)	↓	↑	(Di Buono et al., 1999b; Miettinen et al., 2000a; Stahlberg et al., 1997)

SREBP-1: Sterol regulatory element binding protein-1, ACAT-2: Acyl CoA:cholesterol acyltransferase 2, NPC1L1: Niemann-Pick C1 Like 1 protein, BMI: body mass index.

Table 1-3. Therapeutic factors that play a role in modulating cholesterol absorption and synthesis.

Therapeutic factors	Cholesterol absorption	Cholesterol synthesis	Reference
Plant sterol/stanols			
Plant sterol	↓	↑	(Jones et al., 2000a; Vanstone et al., 2002a)
Plant stanol	↓	↑	(Gylling et al., 1997; Jones et al., 2000a; Vanstone et al., 2002a)
Ezetimibe therapy			
Short term 10 mg/d for 2 weeks	↓	↑	(Sudhop et al., 2002)
Statin therapy			
Short term 20 mg/d for 3 weeks	↔	↓	(Smith et al., 2000)
Long term 20-40 mg/d for 5 years	↑	↓	(Miettinen et al., 2000b)
Weight loss			
3-8 kg weight loss	?	↓	(Di Buono et al., 1999b)

CHAPTER 2. Manuscript 2.

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An Effective Self-Directed Protocol for Moderate Weight Loss through Diet and Exercise in Women

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2.1 Abstract

Objective: To examine the efficacy of a self-directed diet and physical activity program as a prospective method for moderate weight loss in women.

Design: Twenty-four week weight intervention study.

Subjects/setting: Thirty-five overweight and obese hyperlipidemic women completed a 24-week weight loss study. The women were between the ages of 35-60 years with body mass indices between 28-39 kg/m^2.

Intervention: The weight loss intervention consisted of a 20% decrease in energy intake through diet and a 10% increase in energy expenditure through physical activity. The diet was based on an exchange system and consisted of 50-60% carbohydrates, 20% protein, and 20-30% fat. A personal trainer prescribed physical activity regimens. A star system and monthly group session were applied to maintain subject motivation throughout the weight loss period.

Results: Participants lost an average of 11.7 ± 2.5 kg ($P<0.001$). The pattern of weight loss was linear ($P<0.001$) throughout the study period. Average weight loss per week was 0.59 ± 0.55 kg. There was no significant relationship between age and weight loss ($r = -0.180$; $P = 0.302$), and initial weight and weight loss ($r = -0.115$; $P = 0.509$).

Conclusions and Applications: A program combining both structured self-selected diet and independent pre-planned physical activity with a successful motivational strategy resulted in weight loss comparable to that observed in more controlled interventions. The lower cost, ease of use, and outcome success makes this approach a potential option for the clinical setting.

2.2 Background

With about 60% of Canadians and 2 out of 3 Americans classified as overweight or obese, it is no surprise that billions of dollars are spent on weight loss products and services (Cleland R, 1997; Hedley et al., 2004; Tjepkema, 2004). Diet books, pills, programs, and even surgeries are among the products used to offer consumers ways to lose weight. However, few weight loss methods have demonstrated consistent and sustained results.

The life expectancy of an obese individual has been found to be about seven years shorter than their normal weight counterparts (Peeters et al., 2003). Obese individuals are at greater risk for numerous metabolic complications including sleep apnea, infertility, dyslipidemia, hypertension, type 2 diabetes mellitus (DM), infertility in women and cardiovascular disease (CVD) (Must et al., 1999). Research examining the extent to which weight loss ameliorates these comorbidities and the mechanism by which weight loss may improve these conditions remains limited. One of the challenges in determining the effects of weight loss on various mechanisms is the achievement of desired weight loss by study participants. Despite the various treatments that exist, the number one recommended weight loss strategy remains changing energy balance by modifying energy intake and energy expenditure (Donnelly et al., 2005).

Many different combinations of diet and physical activity have been used to achieve weight loss in clinical trials. The success of these strategies in losing weight depends on the extent to which diet and exercise are controlled. Participants in studies that have supervised physical activity programs have been reported to lose more weight than those with less structured self monitored programs (Andersen et al. 1999; Andersen et al. 2002; Evans et al. 1999; Janssen et al. 2002; Jeffery et al. 2003; Miller et al. 2002; Nicklas et al. 2003; Ross et al. 1995; Volek et al. 2002; Wing et al. 1998). Additionally, when the diet component of these programs is closely monitored by weekly meetings, daily diet records or through the provision of study meals, achieved weight loss is also greater (Andersen et al. 1999; Andersen et al. 2002; Evans et al. 1999; Janssen et al. 2002; Jeffery et al. 2003; Miller et al. 2002; Nicklas et al. 2003; Ross et al. 1995; Volek et al. 2002; Wing et al. 1998). These results suggest that motivation is a big factor determining the success. Without tight control, and without

motivation tools, weight loss success is less likely. Since supervising diet and exercise is costly, time consuming, and labour intensive few programs can afford to offer such controls.

In the clinical setting, the monitoring of diet and physical activity is not as rigorous as in a structured and supervised research setting. There is more freedom of choice in the diet and physical activity patients will conduct to achieve weight loss success. Thus, it is important to establish a weight loss protocol in which moderate weight loss can be obtained by diet and exercise in the absence of rigorous treatment control. It has been suggested in the literature that successful motivational strategies for weight loss should incorporate behavioural goals, emphasize personal contact, personalize intervention, provide feedback, and multiple contacts over a substantial period of time (Anderson, 2000). As a result, we developed motivational strategies that were incorporated in a weight loss program that combined a self-selected diet and independently conducted exercise. The objective of this study is to examine the effect of a lower cost method on altering energy balance in a group of overweight and obese women under a typical clinical setting.

2.3 Methods

Experimental design

The trial was 24 weeks in duration and consisted of a two week pre-loss stabilization period, a 20 week weight loss period, and a two week post-loss stabilization period. Subjects served as their own controls in a longitudinal before and after study design.

Participants were asked to maintain a stable weight by keeping usual food and exercise habits during the two week pre- and post-loss stabilization periods. During the two week stabilization periods subjects were weighed at days 1, 5, 8, 12, and 15 to ensure stable weight.

Subjects

Forty-two overweight and obese hyperlipidemic women were recruited by newspaper advertisements and word of mouth from the Montreal area to participate in a 24-week weight loss trial. The number of women included in the trial was based on a power calculation that accounted for parameters that were measured for other separate analyses specifically, cholesterol absorption, synthesis and turnover based on an anticipated difference of 20% using a within group coefficient of variation (CV) of 15-25% at an α level of 0.05 and a β level of 0.80. Inclusion criteria required that the women were 35 and 60 years of age with body mass indices (BMI) of 28-39 kg/m^2. Women with BMI less than 28 kg/m^2 were not included because of the extent of weight loss that was targeted, which was 0.75 kg/wk or 15 kg. Since other analyses were being conducted, subjects were also screened for fasting plasma low density lipoprotein cholesterol (LDL-C) concentrations of 3.4-6.7 mmol/L and triacylglycerol (TAG) concentrations >1.5 mmol/L. Use of oral anti-hypertensive agents and thyroid hormones were permitted, provided they were stable and continued throughout the duration of the study. The women were included regardless of menopausal status, provided they had been on a consistent dose of hormone replacement therapy at least three months prior to commencement and remained on the same dose throughout the study if it was being taken. Fasting blood samples were collected for serum biochemistry and haematology and subjects were also required to undergo a complete physical examination by a certified medical doctor before acceptance into the study. Subjects were excluded if they had taken oral hyperlipidemic therapy less than six months prior to commencement or if they had a history of chronic illness, including

hepatic, renal, gastrointestinal, and cardiac dysfunction. Additionally, those who reported previous history of eating disorders, chronic laxative use, or exercise in excess of 4000 kcal/week were excluded.

Before commencing, a copy of the study protocol was given to the subjects and the primary investigator or the study co-ordinators answered any questions before signing the consent form. The study protocol was carried out in compliance with HIPPA guidelines. Ethical approval for the experimental protocol was obtained from the Faculty of Medicine Ethics Review Board at McGill University, Montreal, QC, Canada and the Human Investigation Review Committee of Tufts University, Boston, MA, USA.

Weight loss protocol

A 20 week weight loss period was undertaken after the two week pre-loss stabilization period. Resting energy expenditure (REE) was estimated using the Harris-Benedict equation (HBE) adjusted for habitual activity levels (Harris, 1919). Since the HBE may overestimate REE (Frankenfield et al., 2005), we compared the values we obtained to what would have been indicated using 25 kcal/kg. The weight loss protocol required subjects to decrease energy intake 20% by diet and increase energy expenditure 10% by exercise, resulting in a 30% total energy deficit.

Diet

In general, individually prescribed energy intake was between 1400-1600 kcal/d. Participants were instructed to follow a diet composed of 50-60% of energy from carbohydrates, 20% of energy from protein, and 20-30% of energy from fat. Principles of the diet were taught to each volunteer on an individual basis using an established protocol by a dietitian or nutritionist, depending on the spoken language (ie. French or English) of the participant. To help subjects adhere to dietary principles, we used the exchange lists for meal planning, consisting in the following groups: (1) starch/bread, (2) fruits and vegetables, (3) meat and alternates, (4) milk foods, (5) fat, (6) free foods and (7) foods for occasional use (The American Dietetic Association and American Diabetes Association, 1995; Canadian Diabetes Association, 1999; Health Canada, 1992). More specifically, subjects were given exchange units for each food group and taught the serving sizes of common foods

within each group. To further help apply the dietary principles, participants received pamphlets containing basic nutrition information, as well as sample recipes and examples of menus. A group dinner was organised to show the subjects examples of healthy meals that met the criteria of the exchange system. Monthly individual consultations, usually lasting 15-30 minutes, with the dietitian or nutritionist aided the participants in ensuring dietary adherence throughout the weight loss period. A grocery store tour where subjects learned to read labels was also undertaken.

Exercise

Semi-private meetings of 2-3 participants were conducted with a personal trainer at the beginning and midway through the weight loss period. Sessions covered the teaching of general concepts to the group, individual coaching for practicing the exercises, and adjustment of the routine according to individual needs. The trainer worked with subject exercise preferences in designing exercise routines that would help individuals achieve a 10% increase in energy expenditure. Though both aerobic and strength training exercises were included in the routines, emphasis was on aerobic activity to increase energy expenditure. Proper techniques were also demonstrated to ensure exercises were carried out safely. Participants were given the option to perform the exercises independently or using the exercise equipment at the CNRU at McGill University.

Compliance and motivation

Subjects were weighed once a week throughout the weight loss period. A star system was created to help the subjects keep track of their progress and increase motivation. A chart was put up with each subject's number to conceal their identity and a weekly weight loss goal was assigned to each participant, calculated based on the 30% energy deficit. When the weekly weight loss goal was met, the individual was given a star sticker to put up by her number, if she desired. Each star stood for the weight loss goal for one week. Thus, if half of the weight loss goal was achieved, the subject was awarded half a star. Accordingly, if the participant lost two weeks worth of weight, then two stars were allocated. Participants were also provided with a graph on which they were able to plot their weekly progress. To further increase motivation and adherence to the weight loss protocol, monthly 60 minute group sessions were held where subjects were provided with additional guidance about diet and physical

activity from guest speakers. Small rewards, such as movie passes and gift certificates, were also distributed at the meetings to individuals who achieved their monthly weight loss goal.

Statistics

All data are expressed as means ± SDs. Changes in body weight between the initial and final stabilization periods were assessed using a Student's paired t-tests. An independent sample t-test was used to determine whether the initial weight of dropout subjects were different than the initial weight of subjects who completed the study. Repeated measures ANOVA was used to determine whether the decrease in weight over the 20 week weight loss period was linear. Pearson's correlations were used to examine potential relationships between age or initial weight and weight loss. All values were defined to be statistically significant at $P < 0.05$. Data were analysed using SPSS for Windows (version 12.0.0; SPSS Inc., Chicago, IL).

2.4 Results

Of the 42 subjects recruited to participate, 35 completed the study. Two participants withdrew from the study before the weight loss period due to inability to undergo investigative procedures and five participants dropped out because of difficulty in adhering to the scheduled study visits. Therefore, of the subjects that began the weight loss program, 87% completed the study. Of the participants who completed the study, two decided to come in to use the exercise facilities at the Mary Emily Clinical Nutrition Research Unit (CNRU) at McGill University, while others decided to conduct their exercises independently.

All results shown are for subjects that completed the trial. Initial subject characteristics are shown in **Table 2-1**. The average age of the subjects was 49.4 ± 6.7 years, mean initial weight at the end of the pre-loss stabilization period was 81.4 ± 9.5 kg and average BMI was 31.4 ± 2.8 kg/m^2. There was no difference between the initial weight of dropout subjects and the initial weight of subjects who completed the trial ($P = 0.641$). At the end of the weight loss period, average weight of the subjects was 69.7 ± 9.1 kg. At the end of the post-loss stabilization period average weight was 69.9 ± 9.3 kg. Overall, participants lost an average of 11.7 ± 2.5 kg (14.5 ± 3.1% of original body weight) ($P < 0.001$) between the last day of the pre-loss stabilization

period and the first day of the post loss stabilization period (see **Figure 2-1**). **Figure 2-2** shows the average weight for each week throughout the weight loss period. Decrease in weight throughout the 20 week weight loss period was linear ($P < 0.001$). Average weight loss per week was 0.59 ± 0.55 kg. There was no significant relationship between age and weight loss ($r = -0.180$; $P = 0.302$), and initial weight and weight loss ($r = -0.115$; $P = 0.509$).

2.5 Discussion

In this study, participants were able to steadily lose 12 kg over a 20 week period. To achieve weight loss we incorporated motivational strategies with a healthy, well balanced diet, and independently conducted exercise routine. Weight loss achieved in our program was not related to subject age or initial weight, indicating a potential for this method to be used successfully in a wide range of overweight and obese individuals. The weight loss protocol is appropriate for application to a clinical setting since, participants are able to choose the types of food to be consumed and the kinds of exercises they want to do.

Other studies that used self selected diet and independent exercise often incorporated sessions led by behaviour therapists or psychologists in order to increase weight loss (Andersen et al., 2002; Andersen et al., 1999; Jeffery et al., 2003; Wing et al., 1998). The use of this method has been reported to result in weight losses of between 8-10 kg over a 24-week period (Wing et al., 1998). Other studies that incorporated behavioural therapy as an adjunct to diet and exercise reported somewhat greater rates of weight loss of 6 kg over 12 weeks and 8 kg over 16 weeks (Andersen et al., 2002; Andersen et al., 1999). We achieved at least comparable or greater rates of weight loss using an approach that was less resource dependent since it required minimal contact with a personal trainer, and limited contact with the dietitian/nutritionist. Since the energy deficits and physical activity levels were comparable among the current and prior studies, we suggest the motivational techniques, specifically frequent contact with study investigators, monthly group meeting, and the award point system, we employed contributed to the success of our subjects (Andersen et al., 2002; Andersen et al., 1999; Wing et al., 1998). In contrast, a meta-analysis of weight loss studies that examined the effect of structured diet and aerobic exercise programs on weight loss reported an average weight loss of 11 kg over an average study period of

13 weeks (Miller et al., 1997). This was greater than that attained in the present study. However, the meta-analysis did not control for the types of diet and exercise protocols that were used (Miller et al., 1997). As a result, it is difficult to make further comparisons in the weight loss protocol.

Other more recent studies which used structured diet and exercise interventions without behavioural therapy had comparable weight loss to that found in the present study (Evans et al., 1999; Janssen et al., 2002; Miller et al., 2002; Ross et al., 1995; Volek et al., 2002). A shorter experiment by Volek et al. (2002) observed a decrease in weight of 4-5 kg in 8 weeks after a moderately decreased energy diet and supervised exercise over 4-5 weeks. Another short trial that lasted 9 weeks, provided meals to participants and supervised moderate intensity exercise, achieved a weight loss of 5.5 kg (Miller et al., 2002). Longer trials, such as that conducted by Ross' group (Janssen et al., 2002; Ross et al., 1995), saw decreases in weight of 10-11 kg after a 16-week intervention of self-selected diet which provided 1000 kcal/d energy deficit and supervised aerobic exercise.

One of the limitations of the present study is that it was only conducted in women and therefore, the success of the protocol on weight loss in men is unknown. However, a review by Donnelly and Smith (2005) found that men had larger decreases in body weight in response to exercise than women. Thus, it may be hypothesized that the use of the present weight loss protocol in men would result in increased weight loss. Further studies will need to be conducted to determine whether this would be the case. Testing the protocol in larger more diverse populations would provide more information on its potential to succeed in other subject groups. The inclusion of a control group to which the motivational strategies are not applied would help to better assess their effect on weight loss. Another limitation in this study is that participants had pre-existing hyperlipidemia. This may have added an additional motivational factor, resulting in larger weight loss. However, the majority of overweight and obese individuals have been shown to have associated comorbities (Must et al., 1999). Therefore, the additionally motivational effect of hyperlipidemia in our subjects may not be much different than in other overweight and obese individuals who likely have other comorbidities.

There are many advantages in using the described protocol. The amount of time and manpower required to carry out the intervention is much less than that with a more controlled diet and exercise program, while achieving similar decreases in weight. As a result, it is cheaper and easier to implement our protocol. Because of this, our protocol can feasibly be used in the clinical setting. After an initial period, it is natural that the difficulty in losing weight increases. As seen in Figure 2-2, weight loss throughout the 20-week intervention period was proportional across time. Therefore, the protocol is effective in reducing weight in a longer run. The weight loss in this trial was achieved through moderate calorie reduction and exercise which the participants selected and enjoyed. As a result, patients are more likely to adhere more closely to the protocol. Lastly, our protocol follows dietary and physical activity principles set out in guidelines defined by many associations including the Heart and Stroke Foundation of Canada, Laboratory Center for Disease Control at Health Canada, American Heart Association, and the American Dietetic Association (Cleroux et al., 1999; Cummings et al., 2002; Krauss et al., 2000). Thus, it can be safely used in the clinical settings for weight loss in a diverse group of patients.

2.6 Conclusions and applications

We have introduced a protocol that combines self-selected diet and independent exercise with a successful motivational strategy to produce weight loss comparable to that obtained in more controlled interventions. The lower cost, ease of use, and outcome success of our protocol make it a good candidate for use in the clinical setting.

2.7 Acknowledgements

This project was funded by a grant from the Canadian Institute for Health Research. SS received funding from the Natural Science and Engineering Research Council. We would like to thank Iwona Rudkowska and Patric Michaud for their valuable professional advice. We would also like to thank our subjects for their commitment to the study.

2.8 Figure legends

Figure 2-1. Individual weights (kg) before and after weight loss period.

Figure 2-2. Average weekly weight during the 20 week weight loss period.

Table 2-1. Baseline characteristics of the subjects.

Variables	Participants (n = 35)
Age (y)	49.4 ± 6.7
Weight (kg)	81.4 ± 9.5
BMI (kg/m^2)	31.4 ± 2.8

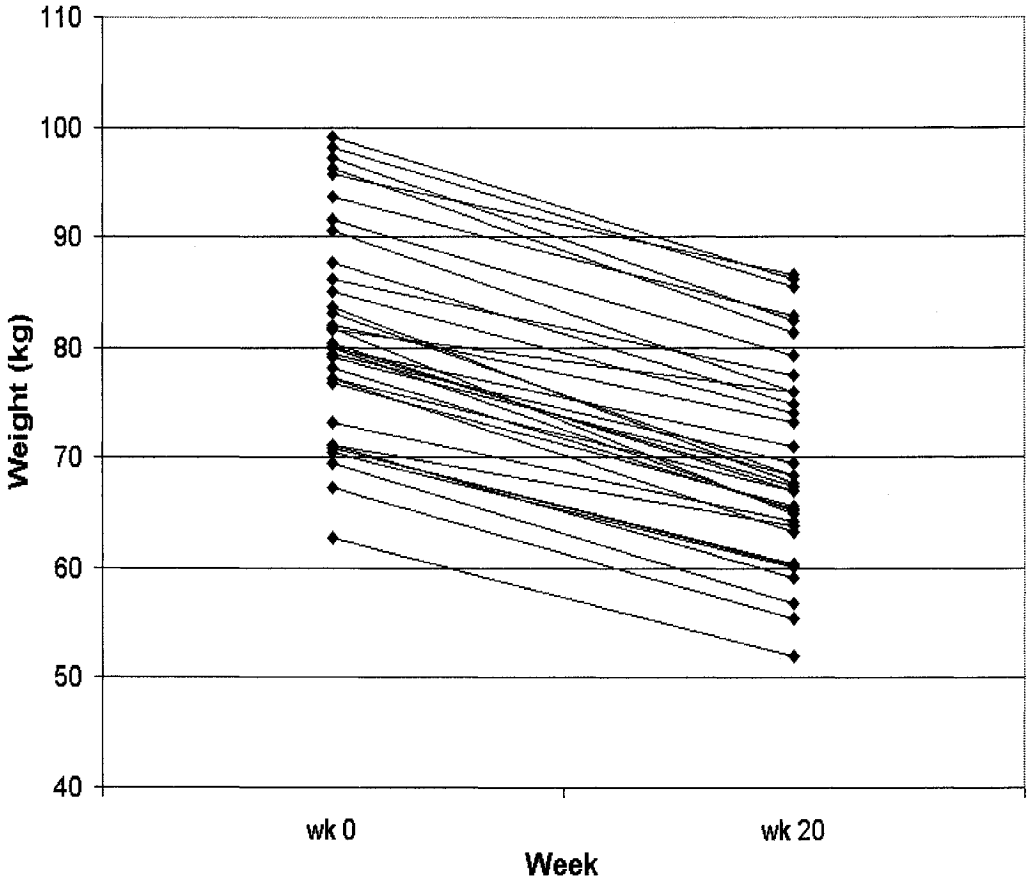

Figure 2-1. Individual weights (kg) before and after weight loss period.

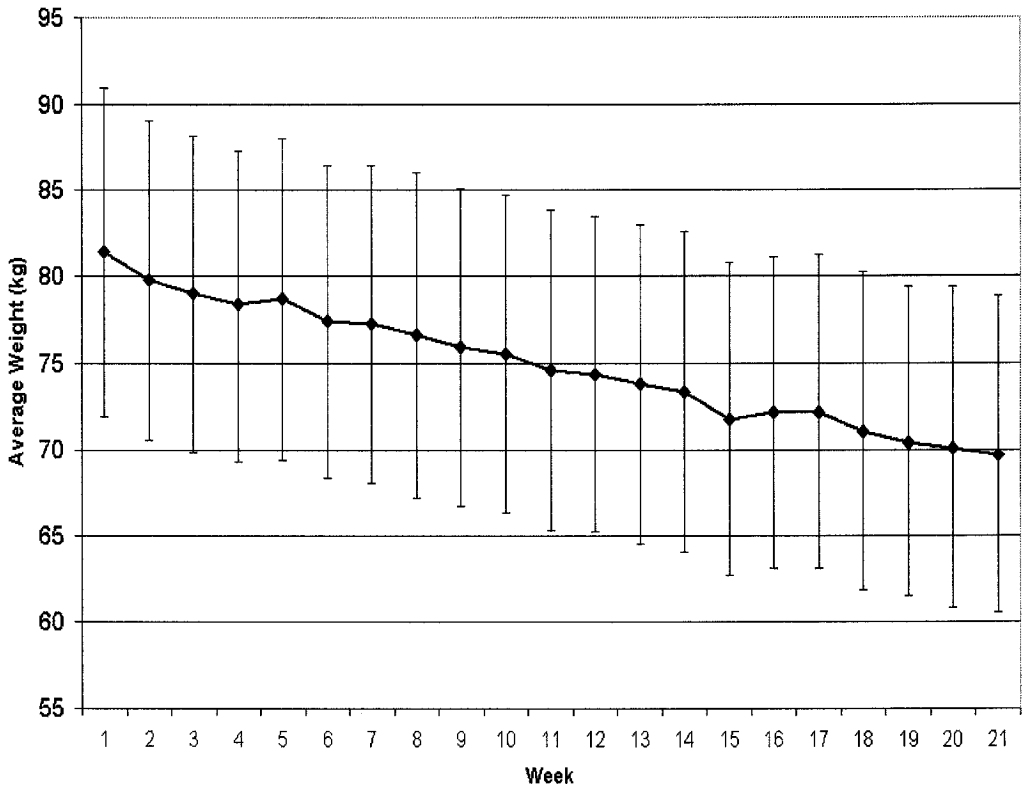

Figure 2-2. Average weekly weight during the 20 week weight loss period.

BRIDGE 1.

Various hormones that play a role energy metabolism may have influenced the weight loss that was achieved with the self-directed protocol from the previous study. More specifically, insulin, adiponectin, leptin, and ghrelin have been shown to be important hormones in maintaining energy homeostasis (Arita et al., 1999; Berg et al., 2002; Halaas et al., 1995; James et al., 1994; Lee et al., 1996; Maffei et al., 1995; Spranger et al., 2003; Weyer et al., 2001; Wu et al., 2004). Weight loss has been shown to result in individual changes in these hormones, as well as reductions in TC, LDL-C, and TAG, and increases in HDL-C (Baratta et al., 2004; Dattilo et al., 1992b; Esposito et al., 2003; Hansen et al., 2002; Ryan et al., 2003; Xydakis et al., 2004). However, no study has examined the asscіations between changes in lipids and hormones as a result of weight loss. Additionally, we also set out to investigate whether baseline metabolic characteristics affected the ability of subjects to loose weight.

All measures were taken at the end of the two-week stabilization periods, which preceded and succeeded the 20-week weight loss period. During these stabilization periods, subjects returned to their usual food and exercise habits. The aim of taking measurements at the end of these stabilization periods was to minimize the effect of changes in diet and exercise so that weight loss itself could be measured. Use of female participants in this study added another unique aspect, since most of the existing studies that examine the effects of weight loss on hormones and lipids are conducted in males.

CHAPTER 3. Manuscript 3.

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An Investigation of Hormone and Lipid Associations after Weight Loss in Women

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3.1 Abstract:

Objective: The objectives of this study were to determine 1) whether the extent of weight loss is predictive of the degree of changes in hormone and lipid levels; 2) the interactions between energy regulating hormones after weight loss through an energy deficit/exercise protocol diet and exercise; 3) whether initial metabolic parameters are indicative of the extent of weight loss.

Methods: Thirty-five hyperlipidemic females (BMI 28-39 kg/m^2) 35-60 years old participated in a six month weight loss trial. Weight loss resulted from a diet and exercise program that when combined produced a 30% energy deficit. Fasting plasma taken during 2 wk stabilization periods at the beginning and end of the study was analysed for lipids, hormone and glucose levels.

Results: Average weight loss was 11.7±2.5 kg ($p < 0.0001$). TC, LDL-C, and triacylglycerols decreased 9.3±9.5% ($p < 0.0001$), 7.4±12.2% ($p < 0.001$), and 26.8±19.6% ($p < 0.05$), respectively, while HDL-C increased ($p < 0.05$) by 8.2±16.3%. Leptin levels declined ($p < 0.001$) 48.9±16.0% and ghrelin levels rose ($p < 0.001$) 21.2±26.7%. While overall levels of adiponectin did not differ, individual values changed such that weight loss predicted increases in adiponectin levels. Though initial weight did not predict weight loss, baseline lipid and insulin levels positively predicted weight loss.

Conclusion: Initial metabolic parameters may be predictors of weight loss. Beneficial effects of weight loss as achieved through diet and exercise on measured parameters indicate moderate weight loss reduces key risk factors of cardiovascular disease in overweight individuals.

Keywords: weight loss, women, cholesterol, adipocyte hormones, ghrelin

3.2 Introduction

The most recent revisions of dietary guidelines by the American Heart Association emphasize the importance of weight management to decrease CVD risk (Krauss et al., 2000). Similarly, weight management is emphasized by the American Diabetes Association (Franz et al., 2004). Since CVD and diabetes are of the primary consequence to obesity and major causes of death and disability in the Western world, it is important to understand the role of weight loss on management and prevention of these diseases.

One of the main reasons that overweight individuals are at increased risk of developing CVD is due to the dyslipidemia that often accompanies this condition. Many trials have been conducted that examine the effects of weight loss on plasma lipid concentrations (Dattilo et al., 1992b; Wing et al., 1995). However, few studies have combined moderate energy expenditure and intake reduction in a highly controlled manner in females. Additionally, the relationship between extent of change in weight versus lipid levels has not been fully elucidated.

Ghrelin, adiponectin, and leptin are three hormones that are likely affected by weight loss. Adiponectin and leptin are adipocytokines, while ghrelin is predominantly released from stomach. Ghrelin and leptin affect energy homeostasis through opposing effects. Leptin decreases feeding by signalling satiety and stimulating energy expenditure, while ghrelin stimulates food intake and energy storage (Maffei et al., 1995; Wu et al., 2004). Studies have shown that leptin is often elevated in people with higher body fat, as is found in obesity (Maffei et al., 1995; Ostlund et al., 1996). In contrast, adiponectin levels have been shown to be reduced in obese subjects (Arita et al., 1999). Adiponectin is also attenuated in patients with insulin-resistance and type 2 diabetes and thus, may have some function in regulation of blood glucose homeostasis (Spranger et al., 2003; Weyer et al., 2001). Levels of adiponectin have also been shown to be lower in those with CVD (Diez et al., 2003; Pischon et al., 2004). However, whether changes in adiponectin reduce risk of CVD and type 2 diabetes is uncertain. Despite the implications of ghrelin, adiponectin, and leptin in weight loss, no studies yet exist that examine changes in these hormones combined in the context of weight loss through diet and exercise.

Previous research has shown that initial metabolic status may influence extent of weight loss. However, studies examined have only accounted for the effect of initial insulin levels and weight (Hansen et al., 2001; McLaughlin et al., 2001). Baseline levels of other hormones, specifically leptin, adiponectin, and ghrelin, that regulate appetite may affect ability to loose weight. Thus, determining the extent to which baseline metabolic characteristics result in successful weight loss may help in deciding the most effective course of treatment of overweight individuals.

The present study uses energy restriction to investigate the potential effects and associations between lipid and hormone parameters, which have not previously been examined in combination after moderate weight loss. Though these parameters have been studied individually after weight loss in men, few studies examine women. In this study, only women were included to investigate the potential of weight loss to affect hormone and lipid risk factors of CVD and type 2 diabetes in this population. Additionally, the novel incorporation of pre and post periods of stabilization will minimize variation and enhance the isolation of the effect of weight loss. In this study, we hypothesized that significant weight loss in overweight and obese hyperlipidemic women results in favourable changes to blood lipid profiles, glucose, insulin, and adiponectin, while decreasing levels of leptin and increasing levels of ghrelin. It is also hypothesized that changes in body weight will be reflected in the degree of changes in the measured parameters. Furthermore, we hypothesized that baseline weight and initial levels of blood lipid, c-reactive protein (CRP), insulin, glucose, adiponectin, leptin, and ghrelin would be predictive of weight loss.

3.3 Materials and methods

Subjects

Hyperlipidemic, but otherwise healthy, free-living overweight and obese female volunteers were recruited. The subjects, aged between 35 and 58 y, had initial body mass indices of 28-39 kg/m^2. Premenopausal and postmenopausal subjects were included, as well as those who were perimenopausal provided they were either not taking or on stable hormone replacement therapy (HRT) for at least 6 months prior to the commencement of the study. Subjects were screened for circulating LDL-C and TAG concentrations. Inclusion criteria included fasting plasma LDL-C concentrations of 3.4-6.7 mmol/L and TAG concentrations >1.5 mmol/L. Use of oral anti-

hypertensive agents and thyroid hormones were permitted, provided they were stable and continued throughout the duration of the study. Before acceptance, subjects were required to provide a medical history and to undergo a complete physical examination. Fasting blood samples were collected for serum biochemistry and hematology. Subjects were screened for chronic illness, including hepatic, renal, gastrointestinal, and cardiac dysfunction, before admission into the study. Additionally, those who were found to have previous history of eating disorders, chronic laxative use, or excessive exercise were excluded. Those undergoing oral hyperlipidemic therapy less than six months prior to commencement of the study were also excluded. Before the start of the study, subjects received the study protocol and discussed any queries with the primary investigator or the study co-ordinators before signing the consent form. The experimental protocol was approved by the Faculty of Medicine Ethics Review Board at McGill University, Montreal, QC, Canada and the Human Investigation Review Committee of Tufts University, Boston, MA, USA.

Experimental design

Subjects served as their own controls in a longitudinal before and after study design. Overall, the trial consisted of three dietary periods; pre-loss stabilization, weight loss and post-loss stabilization periods.

The first and third dietary periods were identical, with each lasting two weeks in duration. During these periods subjects were required to maintain a stable weight, as well as, usual food and exercise habits. To ensure weight maintenance during the stabilization periods, subjects were weighed at days 1, 5, 8, 12, and 15 during period 1 and days 155, 159, 162, 166, 169 during period 3. Compliance during the stabilization period was defined as average weight during the stabilization periods $\pm$ 1 kg. Data are reported for days 15 and 169.

Following the initial two week stabilization period, subjects underwent the 20 week weight loss period, during which, they were counselled to decrease energy intake by 20% and increase energy expenditure by 10%. To achieve the 20% decrease in energy consumption, each subject was provided with individual dietary instruction at the start and for the duration of the weight loss period. Diets were formatted for each volunteer using an exchange system that provided 50-60% of energy from

carbohydrates, 20% of energy from protein and <30% of energy from fat. Subjects also received pamphlets containing basic nutrition information, as well as, sample recipes and menus to aid them in the application of dietary principles.

To achieve a 10% increase in energy expenditure, subjects worked with a personal trainer who demonstrated and taught proper exercise techniques, and assigned exercise routines. Subjects were given the option to perform exercise independently, or within the gym facility at the Mary Emily Clinical Nutrition Research Unit at McGill University.

Compliance to the protocol was monitored by weight loss at weekly weigh-ins. Additionally, participants were encouraged through the use of an award point system and visual graphs on which they were able to plot weight loss.

Fasting blood samples were taken at day 1, 8, and 15 during the first stabilization period and at day 155, 162, and 169 during the second stabilization period for measurement of circulating lipid concentrations. Baseline and endpoint lipid measurements, as well as apolipoproteins (apo) A1 and B100 concentrations, were analysed at days 15 and 169. Glucose, insulin, adiponectin, and leptin and ghrelin were measured at days 14, 15, 168 and 169. Days 14 and 15, and days 168 and 169 were then averaged to reduce day-to-day variation.

Plasma analyses

Fasting plasma was separated from red blood cells (RBC) by centrifugation at 20°C for 15 min at 520 x *g* within 30 minutes of phlebotomy. Samples were then stored at -20°C until analysis. Plasma TC, HDL-C, and TAG concentrations were analysed in duplicate by automated methods through a Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN) using enzymatic or immunoturbidometric reagents (McNamara et al., 1987b). LDL-C was determined directly by the dextran/magnesium sulfate method in order to separate it from HDL-C (N-geneous LDL C assay, Equal Diagnostics) (Rifai et al., 1998; Wang et al., 2001b). Apo A1 and B100 were measured using an Abbott Spectrum CCX Analyzer (Abbott Laboratories, Dallas, TX) using reagents and calibrators from INCSTAR (Stillwater, MN). Immunoprecipitates

of antibody-apoprotein complexes were quantified by turbidimetry (Contois et al., 1996a; Contois et al., 1996b). The assays are standardized through the Lipid Standardization Program of the Centers for Disease Control (Atlanta, GA). C-reactive protein (CRP) was measured using a Tina-quant CRP (Latex) High Sensitive immunoturbidimetric assay (Roche Diagnostics). In brief, anti-CRP antibodies coupled to latex microparticles react with CRP to form a complex which is measured turbidimetrically using a Hitachi 911 automated analyzer (Roche Diagnostics). Glucose was analysed by enzymatic method using the glucose (trinder) reagent from Sigma Diagnostics (St Louis, MO, USA). Concentrations of insulin, adiponectin, leptin, and ghrelin were determined by the RIA method using kits from Linco Research Inc. (St. Charles, MO) (Morgan et al., 1962).

Statistics

All data are expressed as means $\pm$ SDs. The statistical significance of changes in body weight, and lipoprotein cholesterol, TAG, apo A1, apo B100, and CRP was determined using a Student's paired t-tests on values obtained after the two stabilization periods on days 15 and 169. Log transformations were conducted on apo A1 and apo B100 before applying Student's paired t-tests. A Wilcoxon signed rank test was used to compare changes in CRP. Days 14 and 15 and days 168 and 169 were first averaged for glucose, insulin, leptin, adiponectin, and ghrelin concentrations before a Student's paired t-test was carried out on their averages. Log transformations were conducted on leptin and adiponectin before applying Student's paired t-tests. Stability of plasma lipid cholesterol concentrations during the stabilization periods was verified using repeated measures analysis of variance (ANOVA). Linear regression analysis was used to determine the relationship between weight loss and changes in lipid, CRP, glucose, and hormone measures. Forward stepwise regression and best subset analyses were used to determine the predictive values of baseline weight and initial biochemical parameters to weight loss. Pearson correlations were used to determine the relationship between lipid parameters, glucose, insulin, leptin, adiponectin, and ghrelin. All values were defined to be statistically significant at $p < 0.05$. Data were analysed using SPSS for Windows (version 12.0.0; SPSS Inc., Chicago, IL).

3.4 Results

Forty-two subjects were enrolled in the study. Two dropped out at the beginning of the study due to inability to fulfill study requirements and five dropped out because they had difficulty adhering to the weight loss regime and could not adhere to the study visit schedules. Therefore, complete data were collected for 35 participants and analysed as per the study protocol. The average age of the subjects who completed the study was 49.4 $\pm$ 6.7 years. Individual weight loss throughout the study is shown in **Figure 3-1**. Overall, participants lost an average of 11.7 $\pm$ 2.5 kg (14.5 $\pm$ 3.1% of original body weight) ($p < 0.001$).

Changes in biochemical parameters in response to weight loss

No significant differences in lipoprotein cholesterol were found between days 1, 8, and 15, and between days 155, 162, and 169 of the stabilization periods (data not shown). Average circulating plasma lipoprotein cholesterol, TAG, apo A1, apo B100, CRP, glucose, insulin, leptin, adiponectin, and ghrelin levels before and after the study period, as well as, mean changes after weight loss are shown in **Table 3-1**. Both TC and LDL-C levels decreased ($p < 0.001$) 0.62 $\pm$ 0.65 mmol/L, and 0.32 $\pm$ 0.51 mmol/L ($p = 0.001$), respectively. Weight loss also resulted in a reduction ($p < 0.001$) of TAG levels by 0.55 $\pm$ 0.46 mmol/L and an increase ($p = 0.03$) in HDL-C levels by 0.08 $\pm$ 0.21 mmol/L. Additionally, there was a decline ($p = 0.001$ and $p < 0.001$) in both apo A1 and apo B100 levels of 0.06 $\pm$ 0.11 mmol/L and 0.12 $\pm$ 0.12 mmol/L, respectively. Further examination of the LDL-C:apo B100 ratio indicated a rise ($p = 0.002$) of 0.13 $\pm$ 0.23.

After weight loss, glucose levels were lowered ($p = 0.02$) by 0.24 $\pm$ 0.59 mmol/L and insulin by 5.43 $\pm$ 5.00 mUnits/L ($p < 0.001$). There was a reduction ($p < 0.001$) in leptin of 16.0 $\pm$ 7.8 ng/L and ghrelin rose ($p <0.001$) 204.9 $\pm$ 274.9 pg/mL. In contrast, adiponectin levels were not significantly altered by weight loss taking the group as a whole.

Ability of weight loss to predict changes in biochemical parameters

No significant relationship was observed ($p = 0.43$) between changes in weight and changes in total cholesterol ($\beta_1 = -0.04$; $r^2 = 0.02$) nor between changes in weight and changes in LDL-C ($\beta_1 = -0.01$; $r^2 = 0.00$, $p = 0.88$) and changes in weight and changes

in TAG (β_1 = -0.06; r^2 = 0.10, p = 0.07). Weight loss was also not predictive (p = 0.50) of changes in HDL-C (β_1 = -0.01; r^2 = 0.01).

Higher weight loss resulted in larger decreases ($p < 0.001$) in leptin levels (β_1 = -1.82; r^2 = 0.35) **(Figure 3-2a)** and increases (p = 0.009) in adiponectin levels (β_1 = 0.62; r^2 = 0.19) **(Figure 3-2b)**. Changes in weight did not predict changes in glucose, insulin, or ghrelin levels.

Ability of initial biochemical parameters to predict weight loss

Change in weight was best explained by initial apoB (36.2%) and LDL-C levels (13.1%) to a final r = 0.702 in forward stepwise regression. Best subset analyses indicated the same initial parameters explained 52 % of the variation in weight loss (LDL-C p = 0.004 and apoB $p < 0.001$). Initial insulin (p = 0.021), TC ($p < 0.001$), TG (p = 0.004), HDL-C ($p < 0.001$) and LDL-C (p = 0.002) collectively explained 63% of the variation in weight loss. Only the initial leptin level contributed (both in forward stepwise and best subset) to final weight, where r^2 = 0.208 (i.e. 20.8% variation) for p = 0.007. The analyses indicated that baseline weight and initial levels of adiponectin, leptin, and ghrelin were not as strongly associated with the extent of weight loss.

Relationship between lipid parameters, glucose, insulin, leptin, adiponectin, and ghrelin

Calculation of Pearson correlations indicated some relationships between lipid parameters, glucose, insulin, leptin, adiponectin, and ghrelin. Changes in adiponectin levels were positively associated with changes in TAG levels (r = 0.41, p = 0.01) and apo A1 levels (r = 0.38, p = 0.02) **(Figure 3-3a,b)**. Additionally, differences in leptin levels were negatively correlated with changes in TC levels (r = -0.39, p = 0.02), apo A1 levels (r = -0.41, p = 0.01), and apo B100 levels (r = -0.34, p = 0.05) **(Figure 3-3c-e)**. No significant relationships were found between differences in lipid parameters and changes in glucose, insulin, and ghrelin levels. There were also no significant correlations found between adiponectin, leptin, ghrelin, and insulin.

3.5 Discussion

To our knowledge, our study is the only one which attempts to isolate the effect of weight loss through pre- and post- stabilization periods. A steady state was emphasized with the fact that no significant changes were observed during the stabilization periods. Additionally, the exclusion of males in this study is unique because few weight loss trials include only females. This study found that weight loss in females resulted in significant declines in glucose, insulin, leptin, increases in ghrelin, and no changes in adiponectin. The decline in glucose and insulin seen in this study are well established and consistent with findings from other weight loss studies (Esposito et al., 2003; Ryan et al., 2003; Xydakis et al., 2004). However, the effect of weight loss on leptin, adiponectin, and ghrelin levels is not as clear. Only a limited number of existing studies have examined effects of weight loss on ghrelin through diet and exercise on women. The increases in ghrelin found through weight loss in the current study are similar to that found in other weight loss studies (Hansen et al., 2002; Zahorska-Markiewicz et al., 2004).

Although no significant differences in absolute adiponectin levels were found after weight loss in the present trial, individual increases and decreases changed such that weight loss positively predicted changes in adiponectin levels. This finding is consistent with the existing evidence which shows that adiponectin is inversely correlated with fat mass (Kazumi et al., 2004; Xydakis et al., 2004). In addition, a trial that examined the effect of weight loss through surgical procedures found changes in BMI to be negatively associated with adiponectin ($r = -0.20$) (Baratta et al., 2004). The lack of studies examining the consequences of weight loss on adiponectin in women is especially noteworthy since adiponectin concentrations are gender specific; with women having higher concentrations than men (Nishizawa et al., 2002). An investigation of hormonal effects on adiponectin would be useful in further explaining the effect of weight loss on adiponectin levels in females.

Weight loss was also predictive of decreases in leptin in this experiment. Similar relationships were found in other studies that assessed weight loss and changes in leptin levels (Baratta et al., 2004; Hansen et al., 2002). However, these prior studies were conducted in a population that had a much higher initial BMI than was used in

this study, and employed surgical procedures or pharmaceutical methods to promote weight loss (Baratta et al., 2004; Hansen et al., 2002).

Few studies measure all of insulin, adiponectin, leptin, and ghrelin simultaneously and none examine them in the context of weight loss through diet and exercise. Though weight loss was predictive of changes in adiponectin and leptin concentrations, changes in these parameters, as well as changes in insulin and ghrelin, were not correlative. Thus, though adiponectin, leptin, insulin, and ghrelin are all involved in energy expenditure and storage, the present results show that they change independently of each other. This may indicate that each hormone contributes differently to weight loss.

Regression analyses in this study show that the amount of weight loss was not predictive of any change in lipid parameters. A meta-analysis of 70 studies by Dattilo and Kris-Etherton (1992b) found an association whereby TC, LDL-C, and TAG decreases and HDL-C increases for every kilogram loss. However, an earlier study by Andersen et al. (1995), which examined the effects of diet induced weight loss in women, found weight loss to account for no more than 6% of the variation in reductions of TC and TAG. The fact that no relationship was found between lipid parameters could have occurred because there was not enough power with the number of subjects that participated in the trial. However, the meta-analysis examined data from both men and women and the association may not be as strong in women (Dattilo et al., 1992b). Additionally, the associations found between percent weight change and TC, LDL-C, and HDL-C were highly insignificant; indicating an unlikely relationship between the extent of weight loss and changes in lipid parameters. We therefore suggest a threshold effect whereby, after 10% loss such as in the present study, the extent of lipid lowering produced by weight loss in itself is minimized, thus diluting any existing dose-response relationship between weight loss and lipid parameters. This is further supported by the finding by Wing and Jeffery (1995) who found that decreases in weight of 10% to 15% produced cardioprotective changes in blood lipid profiles of both overweight men and women.

A notable finding of the present study was that baseline lipid and insulin levels were predictors of total weight loss, even though initial weight did not predict total weight

loss. Previous research has shown a relationship between initial weight and final weight loss (Hansen et al., 2001). The lack of association between initial weight and final weight loss is unexpected since the higher energy expenditure in those with higher initial weight may result in easier weight loss through energy restriction. As well, higher initial weight may play a motivating role in weight loss. The ability of baseline insulin to predict final weight loss has been seen in other studies (McLaughlin et al., 2001). Trials in Pima Indians and the San Luis Valley Diabetes study have indicated that insulin resistance may limit weight gain (Hoag et al., 1995; Swinburn et al., 1991). Thus, the presence of initial hyperinsulinemia may be predictive of increased weight loss. No study has yet determined that baseline lipids may play a role in predicting weight loss. In this study, participants were screened to have higher baseline TC, LDL-C, and TAG concentrations and thus had knowledge of these levels. The predictive relationship found between initial lipid parameters and final weight loss may indicate the importance of patient knowledge of these risk factors as behavioural motivators for weight loss.

Such favourable changes in lipid profile may be explained by the effect of weight loss on cholesterol metabolism. Although very few studies have examined the effects of weight loss on cholesterol metabolism, those that did have indicated that weight loss may decrease cholesterol synthesis (Di Buono et al., 1999a; Raeini-Sarjaz et al., 2001). Endogenous production of cholesterol occurs in many cells including adipocytes and hepatocytes (Dietschy et al., 1993). Cholesterol synthesis per gram of hepatic tissue has been found to be equivalent in obese and non-obese individuals (Miettinen et al., 2000a). However, in obesity, both adipocytes and hepatocytes increase in number, explaining the higher rates of cholesterol synthesis seen in overweight individuals (Di Buono et al., 1999a; Raeini-Sarjaz et al., 2001) . In weight loss, the mobilization of adipose tissue stores of cholesterol during caloric restriction may result in the inhibition of hepatic cholesterol synthesis, and thus explaining a possible mechanism by which changes in blood lipid concentrations occur (Dattilo et al., 1992b). Further studies that examine the effect of weight loss on cholesterol metabolism are needed to strengthen this hypothesis.

3.6 Conclusion

In summary, though present results show decreases in leptin and increases in ghrelin, beneficial effects of weight loss as achieve through diet and exercise were seen on blood lipid, glucose, and insulin levels of overweight and obese hyperlipidemic women. Such favourable changes in blood lipid profile likely results in decreased risk of morbidity from type 2 diabetes and CVD. Additionally, knowledge of initial lipid risk factors may be behavioural motivators that predict weight loss success. Thus, moderate weight loss is a key factor in the prevention of cardiovascular disease in overweight individuals.

3.7 Acknowledgements

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3.8 Figure legends

Figure 3-1. Weekly changes in weight of individual participants throughout the study. Line shown is based on regression lines of each study period. Formulas for the regression lines are $y = -0.01x + 0.22$; $r^2 = 0.016$, $p > 0.05$ for the pre weight loss stabilization period (days 1-15), $y = -0.10x + 0.76$; $r^2 = 0.997$, $p < 0.001$ for the weight loss period (days 0-155), and $y = 0.02x - 17.43$; $r^2 = 0.167$, $p < 0.02$ for the post weight loss stabilization period.

Figure 3-2a. Regression of leptin (ng/L) ($\beta_1 = -1.81$; $r^2 = 0.35$; $p < 0.001$) on weight loss after a 20 week period.

Figure 3-2b. Regression of adiponectin (mcg/L) ($\beta_1 = 0.62$; $r^2 = 0.19$; $p = 0.009$) on weight loss (kg) after a 20 week period.

Figure 3-3a. Correlations between changes in TAG and adiponectin ($r = 0.41$; $p = 0.01$).

Figure 3-3b. Correlations between change in apo A1 and adiponectin ($r = 0.38$; $p = 0.02$).

Figure 3-3c. Correlations between changes in TC and leptin ($r = -0.39$; $p = 0.02$).

Figure 3-3d. Correlations between changes in apo A1 and leptin ($r = -0.41$; $p = 0.01$).

Figure 3-3e. Correlations between changes in apo B100 and leptin ($r = -0.34$; $p = 0.05$).

Table 3-1. Before and after weight and biochemical parameter concentrations. Before and after weight and lipid parameters were determined at days 15 and 169. The average of days 14 and 15, and days 168 and 169 were used for determination of CRP, glucose and hormone levels. Differences of biochemical parameters are shown as integers and percentages.

Variable	Before	After	Change	Change (percent)
Weight (kg)	81.4 ± 9.5	69.7 ± 9.1	11.7 ± 2.5 [b]	-14.5 ± 3.1
BMI (kg/m^2)	31.4 ± 2.8	26.9 ± 2.9	-4.5 ± 0.9[a]	-14.5 ± 3.1
Total cholesterol (mmol/L)	6.08 ± 0.94	5.46 ± 0.64	-0.62 ± 0.65[a]	-9.3 ± 9.6
LDL cholesterol (mmol/L)	3.76 ± 0.68	3.44 ± 0.53	-0.32 ± 0.51[b]	-7.4 ± 12.2
Triacylglycerol (mmol/L)	1.83 ± 0.70	1.28 ± 0.43	-0.55 ± 0.46[d]	-26.8 ± 19.6
HDL cholesterol (mmol/L)	1.16 ± 0.29	1.24 ± 0.31	0.08 ± 0.21[d]	8.2 ± 16.3
Apolipoprotein A1 (mmol/L)	1.27 ± 0.20	1.21 ± 0.18	-0.06 ± 0.11[c]	-4.6 ± 7.9
Apolipoprotein B100 (mmol/L)	1.03 ± 0.17	0.91 ± 0.13	-0.12 ± 0.12[b]	-10.9 ± 9.9
LDL-C:Apo B100	3.64 ± 0.33	3.77 ± 0.27	0.13 ± 0.23[c]	3.8 ± 6.4
CRP (mg/L)	3.45 ± 4.47	2.03 ± 1.66	-1.41 ± 4.55[c]	-24.7 ± 37.3
Glucose (mmol/L)	5.50 ± 0.81	5.26 ± 0.67	-0.24 ± 0.59[d]	-3.6 ± 10.2
Insulin (mUnits/L)	17.15 ± 6.63	11.73 ± 4.24	-5.43 ± 5.00[b]	-28.4 ± 20.3
Leptin (ng/L)	33.43 ± 8.09	17.41 ± 8.88	-16.02 ± 7.77[b]	-48.9 ± 21.9
Adiponectin (mg /L)	17.63 ± 7.29	17.20 ± 7.21	-0.43 ± 3.62	-0.2 ± 21.8
Ghrelin (pg/mL)	1102.0 ± 464.8	1307.0 ± 562.5	204.9 ± 274.9[b]	21.2 ± 26.7

Data are reported as mean +/- SD. [a]$p<0.0001$, [b]$p<0.001$, [c]$p<0.005$, [d]$p<0.05$

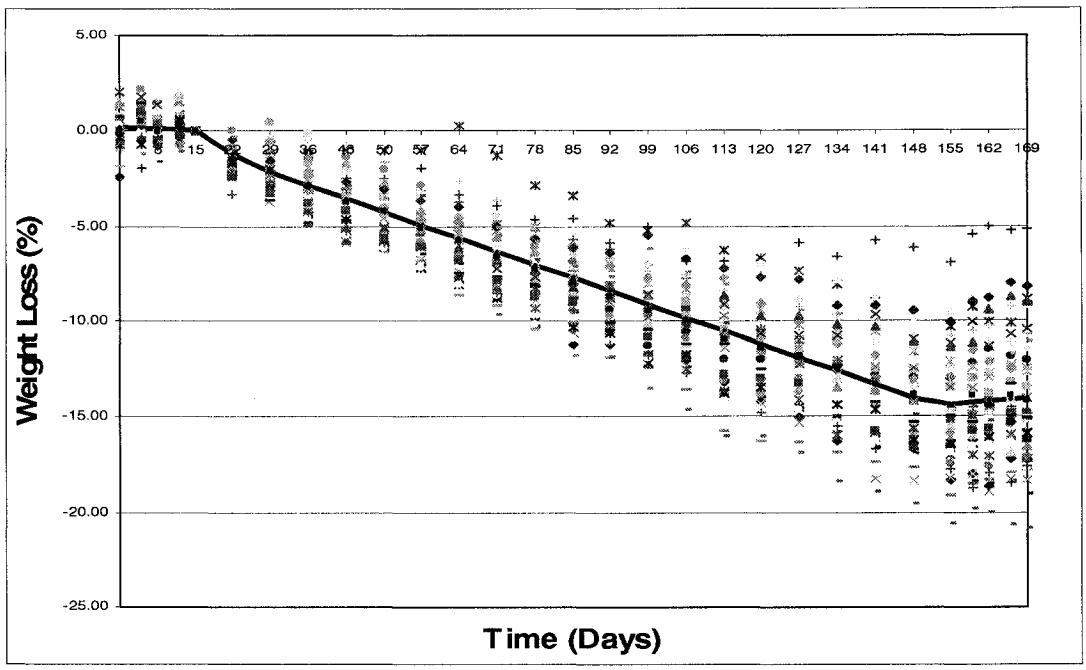

Figure 3-1. Weekly changes in weight of individual participants throughout the study. Line shown is based on regression lines of each study period. Formulas for the regression lines are $y = -0.01x + 0.22$; $r^2 = 0.016$, $p > 0.05$ for the pre weight loss stabilization period (days 1-15), $y = -0.10x + 0.76$; $r^2 = 0.997$, $p < 0.001$ for the weight loss period (days 0-155), and $y = 0.02x - 17.43$; $r^2 = 0.167$, $p < 0.02$ for the post weight loss stabilization period.

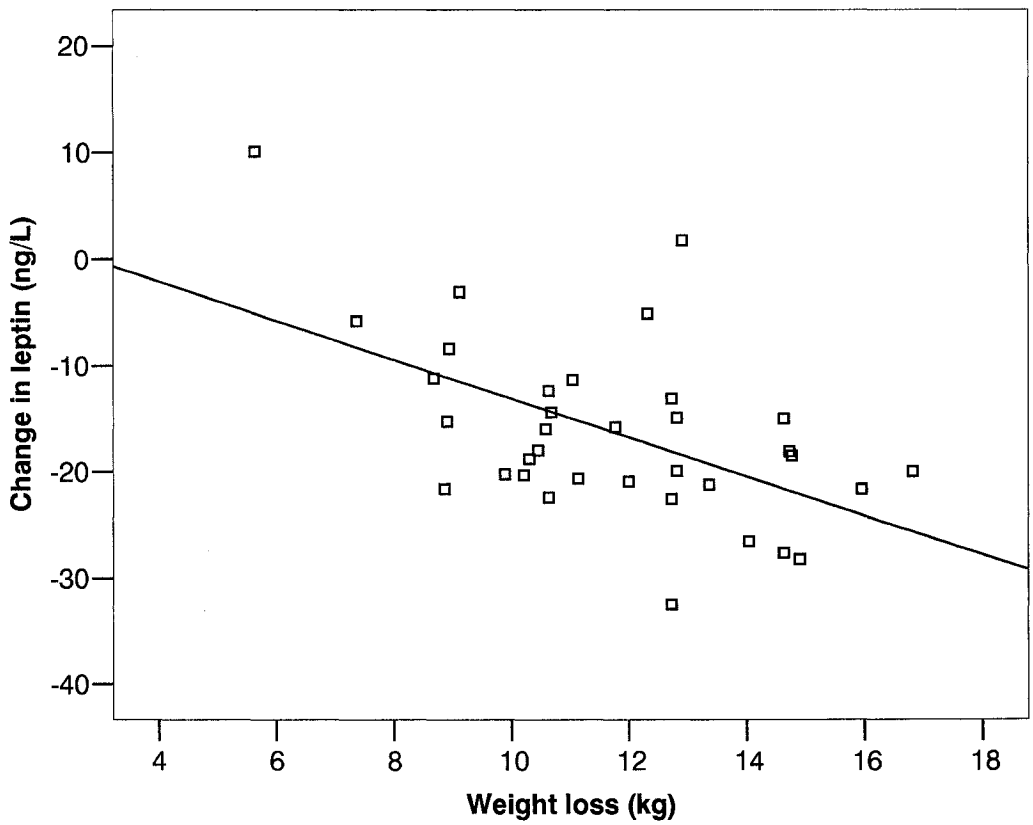

Figure 3-2a. Regression of leptin (ng/L) (β_1 = -1.81; r^2 = 0.35; $p < 0.001$) on weight loss after a 20 week period.

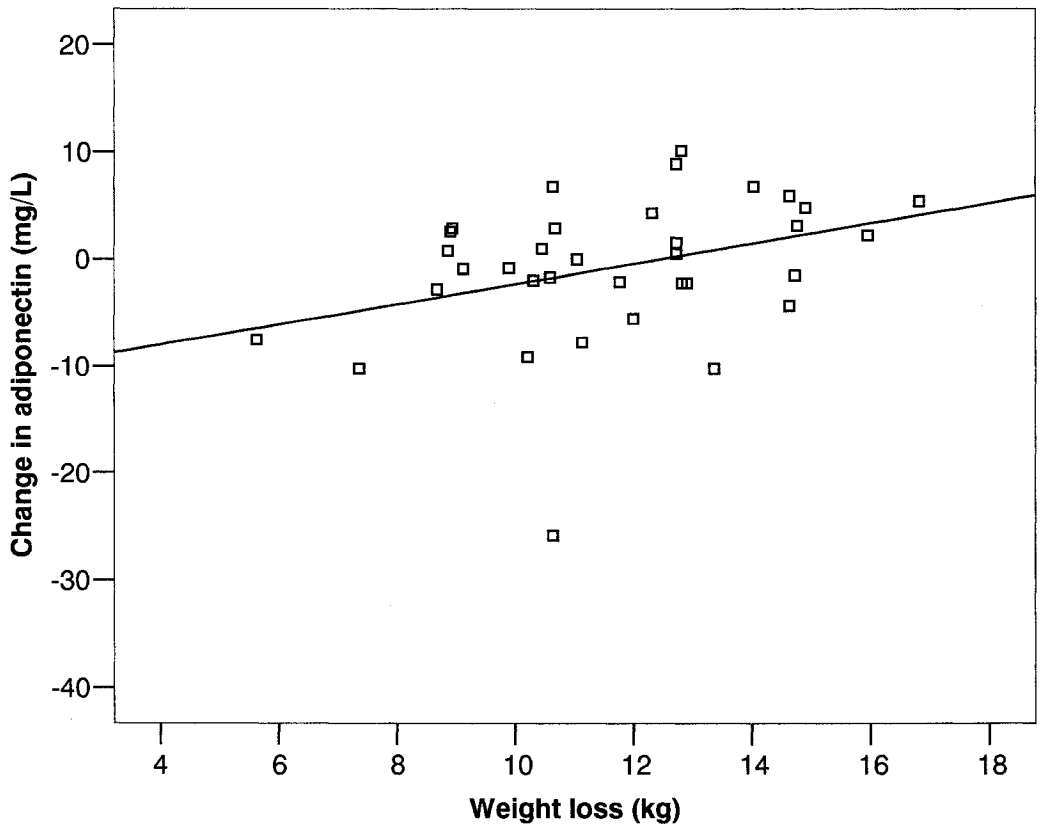

Figure 3-2b. Regression of adiponectin (mcg/L) ($\beta_1 = 0.62$; $r^2 = 0.19$; $p = 0.009$) on weight loss (kg) after a 20 week period.

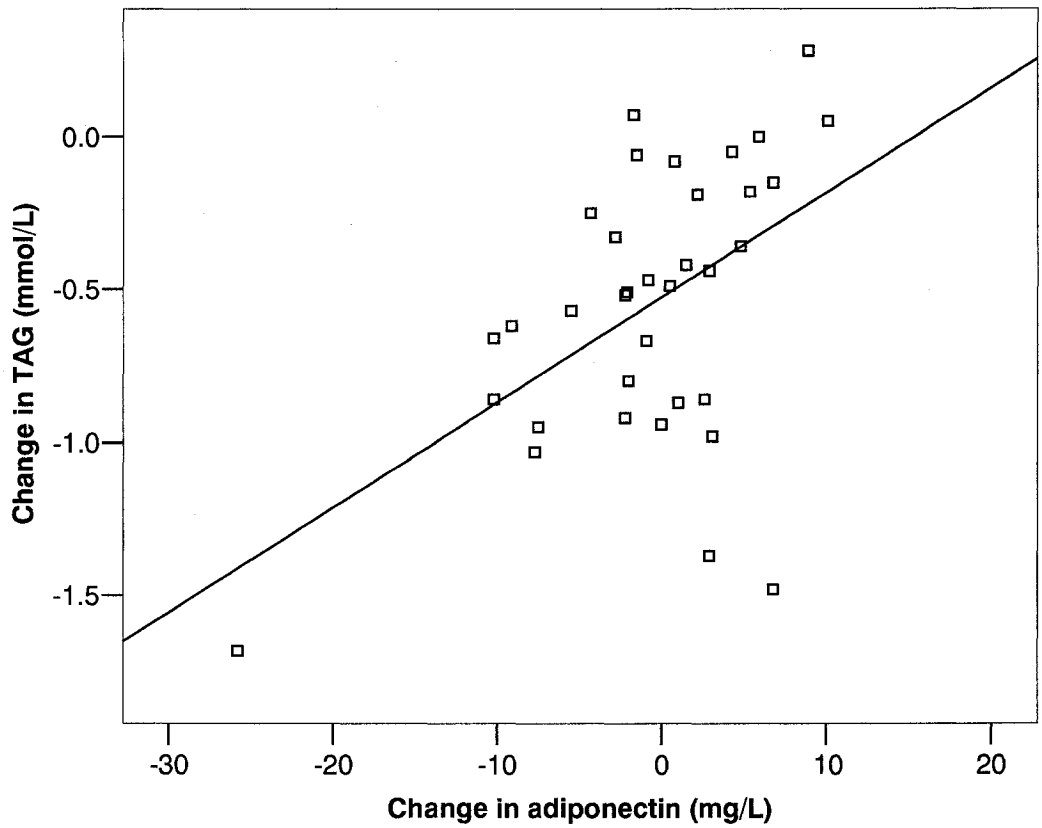

Figure 3-3a. Correlations between changes in TAG and adiponectin (r = 0.41; $p = 0.01$).

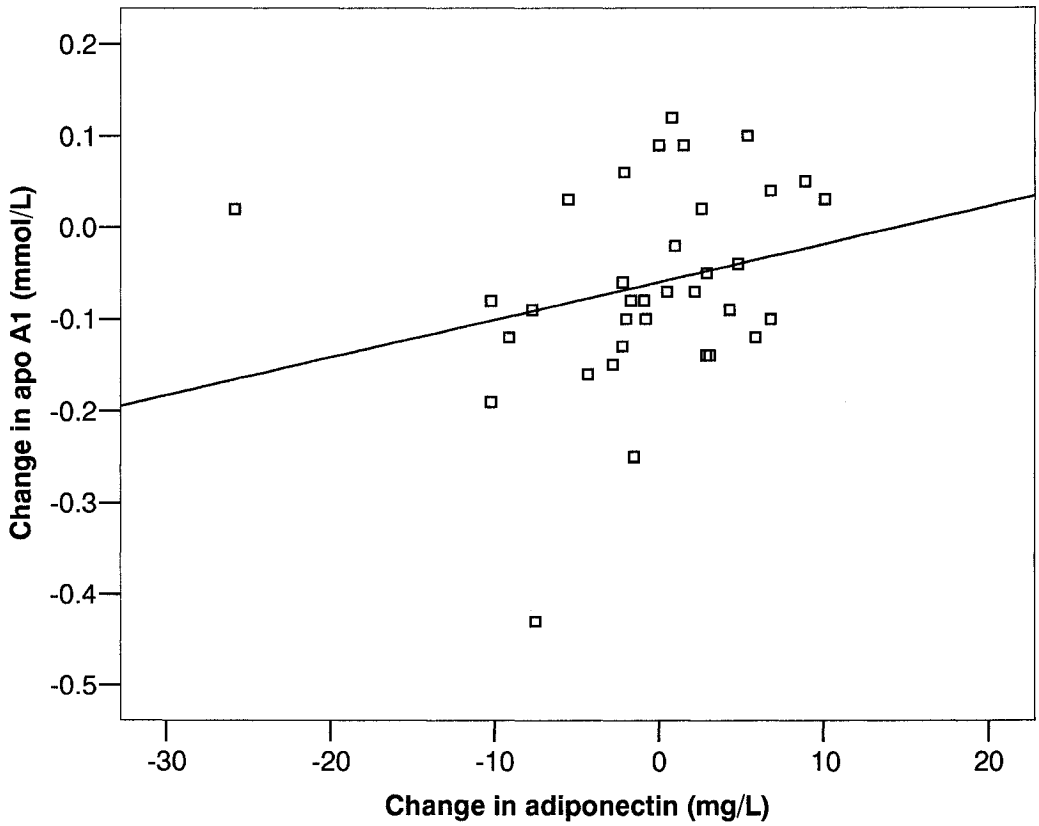

Figure 3-3b. Correlations between change in apo A1 and adiponectin (r = 0.38; p = 0.02).

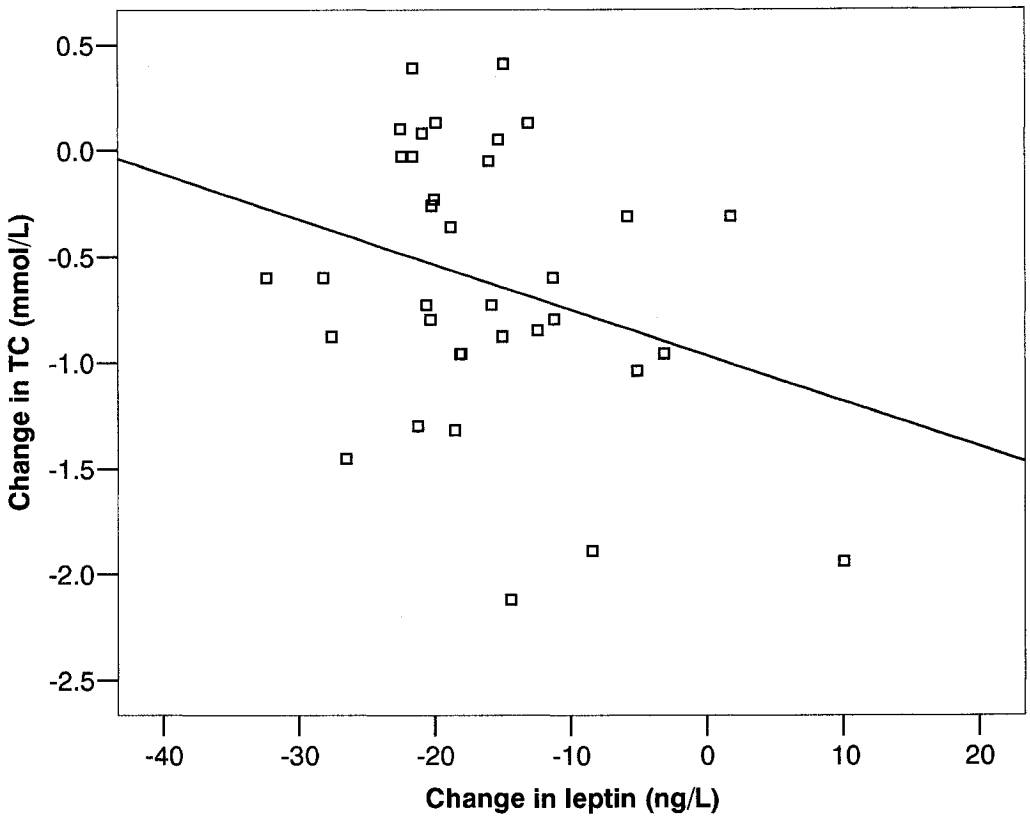

Figure 3-3c. Correlations between changes in TC and leptin (r = -0.39; p = 0.02).

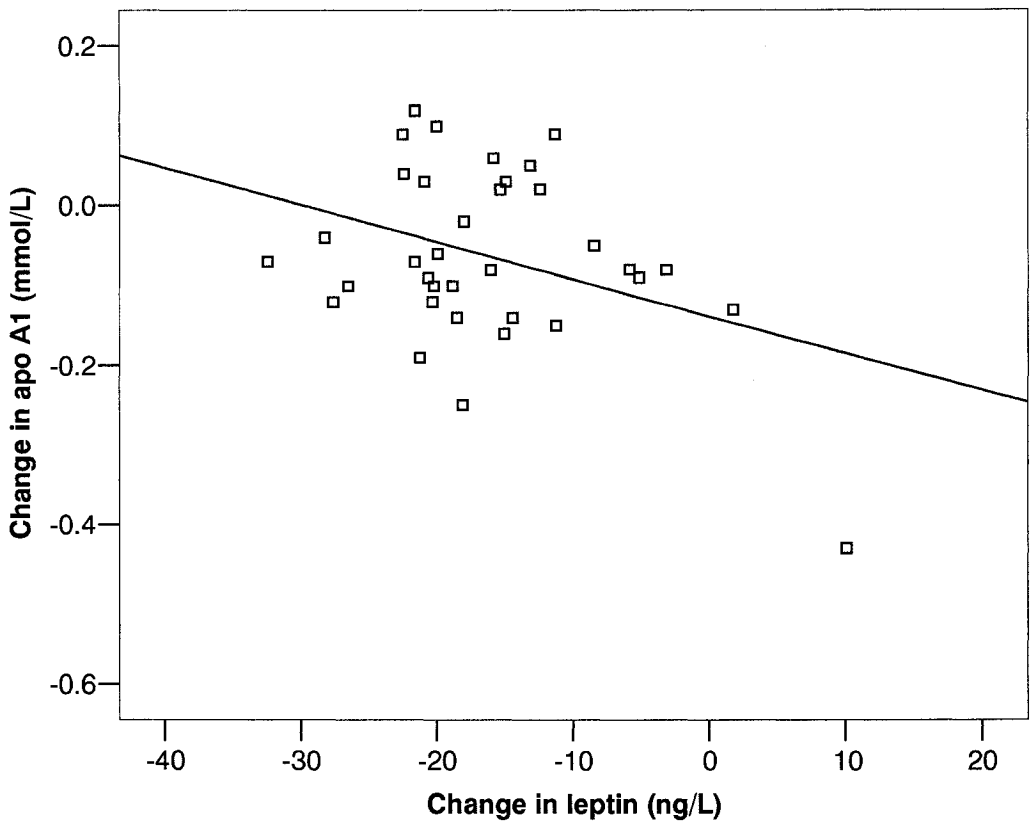

Figure 3-3d. Correlations between changes in apo A1 and leptin (r = -0.41; p = 0.01).

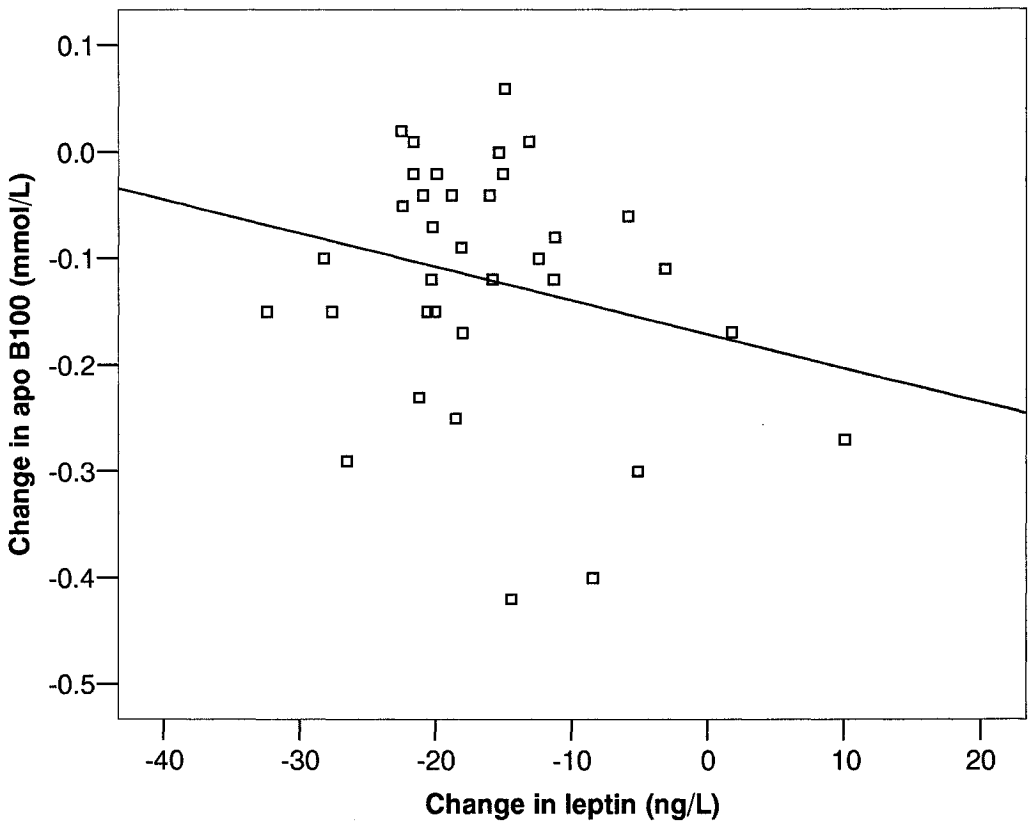

Figure 3-3e. Correlations between changes in apo B100 and leptin (r = -0.34; $p = 0.05$).

BRIDGE 2.

In addition to absolute LDL-C concentration, it has been suggested that LDL particle size and distribution may negatively impact cardiovascular health (Rizzo et al., 2006a). More specifically, small, dense LDL particles are thought to contribute negatively to CVD and health since they are less likely to be taken up by the LDL receptor and therefore, persist in the circulation for a longer duration (Rizzo et al., 2006a). Current evidence indicates that weight loss beneficially modulates LDL size and distribution (Archer et al., 2003; Katzel et al., 1995; Markovic et al., 1998; Purnell et al., 2000). Existing studies, however, have only examined the effect of diet-induced weight loss. Current guidelines recommend energy modification by physical activity, in addition to dietary changes. Thus, the following study was conducted to examine how moderate weight loss, induced by diet and physical activity, affects LDL size and distribution.

CHAPTER 4. Manuscript 4.

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Effect of Weight Loss Resulting from a Combined Low Fat Diet/Exercise Regimen on LDL Particle Size and Distribution in Obese Women

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4.1 Abstract

Background: Weight loss resulting from diet interventions has been shown to favourably affect LDL particle size and distribution, and hence, decrease cardiovascular disease risk. However, the effect of a dietary weight loss strategy when combined with exercise, on LDL electrophoretic characteristics, has yet to be tested.

Objective: This study examined the effect of a weight loss intervention that combined a low fat diet with moderate endurance training, on LDL particle size and distribution in obese women.

Design: Thirty obese, hypercholesterolemic women participated in controlled longitudinal weight loss trial, which consisted of: 1) a 2-wk pre-stabilization phase, 2) a 20-wk weight loss phase, and 3) a 2-wk post-stabilization phase. Weight reduction resulted from a low fat diet (<30% fat, 50-60% carbohydrate, 20% protein) combined with an endurance training program (>40 min moderate training 3x/wk).

Results: Mean weight loss was 14.8% ($P < 0.01$) of initial body weight. Total, LDL-cholesterol, and triacylglycerol concentrations decreased ($P < 0.01$) by 8.9%, 7.5%, and 27.1%, respectively, while HDL-cholesterol concentrations increased ($P < 0.01$) by 9.9%. No significant differences were noted for LDL peak or integrated particle size. The relative proportion of small, medium, and large particles were not significantly different post-treatment. Estimated cholesterol concentrations in large and medium size LDL particles decreased ($P < 0.05$) by 15.3% and 5.9%, respectively, as a result of weight loss. No effect was noted for estimated cholesterol concentrations in small size LDL particles. Increased weight loss over the course of the trial was associated with a decrease in peak particle size ($r = -0.14$, $P = 0.03$).

Conclusions: These findings suggest that weight loss, resulting from a low fat diet/exercise program, has only a minimal effect on LDL particle size and distribution.

Keywords: weight loss, LDL particle size, low fat diet, exercise, cholesterol, cardiovascular disease

4.2 Introduction

Results from several recent epidemiological and clinical intervention trials indicate that obesity is a major independent risk factor for cardiovascular disease (Scaglione et al., 2004). Accordingly, weight loss has been shown to favourably effect several indicators of cardiovascular risk, such as plasma lipid (Gerhard et al., 2004; Yancy et al., 2004), homocysteine (Noakes et al., 2004), and C-reactive protein (Brook et al., 2004) concentrations. In addition to these commonly investigated parameters, weight loss has also been shown to beneficially modulate low-density lipoprotein (LDL) size and distribution (Archer et al., 2003; Katzel et al., 1995; Markovic et al., 1998; Purnell et al., 2000). More specifically, Archer et al. (2003), demonstrated that a low fat/high carbohydrate diet reduced the average body weight of overweight men by 2%, while causing reductions in the cholesterol content of small and medium size LDL particles. Similarly, Markovic et al. (1998) found that after consuming a low fat diet for 28 days, mildly obese patients lost an average of 6.2 kg and increased the proportion of large LDL particles in plasma. Furthermore, in a study by Katzel et al. (1995), it was shown that an average loss of 10 kg of body weight, induced by a low fat diet, resulted in a significant increase in LDL peak particle diameter. Although these data indicate that diet-induced weight loss positively alters LDL electrophoretic characteristics, the effect of a low fat diet when placed in combination with exercise, another commonly implemented weight loss strategy, on LDL particle diameter, has yet to be tested. Additionally, the effect of substantial weight loss, i.e. loss of more than 10% of initial body weight, on LDL size and distribution still needs further clarification.

Thus, the objective of the present study was to examine the effect of a 24-week weight loss intervention that combined a low fat diet with moderate endurance training, on LDL particle size and distribution in obese, hypercholesterolemic women. In addition, the extent to which substantial changes in body weight modulated LDL electrophoretic was also examined.

4.3 Subject and methods

Subjects

Subjects were recruited from the greater Montreal area by means of newspaper advertisements. After a preliminary questionnaire, blood screening, and physical exam, 42 subjects were deemed eligible to partake in the trial. Key inclusion criteria

were as follows: non-pregnant females; age 35 to 60 years; body mass index (BMI) between 28 and 37 kg/m^2; LDL-cholesterol concentrations greater than 4.5 mmol/L; triacylglycerol concentrations greater than 1.5 mmol/L; free of cardiovascular disease; free of gastrointestinal, renal, pulmonary, hepatic or biliary disease; free of cancer; no history of disordered eating; alcohol intake of less than 2 drinks/d; use of fiber or stimulant laxatives less than 2 doses/wk; not taking lipid lowering medications for the past 6 months; less than 4000 kcal/wk expended by endurance training. The protocol was approved by the Human Ethical Review Committee of the Faculty of Medicine at McGill University. Prior to the commencement of the study, all volunteers gave their written informed consent to participate in the trial.

Experimental design

A 24-week longitudinal design was implemented as a means of testing the study objectives. Each subject acted as her own control, and therefore, no control group was required (Donnelly et al., 2000). The trial consisted of three consecutive dietary periods: 1) a 2-week pre-loss stabilization phase, 2) a 20-week weight loss phase, and 3) a 2-week post-loss stabilization phase. During the first and third phases, subjects were required to maintain a stable weight, and were instructed to continue with their usual food habits. In contrast, during the second phase, subjects were required to reduce their energy intake by 20% and increase their energy expenditure by 10%. As a result, target weight loss was approximately 0.75 kg/wk. Pre-menopausal women started each of the three phases at identical points during their menstrual cycles.

Dietary protocol

In order to decrease energy intake by 20% throughout the weight loss phase, each subject attended individual dietary counselling sessions with a Registered Dietician or Nutritionist on a bi-weekly basis. During these sessions, volunteers were taught how to attain daily caloric goals using an exchange system that provided 50-60 % of energy from carbohydrates, 20% of energy from protein, and <30% of energy from fat. Teaching aids, which outlined the caloric contents of commonly ingested food items, as well as sample menus and recipes, were distributed to the volunteers.

Exercise protocol

As a means of increasing energy expenditure by 10% throughout the 20-week weight loss phase, subjects were instructed to perform moderate aerobic training 3 times per week, for a minimum of 40 minutes. During the trial, each subject met with a personal trainer and was taught proper training techniques and routines. The exercise was performed independently at home, or at the Mary-Emily Clinical Nutrition Research Unit.

Weight loss assessment and compliance

Compliance with both the dietary and exercise protocol was determined by way of weigh-ins that took place once per week throughout the trial. Continuous, regular weight loss throughout the second phase of the study was encouraged by means of a point system as well as visual graphs which plotted weekly weight changes.

Blood collection protocol

Twelve-hour fasting blood samples were collected on the mornings of day 0, 13, 14, 15 (phase 1), and 167, 168, and 169 (phase 3) of the trial. Blood was centrifuged for 15 min at 520 x g and 4°C to separate plasma from RBCs, and was stored at –20°C until analysed.

Analyses

Plasma lipid profile determination

Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglycerol concentrations were measured in duplicate by automated methods through Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN) using enzymatic or immunoturbidometric reagents (McNamara et al., 1987b). LDL-cholesterol was determined directly by the dextran/magnesium sulfate method in order to separate it from HDL-cholesterol (N-geneous LDL C assay, Equal Diagnostics) (Rifai et al., 1998; Wang et al., 2001b).

LDL particle size determination

LDL particle diameter analysis was performed on whole plasma using nondenaturing 2–16% polyacrylamide gradient gel electrophoresis (St-Pierre et al., 2001). Plasma

samples (3.5 μL) were mixed with a sampling buffer containing 20% sucrose and 0.25% bromophenol blue in a 1:1 (v/v) ratio. The 6 samples, representing day 13, 14, 15, 167, 168, and 169, for each individual subject were placed on one unique gel. After a 15 min pre-run, electrophoresis was performed at 150 V for 3 h. Gels were then stained for 1 h with Sudan black (0.07%) and stored in a 0.81% acetic acid/4% methanol solution until analysis. The Imagemaster 1-D Prime computer software (Amersham Pharmacia Biotech, Piscataway, NJ) was used to analyze the gels. Mean LDL particle size was computed by integrating the relative contribution of each LDL particle subfraction within a sample and corresponded to the weighted mean of all LDL subfractions. Integrated LDL particle size was calculated as the sum of LDL subspecies' diameter multiplied by its relative proportion. The relative proportion of LDL having a diameter <255 Å (termed LDL% $_{<255\ Å}$) was obtained by computing the relative area of the densitometric scan <255 Å. The absolute concentration of cholesterol within the LDL subfraction characterized by a diameter <255 Å (termed LDL-C $_{<255\ Å}$) was crudely estimated by multiplying total plasma LDL-cholesterol concentrations by LDL% $_{<255\ Å}$ as previously described (St-Pierre et al., 2001). In order to assess the relative and absolute concentrations of cholesterol in the LDL subfractions with a diameter >260 Å (LDL% $_{>260\ Å}$ and LDL-C $_{>260\ Å}$, respectively) and in those with a diameter between 255 and 260 Å (LDL% $_{255–260\ Å}$ and LDL-C $_{255–260\ Å}$, respectively), a similar approach was employed.

Statistics

Results are presented as means ± standard error of the mean (SEM). A paired t-test was implemented as a means of comparing baseline and post-treatment weight, lipid, LDL particle size and distribution values. Pearson's correlation coefficients were calculated to test for associations between treatment-induced changes in body weight, plasma lipid concentrations, and LDL electrophoretic characteristics. A level of statistical significance at $P < 0.05$ was used in all analyses. Data were analyzed using SAS software (version 8.0; SAS Institute Inc, NC).

4.4 Results

Subject baseline characteristics and compliance

Forty-two subjects commenced the study, with 35 completing the entire 24-week trial. The seven subjects who did not complete the trial dropped out due to an inability to

comply with the study protocol. In addition, data from five subjects were not analysed due to plasma sample loss. Therefore, after accounting for these loses, a total of 30 complete subject data sets were used for LDL particle size and distribution analysis. On day 0 of the trial, the mean age, body weight, and BMI of the 30 participants was 48.9 ± 1.24 years, 82.1 ± 1.74 kg, and 31.5 ± 0.52 kg/m^2, respectively. During the weekly meetings with the Registered Dietician/Nutritionist, compliance with the calorie-reduced, low fat diet was reported to be adequate, as demonstrated by proper use of the checklist exchange system. Also during these meetings, reported increases in energy expenditure during the weight loss phase of the study were shown to be satisfactory, as the subjects reported increasing their physical activity levels by a minimum of 120 minutes per week.

Body weight and plasma lipid profiles

Changes in body weight, BMI, and plasma lipid profiles over the course of the 24-week trial are presented in **Table 4-1.** The mean rate of weight loss over the 20-week weight loss phase was 0.59 kg/week. From the beginning to the end of the trial, the 30 volunteers experienced a significant ($P < 0.01$) mean reduction in body weight of 12.0 ± 0.41 kg. When expressed as the difference between day 15 and day 169 values, subjects were shown to have decreased their overall body weight by 14.8 %. Body mass index decreased significantly ($P < 0.01$) from 31.5 kg/m^2 to 26.9 kg/m^2 from baseline to post-treatment.

As reported previously (Santosa S, 2006), plasma lipid parameters were significantly altered as a result of weight loss. Total and LDL-cholesterol concentrations decreased significantly ($P < 0.01$) by 8.9% and 7.5%, respectively, after the intervention period. In addition, HDL-cholesterol concentrations increased ($P < 0.01$) by 9.9%, while triacylglycerol concentrations decreased by 27.1% from baseline to the end of the trial.

LDL peak particle size, integrated size, and distribution

LDL peak particle size, integrated size, and distribution over the course of the trial are presented in **Table 4-2**. No differences were noted with respect to LDL peak particle size from the beginning to the end of the trial. Similarly, no significant differences were noted for LDL integrated size when baseline values were compared to post-

treatment values. Furthermore, the relative distributions of cholesterol among small (<255 Å), medium (255-260 Å), and large (>260 Å) LDL particles showed no significant differences from baseline to post-treatment. However, with respect to the absolute distribution of the different LDL particle subfractions, the estimated cholesterol concentration in large size LDL particles was shown to decrease significantly ($P < 0.05$) by 15.3% (0.80 to 0.68 mmol/L) from the beginning to the end of the trial. In addition, the estimated cholesterol concentration in medium size LDL particles was shown to decrease ($P < 0.05$) by 5.9% (1.39 to 1.31 mmol/L) post-treatment. No significant changes were noted for the estimated cholesterol concentration in small LDL particles.

Correlation between body weight and LDL particle size

Body weight has been shown to correlate with variations in LDL particle size and distribution. For this reason, analyses were performed to see if the substantial decrease in body weight resulting from the diet/exercise intervention had an effect on LDL electrophoretic characteristics. Correlational analysis revealed that the greater change in body weight over the course of the trial was associated with increased LDL peak particle size post-treatment ($r = -0.14$, $P = 0.03$).

Correlation between triacylglycerol concentrations and LDL particle size

Post-treatment triacylglycerol concentrations were not significantly correlated with any of the post-treatment LDL particle size or distribution parameters measured. In addition, post-treatment triacylglycerol concentrations showed no significant associations with either percent body weight change or post-treatment body weight.

4.5 Discussion

The results of the present study indicate that a substantial amount of weight loss, i.e. loss of approximately 15% of initial body weight, resulting from a low fat diet combined with exercise, decreases the estimated cholesterol concentrations of large and medium LDL particles. The relative proportion of small, medium, and large particles, however, were not significantly altered by this decrease in body weight.

Results of several recent clinical trials show that losing a considerable amount of body weight has favourable effects on certain indicators of cardiovascular disease risk

(Anderson et al., 2001; Janssen et al., 2002; Tanaka et al., 2004). Therefore, it would be expected that in the present intervention trial, which produced a substantial amount of weight loss, certain cardiovascular risk factors such as LDL particle size would be ameliorated. Although such favourable alterations have been previously reported (Archer et al., 2003; Katzel et al., 1995; Markovic et al., 1998), the results of the present study demonstrate that this magnitude of weight loss has a minimal effect on LDL particle distribution. More specifically, this study reports a decrease in cholesterol concentrations in both large and medium LDL particles, as a result of a 15% diet/exercise-induced weight reduction. No increase in the cholesterol content of small LDL particles, however, was noted in response to these cholesterol shifts between subfractions. There are several possible explanations that may account for these unexpected findings. First and foremost, these results could partially be explained by the low-fat diet regimen. Recent findings suggest that LDL particles shift from larger, less atherogenic, to smaller, more atherogenic, particles in direct proportion to the degree to which dietary fat is replaced by carbohydrate (Desroches et al., 2004). Thus, since the subjects were required to lower their fat intake to <30% of total energy, and hence, increase their carbohydrate intake to approximately 60% of total energy, it can be assumed that this shift in macro-nutrient distribution may be responsible for the present findings. Secondly, it is possible that the decrease in cholesterol within large and medium LDL subfractions may be attributed to the physical activity component of the study. More specifically, in a recent study by Varady et al. (2005), it was demonstrated that participation in a moderate intensity exercise program decreased the peak particle size of previously sedentary, hypercholesterolemic adults. However, contradictory to these findings (Varady et al., 2005), other studies that have examined this relationship have reported either an increase in LDL peak particle size as a result of exercise (Beard et al., 1996; Halle et al., 1999; Kang et al., 2002; Williams et al., 1990), or no effect (Elosua et al., 2003). For instance, in two trials (Halle et al., 1999; Williams et al., 1990) that examined the effect of endurance training on LDL particle size in obese men, the number of small, dense LDL particles was shown to decrease as a result of training. In line with these findings, Kang et al. (2002), demonstrated that physical activity had a beneficial effect on LDL particle diameter in obese adolescents. In contrast, Elosua et al. (2003), demonstrated that after a 16-week training period, no changes were observed with regards to LDL particle diameter in previously sedentary men and women. In view of

these findings, since the majority of the studies in the area suggest a beneficial effect of exercise on LDL electrophoretic characteristics, it is unlikely that the exercise intervention produced these slightly deleterious changes in LDL particle size. Thus, before it can be concluded that exercise is responsible for these unfavourable effects, it is essential that the results of the Varady et al. (2005) trial be supported by other independent studies testing similar objectives.

Interestingly, correlational analysis revealed a weak but significant association between greater weight loss and an increase peak LDL particle size ($r = -0.14$, $P = 0.03$). These associative findings are, evidently, contrary to the causal findings reported in the present paper. The reason for these conflicting results is not clear. However, one possible explanation for these contradictory findings may again be the change in diet composition. Since replacing fat with carbohydrate leads to a decrease in particle size, it can be hypothesized that, if this change in diet was not implemented, LDL size may have increased in response to weight loss. Then again, since these findings are merely associative, and also quite weak, these correlational results should not put into question the results of the causative findings. Nevertheless, these contradictory results may suggest that, when attempting to assess the effect of weight loss on vascular disease risk, LDL particle size analysis should be performed in conjunction with other more well-established indicators (Sacks et al., 2003).

In summary, results of the present study demonstrate that a substantial amount of weight loss resulting from a low-fat diet and exercise regimen has no effect on the distribution of small, medium and large LDL particles. Additionally, these data suggest that this weight loss regimen decreases the estimated cholesterol concentrations of large and medium size LDL particles. However, no increase in cholesterol within small LDL particles was observed in response to these cholesterol shifts between subfractions. These findings suggest that low fat diet/exercise-induced weight loss only minimally affect LDL particle size and distribution. Therefore, when viewed in terms of modulating LDL electrophoretic characteristics, the effect of low fat diet/exercise-induced weight loss on cardiovascular risk reduction cannot be inferred from the present data.

4.6 Acknowledgements

We would like to thank Annie St-Pierre and Dr. Alice Lichtenstein for their assistance in the analytical aspects of the study, as well as Dr. William Parsons and Catherine Vanstone for their contribution to the clinical components of the trial. This study was supported by the Heart and Stroke Foundation of Canada.

Table 4-1. Body weight, body mass index, and plasma lipid concentrations at baseline and post-treatment.

	Baseline		Post-treatment		% Change		P-value[1]
n = 30	Mean	SEM	Mean	SEM	Mean	SEM	
Body weight (kg)	82.14	± 1.74	70.09	± 1.71	-14.80	± 0.52	< 0.01
BMI (kg/m^2)	31.53	± 0.52	26.90	± 0.52	-14.80	± 0.52	< 0.01
Total cholesterol (mmol/L)	6.05	± 0.16	5.46	± 0.11	-8.94	± 1.78	< 0.01
LDL-cholesterol (mmol/L)	3.77	± 0.12	3.44	± 0.09	-7.54	± 2.21	< 0.01
HDL-cholesterol (mmol/L)	1.17	± 0.04	1.27	± 0.05	9.92	± 3.01	< 0.01
Triacylglycerol (mmol/L)	1.80	± 0.13	1.25	± 0.08	-27.14	± 3.59	< 0.01

[1]P-value within group: Student's paired t-test comparing day 15 values to day 169 values.

Table 4-2. Low density lipoprotein peak particle size, integrated size, and distribution at baseline after a 24-week diet/exercise weight loss intervention.

	Baseline[1]		Post-treatment[2]		% Change[3]		P-value[4]
n = 30	Mean	SEM	Mean	SEM	Mean	SEM	
LDL peak particle size (Å)	256.26	± 0.41	256.23	± 0.26	-0.01	± 0.12	0.913
LDL integrated size (Å)	256.27	± 0.40	256.15	± 0.20	-0.05	± 0.11	0.678
LDL $\%_{>260\ Å}$ (%)	20.93	± 2.34	20.07	± 1.37	-4.12	± 6.20	0.574
LDL $\%_{260\text{-}255\ Å}$ (%)	36.96	± 1.47	37.95	± 1.34	2.68	± 4.07	0.317
LDL $\%_{<255\ Å}$ (%)	42.26	± 2.27	42.10	± 1.81	-0.38	± 5.36	0.909
LDL-$C_{>260\ Å}$ (mmol/L)	0.80	± 0.10	0.68	± 0.05	-15.35	± 7.96	0.043
LDL-$C_{260\text{-}255\ Å}$ (mmol/L)	1.39	± 0.07	1.31	± 0.06	-5.87	± 3.79	0.048
LDL-$C_{<255\ Å}$ (mmol/L)	1.58	± 0.09	1.46	± 0.09	-7.82	± 5.37	0.100

[1] Mean of day 13,14,15 values.

[2] Mean of day 167,168,169 values.

[3] Percent change comparing the mean of day 13,14,15 values to the mean of day 167,168,169 values.

[4] P-value within group: Student's paired t-test comparing the mean of day 13,14,15 values to the mean of day 167,168,169 values.

BRIDGE 3.

The cardioprotective changes in blood lipid levels, specifically decreases in TC, LDL-C and TAG and increases in HDL-C found in the previous manuscript and other studies may be explained by changes in cholesterol metabolism including absorption, synthesis, and turnover. Weight loss studies in men or in both men and women have indicated decreases in cholesterol synthesis (Di Buono et al., 1999a; Griffin et al., 1998; Raeini-Sarjaz et al., 2001), however, none have examined these decreases in relation to changes in cholesterol absorption and turnover. Previous studies have also indicated that changes in lipid levels may be attributed to changes in regional body composition (Janssen et al., 2002; Nieman et al., 2002). No studies yet determine, however, whether changes in regional body composition affect cholesterol metabolism. Therefore, this study set out to investigate how weight loss affects cholesterol absorption, synthesis, and turnover, and the effect that changes in regional body composition may have on these parameters. Understanding how weight loss modulates cholesterol metabolism is important because it allows for better treatment focus and therapy of obesity-associated dyslipidemia.

CHAPTER 5. Manuscript 5.

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Cholesterol Metabolism and Body Composition in Women: The Effects of Moderate Weight Loss

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5.1 Abstract

Objective: To determine how moderate weight loss protocol through diet and exercise may affect changes in body composition, to determine the effects of weight loss on cholesterol metabolism, and to examine the relationship between cholesterol metabolism and changes in body composition.

Design: Thirty-five otherwise healthy, hypercholesterolemic women completed a 24-week weight loss study. A 20% decrease in energy intake through diet and a 10% increase in energy expenditure by exercise were combined with motivational strategies to encourage weight loss. The diet was self selected and comprised of 50-60% carbohydrates, 20% protein, and <30% fat.

Results: Participants lost an average of 11.7±2.5 kg (P<0.001). Whole body and regional losses in tissue mass occurred after weight loss. After weight loss, cholesterol fractional synthesis rate (FSR) decreased (P=0.003) 3.86±9.33%, while rates of cholesterol absorption and turnover did not change. Changes in cholesterol turnover were positively correlated with changes in FSR. Visceral adipose tissue (VAT) and VAT:subcuataneous adipose tissue (SAT) was negatively correlated with cholesterol turnover. Losses in skeletal muscle (SM) and upper body SM predicted decreases in cholesterol absorption.

Conclusions: Decreases in cholesterol synthesis after moderate weight loss is not compensated for by changes in cholesterol absorption or turnover. Changes in VAT and SM were associated with variations in cholesterol turnover and absorption, respectively. Understanding how losses in regional adipose tissue affect cholesterol metabolism will help in developing individualized treatment approaches that would result in more intensive therapy of obesity-associated dyslipidemia.

Keywords: weight loss, women, cholesterol metabolism, body composition, magnetic resonance imaging, isotope kinetics study

5.2 Introduction

The life expectancy of an obese individual has been found to be about seven years shorter than that of a normal weight counterpart (Peeters et al., 2003). If current trends in obesity continue, the present generation may have a lower life expectancy than their parents (Olshansky et al., 2005). Weight loss however, has recently been shown to increase mortality (Allison et al., 1999). One explanation may be that regional changes in body composition, rather than absolute changes in weight, is important in assessing the potential efficacy of weight loss to lower the risk of associated comorbidities and death. Evidence also shows that the presence of large visceral adipose tissue (VAT) depots is associated with increased risk of hypertension, cardiovascular disease (CVD), and type 2 diabetes mellitus (DM) (Pi-Sunyer, 2004; Park et al., 2005). Lapidus et al. (1984) reported that women with the highest WHR, an indicator of visceral adiposity, had elevated relative risk of death from CVD. Thus, body composition is an important measure in determining potential health benefits of a weight loss program.

Decreases in body weight of 10% to 15% have been shown to produce cardioprotective changes in blood lipid profiles of overweight individuals (Wing et al., 1995). Favourable changes in cholesterol levels may be attributed to declines in body fat percent, as shown in previous trials (Janssen et al., 2002; Nieman et al., 2002). Additionally, decreases of VAT, resulting from weight loss, have been related to reductions in blood lipid level (Leenen et al., 1993). Such favourable changes may be explained by the effect of weight loss on cholesterol metabolism. Although very few studies have examined effects of weight loss on cholesterol kinetics, those that did have indicated that weight loss may decrease rates of cholesterol synthesis (Di Buono et al., 1999a; Raeini-Sarjaz et al., 2001). However, an unresolved question is whether weight loss affects other aspects of cholesterol metabolism, specifically cholesterol absorption and turnover. To our knowledge, no studies exist that examine effects of changes in body composition on cholesterol kinetics. The objective of this study is three-fold: 1) to determine how moderate weight loss through diet and exercise may affect changes in whole body and regional body composition, 2) to determine the effects of weight loss on cholesterol absorption, synthesis and turnover, and 3) to examine the relationship between cholesterol metabolism and regional changes in body composition.

5.3 Methods

Subjects

Forty-two hyperlipidemic overweight and obese women were recruited by newspaper advertisements and word of mouth from the Montreal area to participate in a six-month weight loss trial. Inclusion criteria required that the women were 35 to 60 years of age with body mass indices of 28-39 kg/m^2. Since other analyses were being conducted, subjects were also screened for fasting plasma LDL-C concentrations of 3.4-6.7 mmol/L and TAG concentrations >1.5 mmol/L. Use of oral anti-hypertensive agents and thyroid hormones were permitted, provided they were stable and continued throughout the duration of the study. The women were included regardless of menopausal status, provided they were stable on hormone replacement therapy if it was being taken. Fasting blood samples were collected for serum biochemistry and haematology and subjects were also required to undergo a complete physical examination by a certified medical doctor before acceptance into the study. Subjects were excluded if they underwent oral hyperlipidemic therapy less than six months prior to commencement or if they had a history of chronic illness, including hepatic, renal, gastrointestinal, and cardiac dysfunction. Additionally, those who were found to have previous history of eating disorders, chronic laxative use, or reported exercise in excess of 4000 kcal/week were excluded. Before commencing, a copy of the study protocol was given to subjects and any queries were answered by the primary investigator or study co-ordinators before signing the consent form. Ethical approval for the experimental protocol was obtained from the Faculty of Medicine Ethics Review Board at McGill University, Montreal, QC, Canada and the Human Investigation Review Committee of Tufts University, Boston, MA, USA.

Protocol

Experimental design

The trial was 24 weeks in duration and consisted of a two-week pre-loss stabilization period, a 20-week weight loss period, and a two-week post-loss stabilization period. Subjects served as their own controls in a longitudinal before and after study design.

Participants were required to maintain a stable weight, as well as usual food and exercise habits during the two-week pre- and post-loss stabilization periods. During

the two-week stabilization periods subjects were weighed at days 1, 5, 8, 12, and 15 to ensure stable weight. Fasting blood samples were taken on days 1, 8, and 15 of each stabilization phase to measure circulating lipid concentrations. Cholesterol metabolism measurements were done between days 11 and 15 of each stabilization phase using stable isotopes. On day 11 of each stabilization phase, baseline fasting blood samples were collected before subjects received an intravenous injection of 15 mg [25, 26, 26, 26, 27, 27, 27-D]cholesterol and a 75 mg oral dose of [3,4-^{13}C]to assess cholesterol for cholesterol absorption and turnover. Cholesterol fractional absorption rate was determined by dual isotope ratio methodology which compared the ratio of ingested [^{13}C]cholesterol to intravenous [D_7]cholesterol enrichment in red blood cell cholesterol after hours 24, 48, and 72. Cholesterol turnover was measured by the rate of decay in enrichment of [D_7]cholesterol of red blood cell (RBC) cholesterol taken after hours 12, 24, 48, and 72. Deuterium incorporation was used to measure cholesterol synthesis. After a fasting blood sample was taken on day 14 of the stabilization period subjects imbibed 0.7 g D_2O/kg estimated body water (99.8 atom percent excess; CDN Isotopes, Montreal). Body water was estimated at 60% of total body weight. Body composition was determined by MRI scans and dietary records were also taken during the two stabilization periods to ensure usual food habits were followed.

Weight loss protocol

A 20-week weight loss period was undertaken after the two-week pre-loss stabilization period. Energy expenditure was estimated using the Harris-Benedict equation multiplied by an activity factor (Harris, 1919). Weight loss was achieved through a 20% decrease in energy intake by diet restraint combined with a 10% increase in energy expenditure by exercise, resulting in a 30% total energy deficit. In general, prescribed energy intake was between 1400-1600 kcal/d. Dietary composition consisted of 50-60% of energy from carbohydrates, 20% of energy from protein, and <30% of energy from fat. The U.S. Exchange System consisting of grains, fruits and vegetables, meat and alternatives, milk, and fat food groups was used to help subjects adhere to dietary guidelines. Monthly individual consultations with nutritionists aided the participants in ensuring dietary adherence throughout the weight loss period. Semi-private meetings with a personal trainer were conducted at the beginning and midway through the weight loss period to help subjects establish

exercise routines that would achieve a 10% increase in energy expenditure. Proper techniques were also demonstrated to ensure exercises were carried out safely.

Subjects were weighed once a week throughout the weight loss period. An award point system was created to help the subjects keep track of their progress and increase motivation. Additionally, participants were given a graph on which they were able to plot their weekly progress. To further increase motivation and adherence to the weight loss protocol, monthly group sessions were held where guest speakers taught subjects further exercise and nutrition knowledge. Two participants withdrew from the study before the weight loss period due to inability to undergo MRI scans and five participants dropped out because of difficulty in following the weight loss regime and adhering to the scheduled study visits.

Analyses

Plasma lipids

Collected samples were centrifuged at 1500 rpm for 15 min to separate RBC and plasma within 30 min of phlebotomy. Separated aliquots were immediately stored at 20°C until analyzed. Plasma total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C) concentrations were analysed as previously described, using enzymatic or immunoturbidometric reagents and a Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN), as previously described (McNamara et al., 1987b). The dextran/magnesium sulfate method was used to determine low density lipoprotein cholesterol (LDL-C) concentrations (Rifai et al., 1998; Wang et al., 2001b).

Cholesterol absorption

Determination of cholesterol absorption is described elsewhere (Folch et al., 1957; Vanstone et al., 2002b). Briefly, lipids were extracted from RBC in duplicate using a modified Folch extraction procedure and free cholesterol was isolated by thin-layer chromatography (Folch et al., 1957). Free cholesterol was then combusted for 4 hours at 540°C to ^{13}C-enriched CO_2, and D_7 enriched water. ^{13}C-enriched CO_2, and D_7-enriched water was separated by vacuum distillation (Vanstone et al., 2002b). Isotopic enrichment of the [^{13}C]cholesterol and [D_7]cholesterol tracers was verified using nuclear magnetic resonance (CDN Isotopes, Pointe Claire, Canada) and were

found to be >99 atom percent excess. ^{13}C and D_7 enrichment of free cholesterol was assessed by differential isotope ratio mass spectrometry (IRMS) (Vanstone et al., 2002b). Cholesterol absorption was calculated for hours 48 and 72 by the following equation (Bosner et al., 1993):

$$\text{Cholesterol Absorption (\%)} = \frac{\Delta\,^{13}C \times 7/46 \times D_7\text{-cholesterol IV dose (mg)} \times 0.0122}{\Delta D_7 \times 2/27 \times {}^{13}C\text{-cholesterol oral dose (mg)} \times 0.000155} \times 100$$

where Δ for ^{13}C and D_7 is the difference between samples at 48 and 72 hours and the baseline abundance (t=0) in parts per thousand relative to PDB and SMOW standards, respectively. The factors 7/46 and 2/27 reflect the ratio of labeled atoms/mg dose and the constants 0.0112 and 0.000155 are conversion factors of the part per thousand units into atom percent excess for the PDB and SMOW scales, respectively. CAC at 48 h and 72 h were then averaged to determine overall cholesterol absorption.

Cholesterol biosynthesis

Free cholesterol from 72 h and 96 h erythrocytes were extracted, as explained earlier, to measure cholesterol synthesis by deuterium incorporation (Folch et al., 1957; Jones et al., 1993a). Additionally, deuterium enrichment of plasma water was determined, as described elsewhere (Vanstone et al., 2002b). Fractional synthesis rate (FSR) of cholesterol over 24 hours was determined using the following equation that corrects for cholesterol deuterium:protium ratio (Jones et al., 1993a):

$$\text{FSR (\%/d)} = (\Delta_{\text{cholesterol}}/\Delta_{\text{plasma}}) \times 0.478 * 100,$$

where Δ refers to deuterium enrichment above baseline level over 24 hours in parts per thousand relative to a SMOW standard. The factor 0.478 reflects to ratio of labelled H atoms replaced by deuterium during in vivo biosynthesis (Jones et al., 1993a).

Cholesterol turnover

Turnover of plasma free unesterified cholesterol is defined as the rate of influx from synthesis and dietary absorption compared to the rate of efflux from esterification, excretion or transfer from other body pools (Goodman et al., 1973; Nestel et al., 1973; Vanstone et al., 2002b). Therefore, turnover rates of erythrocyte free cholesterol was determined after subtraction of baseline D_7, [D_7]cholesterol enrichment of RBC at 12, 24, 48, and 72 h were fitted to exponential curves to determine the rate of decay.

Body composition measurement by magnetic resonance imaging (MRI)

Magnetic resonance images were obtained using a Siemens 1.5 Tesla MRI scanner (Siemens, Mississauga, Canada) using a T-1 weighted spin-echo sequence with a 322 ms repetition time and a 12 ms echo time (Ross et al., 1996). Scans of the truncal region, defined by the area between the femoral and humoral heads, were acquired with a scan time of 18 s. Appendicular regional scans, defined as the area above below the femoral heads and above the humoral heads, were acquired with a scan time of 24 s. The scan was conducted in two parts, divided into lower body and upper body by the 4th and 5th lumbar (L_4-L_5) vertebrae (Ross et al., 1996). For both parts, subjects laid in prone position inside the magnet. During the upper body scan, arms were extended overhead. Transverse slices of 10 mm thickness were obtained every 40 mm from the intervertebral space of L_4-L_5 with each pass of the magnet obtaining a set of seven slices. The body was divided into four compartments for analysis; the lower body defined as the region below but not including L_4-L_5, the upper body defined as the region above and including L_4-L_5, the abdominal area defined as the slices one below and three above L_4-L_5, and the legs defined as any slice with leg bone (Ross et al., 1996).

All MRI data were analysed using the Slice-O-Matic v. 4.2 rev 8-2e program (Tomovision Inc, Montreal, Canada), as previously described (Ross et al., 1996). The volume for each tissue was calculated with the following equation as defined by Ross et al. (1996):

$$V = \sum_{i=1}^{N} A_i t + h/3 \sum_{i=2}^{N} [A_{i-1} + A_i + (A_{i-1}A_i)^{1/2}]$$

where V represents total tissue volume, A represents tissue area, *t* represents slice thickness, and *h* represents the distance between consecutive slices. Volumes were then divided by 1000 to convert to L and multiplied by the tissue density to convert to kg. The density factors used were 0.94 L/kg for AT and 1.1 L/kg for LT (Katch F, 1993; Martin et al., 1994).

Statistics

All data are expressed as means $\pm$ SDs. Data were tested for normality using the Shapiro-Wilk test. The statistical significance of changes in cholesterol metabolism,

body weight, and tissues within each compartment were determined using a Student's paired t-tests. Data that were found to have a non-Gaussian distribution were log transformed and retested for normality before the paired T-test was applied. The Wilcoxon signed rank test was used for data that did not have a Gaussian distribution after log transformation. Student's t-test was used to examine differences in percent changes in regional tissue distribution. The Mann-Whitney test was used to compare the percent changes in regional tissue distribution in the event that the data were non Gaussian. Spearman's rank correlation was used to determine the interactions between cholesterol absorption, FSR, and turnover. Linear regression analysis was used to examine the effects of regional changes in weight loss on cholesterol metabolism, as well as, the effects of changes in cholesterol metabolism on plasma cholesterol. All values were defined to be statistically significant at $P < 0.05$. Data were analysed using SPSS for Windows (version 12.0.0; SPSS Inc., Chicago, IL).

5.4 Results

Forty-two subjects were recruited to participate with 35 completing the study. Subjects had an average age of 49.4 $\pm$ 6.7 years, intial weight of 81.4 $\pm$ 9.5 kg, and BMI of 31.4 $\pm$ 2.8 kg/m^2. During the pre-weight loss stabilization period, average body composition was found to be 44.2 $\pm$ 3.95% adipose tissue (AT) and 55.8 $\pm$ 3.95% lean tissue (LT). Skeletal muscle (SM) made up 30.39 $\pm$ 2.80% of body composition, while subcutaneous adipose tissue (SAT) and VAT made up 36.98 $\pm$ 4.19% and 3.05 $\pm$ 1.01%, respectively (**Figure 5-1a, b**).

Changes in cholesterol absorption, biosynthesis and turnover in response to weight loss

TC and LDL-C levels declined by 0.62 $\pm$ 0.65 mmol/L ($P < 0.001$), and 0.32 $\pm$ 0.51 mmol/L ($P = 0.001$), respectively, and HDL-C increased ($P = 0.03$) by 0.08 $\pm$ 0.21 mmol/L after weight loss.

After weight loss, FSR was reduced ($P = 0.003$) by 3.86 $\pm$ 9.33 %/d (15.2 $\pm$ 91.0% relative to initial). No significant changes in cholesterol absorption or rates of cholesterol turnover were found. Despite the lack of change found in cholesterol turnover, a relationship was found ($P = 0.01$) between FSR and turnover, where decreases in FSR correlated with declines in turnover rates of free cholesterol (r =

0.44) (see **Figure 5-2**). No relationship was detected between changes in cholesterol absorption and changes in FSR or between changes in cholesterol absorption and turnover rates.

Overall changes in whole body composition after weight loss

Overall, participants lost an average of 11.7 ± 2.5 kg (14.5 ± 3.1% of original body weight) ($P < 0.001$) after following the 20 week weight loss protocol. Figure 5-1a, b shows the weight and percent whole body composition of SM, SAT, LT, and AT before and after the weight loss period. Whole body losses in tissue mass were 1.36 ± 1.00 kg for SM, 8.67 ± 3.38 kg for SAT, 1.74 ± 1.73 kg for LT, and 9.85 ± 4.07 kg for AT ($P < 0.001$ for all). Percent composition of SM and LT increased ($P <0.001$) by 3.35 ± 1.87% and 7.33 ± 3.35% ($P < 0.001$). Both SAT and AT percent composition decreased 6.80 ± 2.87% ($P < 0.001$) and 7.33 ± 3.35% ($P < 0.001$), respectively. Despite significant losses in muscle mass, the LT:AT ratio increased after weight loss by 0.47 ± 0.24 ($P < 0.001$) (**Figure 5-3**).

Regional changes in body composition after weight loss

Reductions in percentage SAT in the upper body (-33.3% ± 12.1%) were greater ($P = 0.038$) than those found in the lower body (-27.9% ± 8.81%). Percent changes in the proportions of SM, LT and AT of upper body and lower body did not significantly differ (**Figure 5-4**).

In the abdominal region, the decline in AT after weight loss was 2.60 ± 1.14 kg ($P < 0.001$), while there was no change in LT. SM, SAT, and VAT decreased ($P < 0.001$) by 0.17 ± 0.18 kg, 1.94 ± 0.86 kg, and 0.61 ± 0.47 kg, respectively. At the end of the weight loss treatment, the percent whole body AT composition of SAT from the abdominal region dropped by 1.18 ± 1.50% ($P < 0.001$), and the percent composition of AT from the abdomen was reduced 0.99 ± 1.63% ($P < 0.001$). No changes in percent composition of VAT were found. There were no differences in the VAT:SAT ratio after weight loss.

Relationship between changes in whole and regional body composition and cholesterol metabolism

Larger reductions in VAT predicted rises in cholesterol turnover rate (β = -5.04, r = 0.383; P = 0.03) (see **Figure 5-5a**). The changes in cholesterol turnover rate was also negatively predicted by greater declines in the VAT:SAT ratio (β = -147, r = 0.399; P = 0.03) (see **Figure 5-5b**). In regards to cholesterol absorption, larger losses in SM predicted greater decreases (β = 6.82, r = 0.357; P = 0.04) (see **Figure 5-6a**). Changes in cholesterol absorption were also positively predicted by changes in upper body SM (β = 14.7, r = 0.413; P = 0.01) (see **Figure 5-6b**). No other changes in whole or regional body composition were predictive of changes in cholesterol metabolism.

5.5 Discussion

The novel findings in this study are that changes in cholesterol turnover after weight loss were negatively predicted by changes in VAT and VAT:SAT. Changes in cholesterol absorption were positively predicted by changes in total and upper body SM. Moreover, percent cholesterol FSR decreases in absence of any changes in cholesterol absorption or turnover rate after moderate weight loss in women, and that decreases in FSR are related to decreases in cholesterol turnover. Weight loss resulted in favourable changes in plasma cholesterol concentrations suggesting an amelioration of CVD risk.

The importance of regional body composition on morbidity and mortality has been well established. To our knowledge, this is the first study in women to examine effects of self-directed diet and exercise on regional body composition using MRI. Mean weight loss of about 12 kg in this trial resulted in whole body reductions in SM, SAT, LT and AT mass. Other trials that saw comparable weight loss achieved similar losses in SAT and AT to those found in the present study (Janssen et al., 2002; Janssen et al., 1999). Additionally, this study observed significant decreases in SM that were not found in similar trials (Janssen et al., 2002; Janssen et al., 1999; Ross et al., 1995). Of particular note, is that these trials also included a diet only group, which had significant and comparable losses in SM as those found in the present study (Janssen et al., 2002; Janssen et al., 1999; Ross et al., 1995). The exercise

intervention however, in these weight loss trials was conducted under supervision, thereby allowing for control of the intensity and type of exercise conducted. The self selected exercises used in the present study may have been conducted at a lower intensity, which might explain differences in body composition changes between this and other studies (Janssen et al., 2002; Janssen et al., 1999; Ross et al., 1995). Despite losses in SM, increases in the LT:AT ratio in this study suggest that the proportion of lean tissue that is preserved is greater than the proportion of adipose tissue that is lost. This is especially important since losses in LT may be related to higher mortality, whereas losses in AT may be related to lower mortality (Allison et al., 1999).

Some of the health benefits derived from weight loss are related to CVD risk factors. The moderate weight loss in this study was associated with decreases in TC, LDL-C, and TAG, as well as increases in HDL-C levels, apparently caused by shifts in cholesterol metabolism. Experiments in men indicate decreases in cholesterol synthesis after weight loss (Di Buono et al., 1999a; Raeini-Sarjaz et al., 2001). However, the declines in synthesis of about 4%/d found in the present study after an approximate 12 kg weight loss in women was less than those found in these other studies in men. One of the reasons for this may be because cholesterol metabolism measurements in this study were taken after stabilization periods that minimize the effect of the diet and exercise weight loss method. Di Buono et al. (1999a) found decreases in FSR of about 5-6%/d after a weight loss of 3-8 kg in six men following the AHA Step I diet with a caloric deficit of 1000 kJ/d. Another study in male participants that were fed a low-fat diet showed that losses of 3 kg of body weight were accompanied by larger reductions in FSR of compared to those found in the present study (Raeini-Sarjaz et al., 2001). Contrary to these findings, a trial by Griffin et al. (1998) that included men and women people with type 2 diabetes, found no differences in fasting cholesterol synthesis measured by C^{14}-acetate after a diet-induced weight loss of about 7-8 kg. Despite the fact that the present study included a larger number of subjects and achieved greater weight loss than previous trials, the decrease in cholesterol synthesis after moderate weight loss was attenuated. This was the only study however, to limit inclusion of subjects to women. Thus, though cholesterol synthesis decreases, this may indicate that its response to weight loss may be less pronounced in women than in men.

This is the first study to use MRI to determine how changes in body composition may affect cholesterol metabolism. While a relationship has been established between abdominal AT compartments and WHR, WHR cannot accurately predict changes in these compartments (Kamel et al., 2000). Accurate assessment of VAT is limited to use of MRI or computed tomography (Ross, 1996). Thus, in addition to assessing whole body composition, the use of MRI in this study allowed for a precise examination of abdominal tissue distribution. As a result, significant decreases in total abdominal AT, abdominal SAT and VAT were found. The loss of VAT in this study has positive implications on health since VAT has been positively associated with increased risk of metabolic aberrations and death (Lapidus et al., 1984; Pi-Sunyer, 2004). Losses of 0.6 kg VAT observed in the present study were comparable to those found in other diet and exercise weight loss studies (Janssen et al., 2002; Janssen et al., 1999). Additionally, larger changes in VAT predicted smaller changes in cholesterol turnover. The ability of the VAT:SAT ratio to negatively predict changes in cholesterol turnover may be attributed mainly to changes in VAT, since changes in SAT did not independently predict changes in cholesterol turnover. A possible explanation for these observations is that cholesterol mobilized from visceral adipocytes is drained by the portal vein, which travel to the liver (Rebuffe-Scrive et al., 1990). Since the liver is a primary metabolic site, a greater influx of cholesterol from larger losses in VAT depots may decrease cholesterol turnover by increasing influx relative to a constant rate of efflux.

The differences in cholesterol turnover were also positively related to cholesterol synthesis. Whether changes in one predict changes in the other cannot be determined since variations in cholesterol turnover are predicted by changes in VAT, which may in turn affect rates of biosynthesis. It is possible that differences in synthesis following weight loss may result in proportional changes in turnover because synthesis itself may be reduced as a direct cause of a decrease in liver size. Cholesterol synthesis per gram of hepatic tissue has been found to be equivalent in obese and non-obese individuals (Angel et al., 1979; Stahlberg et al., 1997). Additionally, liver biopsies from normal and obese individuals indicated that obese individuals had higher expression of the HMG CoA reductase enzyme involved in cholesterol synthesis (Stahlberg et al., 1997). The activity of other enzymes which participate in synthesis were also elevated in obese individuals (Stahlberg et al.,

1997). Thus, enlarged livers found in obese individuals may result in an increased rate of cholesterol synthesis and weight loss may result in decreased liver size (Stahlberg et al., 1997).

Neither a change in cholesterol absorption nor a relationship between change in cholesterol synthesis and absorption was detected after weight loss. However, previous studies in kinetics indicate the potential of a reciprocal relationship in cholesterol absorption and synthesis where change in one parameter oppositely affects the other (Grundy et al., 1969b; Gylling et al., 2002a; Jones et al., 2000b; Miettinen et al., 2003c; Sudhop et al., 2002; Vanstone et al., 2002b). A paucity of studies exists which measure the effects of weight loss on both absorption and synthesis. The only studies found were in subjects with type 2 diabetes mellitus (DM) using plant sterols and cholestanols, precursors as indicators of absorption and synthesis (Simonen et al., 2000; Simonen et al., 2002a). Though, both studies indicated a decrease in synthesis and an increase in absorption after weight loss, the methods offers only a crude estimation of cholesterol kinetics, since plant sterols and cholesterol precursors might be affected by diet and are not quantitative measures of metabolism (Simonen et al., 2000; Simonen et al., 2002a). The use of isotope ratio methodology in this trial offers a more precise quantifiable measure of cholesterol metabolism and thus, may explain the differences in findings between this and other studies.

Despite the absence of change in cholesterol absorption, increases in total body SM predicted increases in cholesterol absorption. The ability of total body SM to predict absorption is likely attributed to increases in upper body SM, since upper and not lower body SM was found to be a predictor of cholesterol absorption. Although the reason that changes in SM predicted changes in cholesterol absorption is unclear, we postulate that it might be due to an elevation in demand of cholesterol, which is needed to build myocytes.

In summary, moderate weight loss achieved in female participants of the present study resulted in decreases in cholesterol biosynthesis in the absence of any changes in cholesterol absorption and turnover. The use of MRIs in this study showed that significant weight loss as a result of a self-directed diet and exercise program resulted in favourable changes in regional and whole body SM, SAT, and VAT, as well as total

lean and adipose tissue. For the first time, we were able to examine how regional changes in regional body composition affected cholesterol metabolism. More specifically, decreases in VAT and VAT:SAT predicted increases in cholesterol turnover, and increases in whole and upper body SM predicted increases in cholesterol absorption. Understanding changes in cholesterol metabolism resulting from weight loss may help identify individuals at increased risk of cardiovascular disease and allow for earlier and more intensive therapy for the associated dyslipidemia.

5.6 Acknowledgements

We would like to thank Catherine Vanstone, Dr. William Parsons, Iwona Rudkowska, Patric Michaud, and Dr. Marie-Pierre St. Onge for their help in this trial. This project was funded by a grant from the Canadian Institute for Health Research MOP57814. SS received funding from the Natural Science and Engineering Research Council of Canada.

5.7 Figure legends

Figure 5-1a. Before and after weight (kg) of each tissue component in whole body. *** $P < 0.001$.

Figure 5-1b. Before and after percent whole body composition (%) of each tissue component. *** $P < 0.001$.

Figure 5-2. Cholesterol FSR vs. cholesterol turnover rate ($r = 0.44$; $P = 0.01$).

Figure 5-3. Individual whole body LT:AT ratio before and after weight loss. Mean difference of after LT compared to before LT:AT is 0.47 + 0.24; $P < 0.001$.

Figure 5-4. Change of each tissue component relative to initial values in the lower body and upper body regions. *$P < 0.05$

Figure 5-5a. Changes in cholesterol turnover in response to changes in VAT ($\beta = -5.04$, $r = 0.383$; $P = 0.03$).

Figure 5-5b. Changes in cholesterol turnover in response to changes in VAT:SAT ($\beta = -147$, $r = 0.399$; $P = 0.03$).

Figure 5-6a. Changes in cholesterol absorption in response to changes in whole body SM ($\beta = 6.82$, $r = 0.357$; $P = 0.04$).

Figure 5-6b. Changes in cholesterol absorption in response to changes in upper body SM ($\beta = 14.7$, $r = 0.413$; $P = 0.01$).

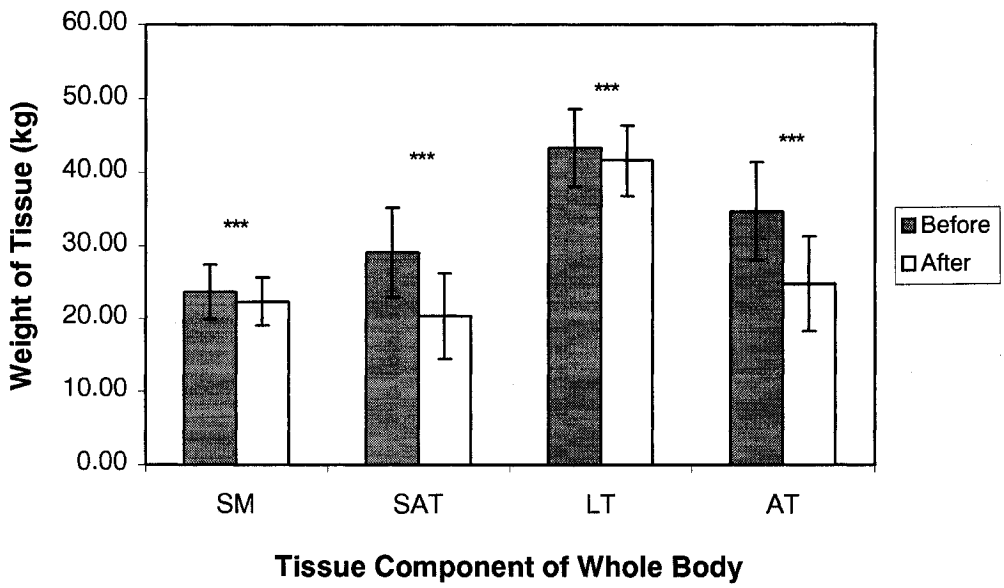


Figure 5-1a. Before and after weight (kg) of each tissue component in whole body. *** $P < 0.001$.

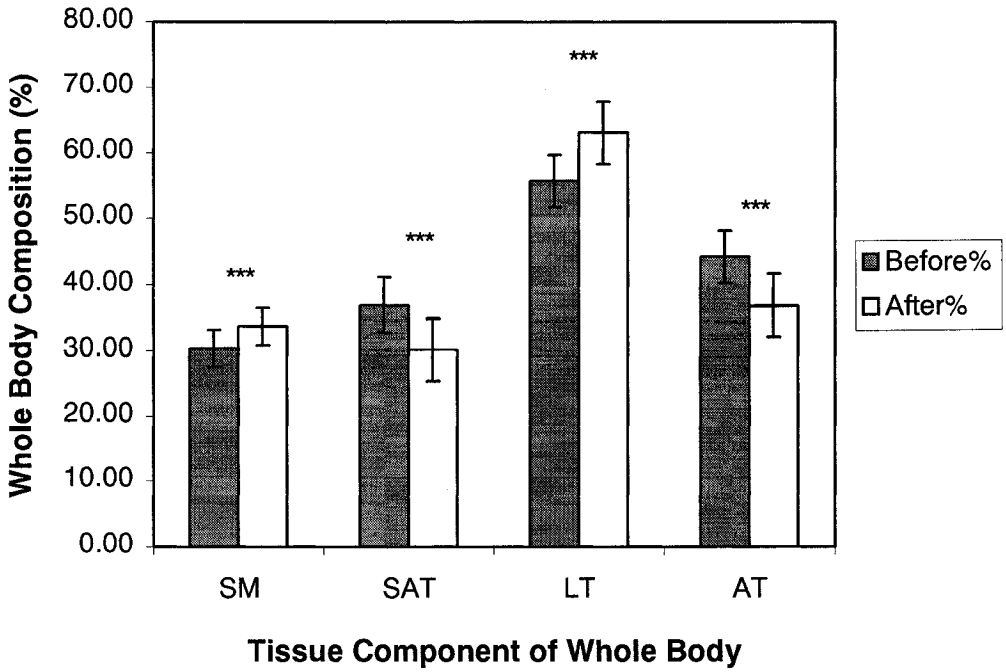
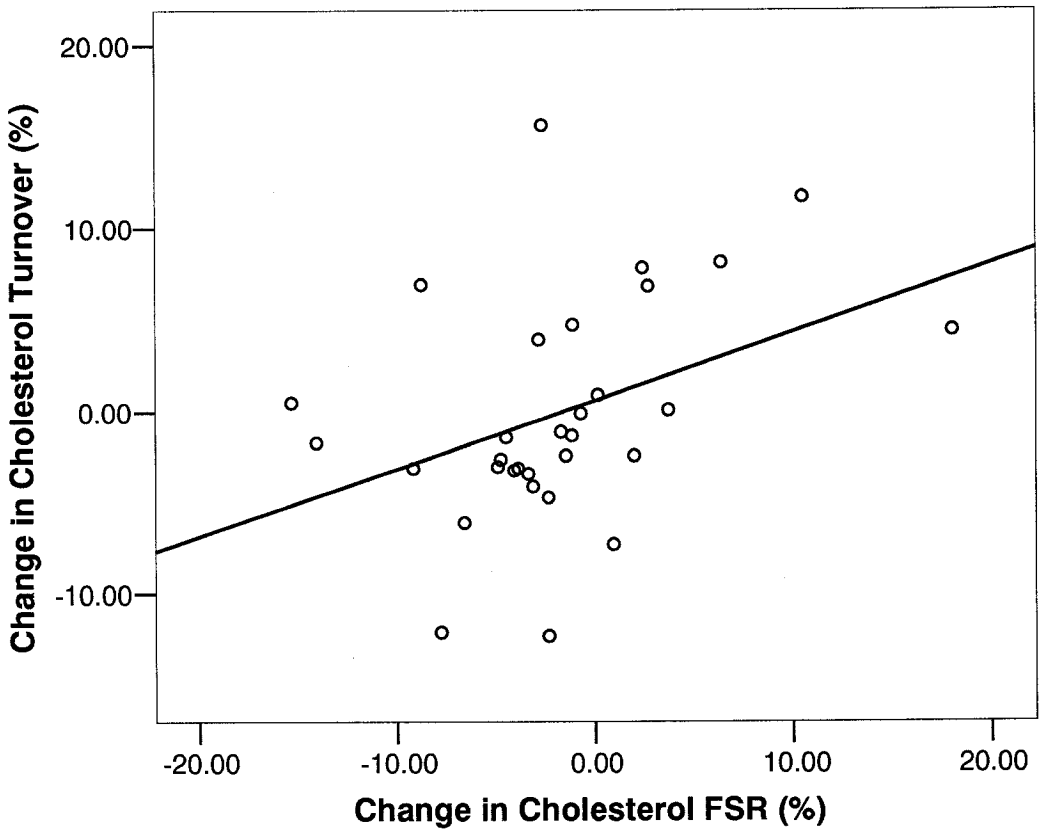


Figure 5-1b. Before and after percent whole body composition (%) of each tissue component. *** $P < 0.001$.



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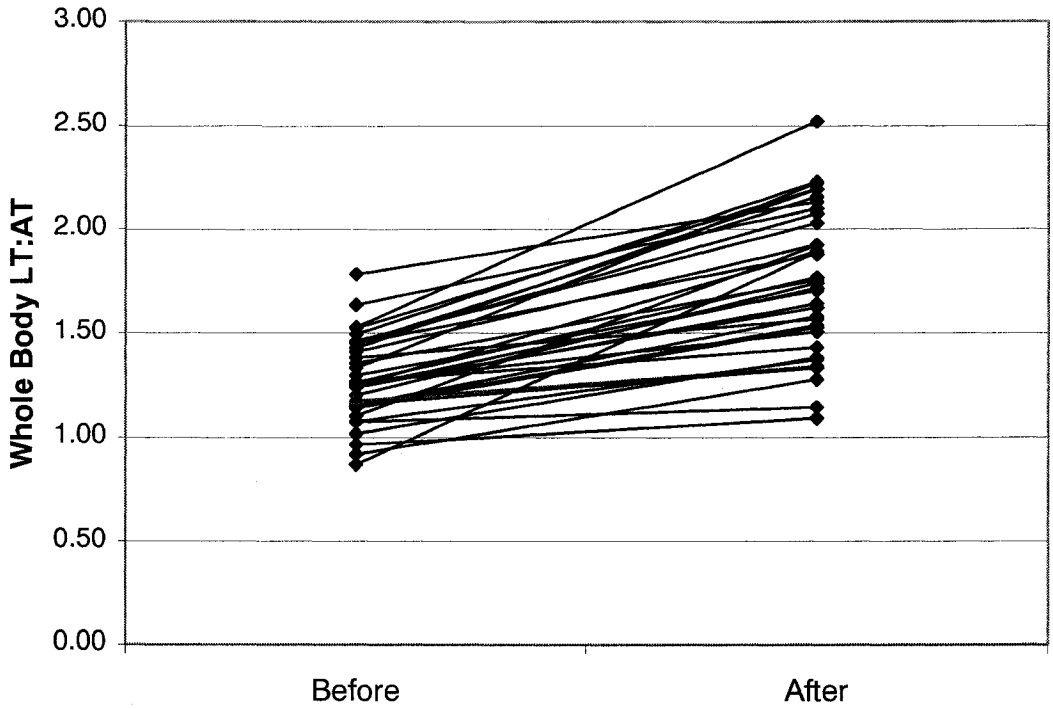

Figure 5-3. Individual whole body LT:AT ratio before and after weight loss. Mean difference of after LT compared to before LT:AT is 0.47 + 0.24; $P < 0.001$.

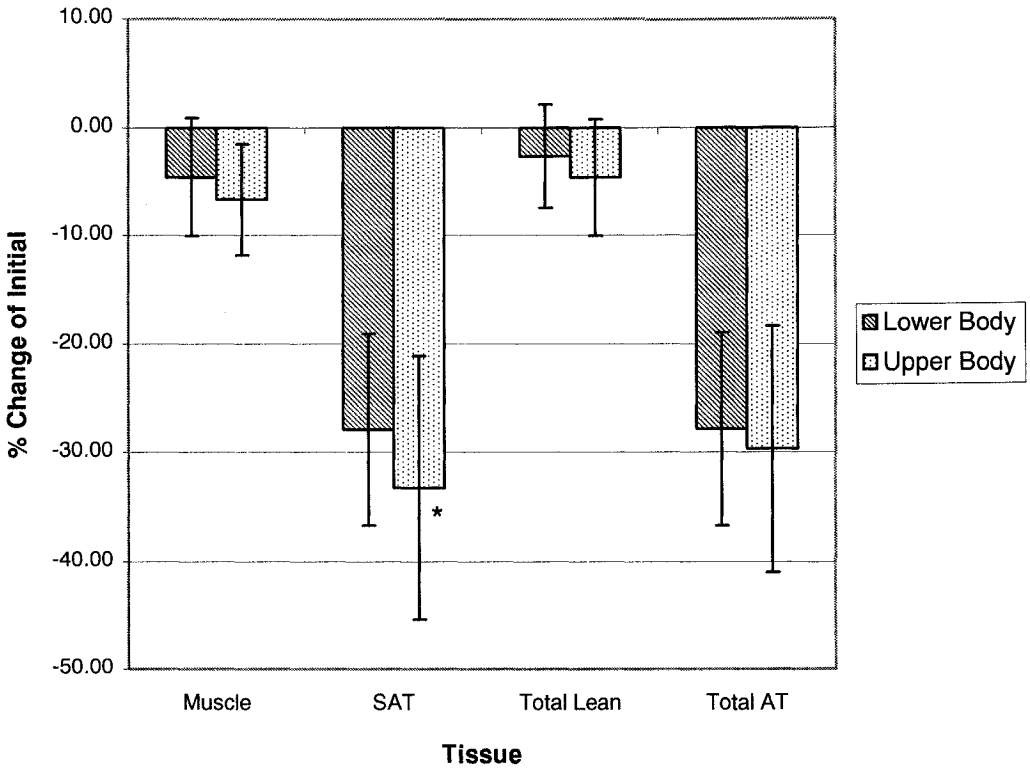

Figure 5-4. Change of each tissue component relative to initial values in the lower body and upper body regions. $^{*}P < 0.05$

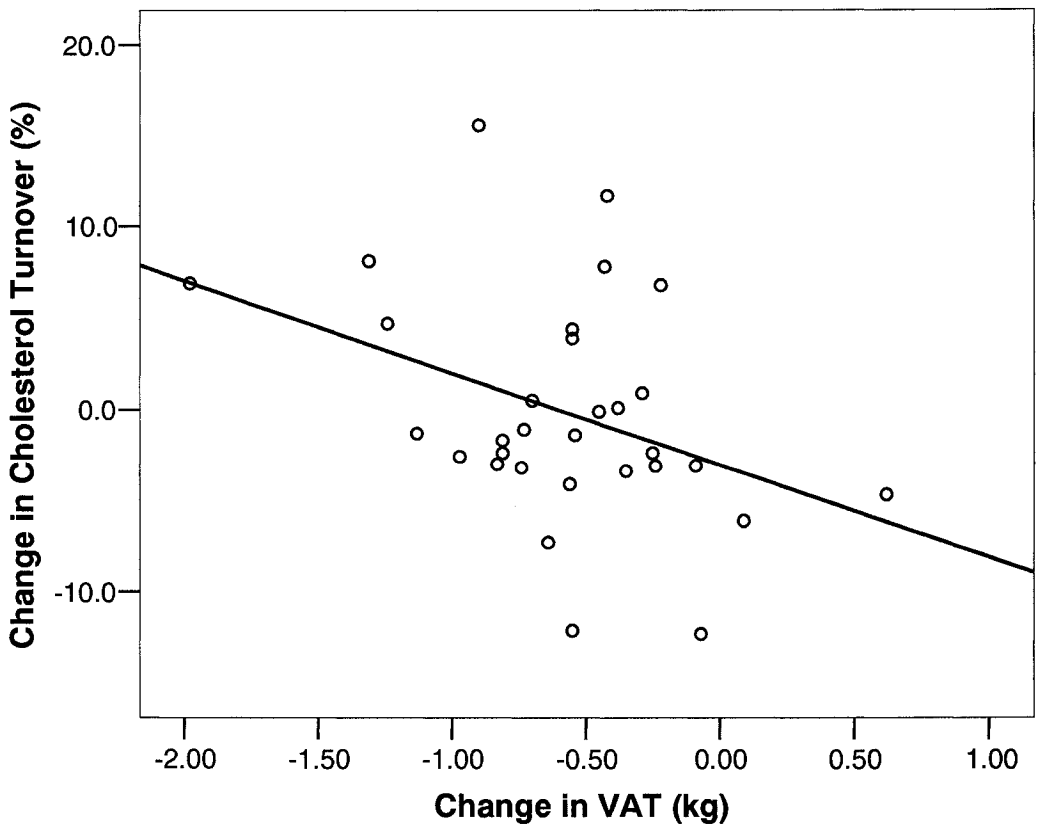

Figure 5-5a. Changes in cholesterol turnover in response to changes in VAT (β = -5.04, r = 0.383; P = 0.03).

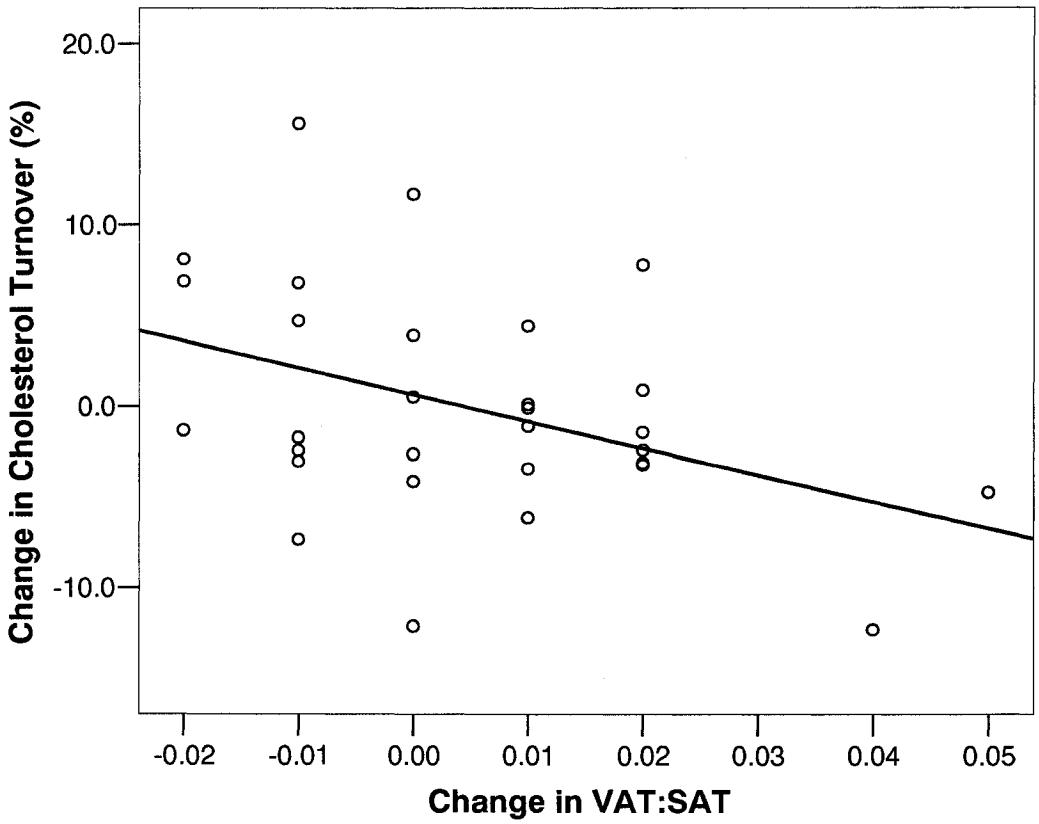

Figure 5-5b. Changes in cholesterol turnover in response to changes in VAT:SAT (β = -147, r = 0.399; P = 0.03).

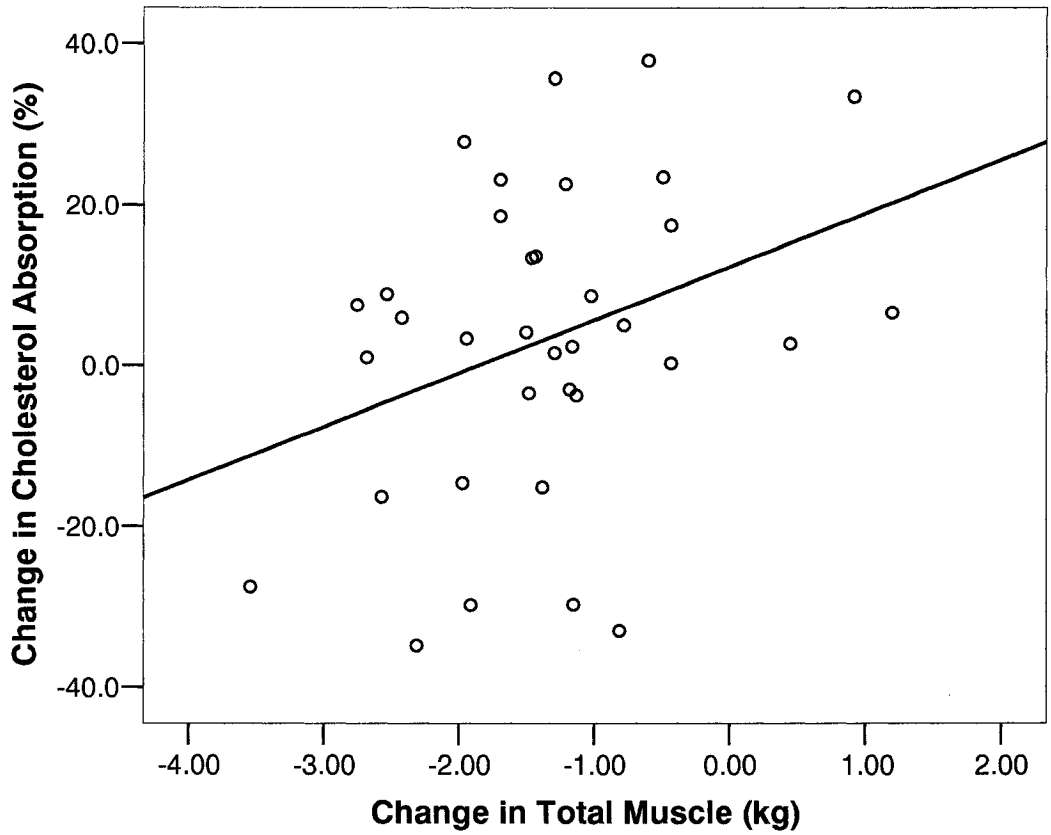

Figure 5-6a. Changes in cholesterol absorption in response to changes in whole body SM ($\beta = 6.82$, $r = 0.357$; $P = 0.04$).

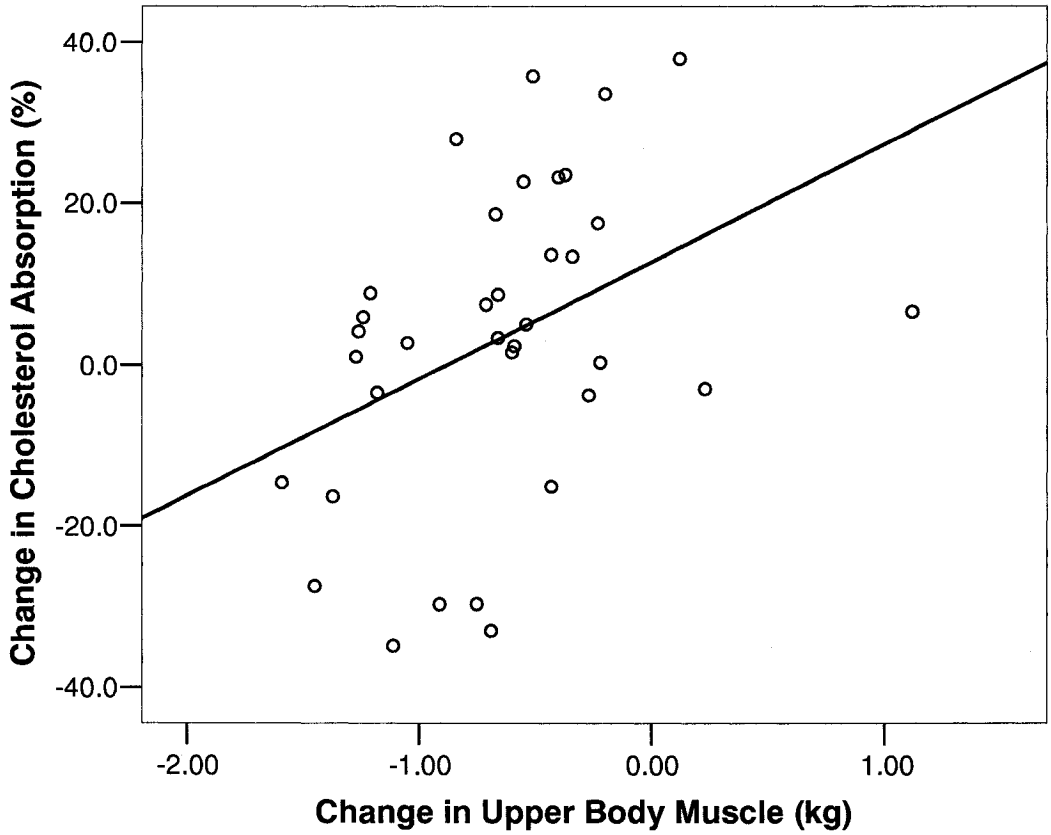

Figure 5-6b. Changes in cholesterol absorption in response to changes in upper body SM (β = 14.7, r = 0.413; P = 0.01).

BRIDGE 4.

The previous chapter found neither a change in cholesterol absorption nor an association between synthesis and absorption after weight loss. Though the methodology used might explain the variance in results between the previous and other studies, genetic factors may be another reason for the lack of change found in cholesterol absorption. Specifically, genetic variations in cholesterol transporters such as ATP binding cassette protein (ABC) G5 and G8 may affect the ability of cholesterol absorption to respond to changes in cholesterol synthesis (Klett et al., 2004). ABCG5 and ABCG8 are heterodimers that promote the efflux of cholesterol from enterocytes and hepatocytes (Berge et al., 2000). Various single nucleotide polymorphisms (SNPs) on ABCG5 and ABCG8 have been indicated to affect lipid levels. However, the effect of SNPs on changes in cholesterol levels and metabolism as a result of weight loss has never been investigated. Therefore, the objective of this last study was to examine the effect of weight loss on changes in lipid levels and cholesterol absorption, synthesis, and turnover in the context of SNPs of ABCG5 and ABCG8.

CHAPTER 6. Manuscript 6.

Submitted in the Journal of Lipid Research

ABCG5 and ABCG8 Single Nucleotide Polymorphisms Contribute to the Responsiveness of Cholesterol Kinetics to Weight Loss

Short Title: Santosa et al ABCG5/G8 and cholesterol kinetics

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6.1 Abstract

Objective: To examine whether changes in cholesterol lowering and metabolism after weight loss were affected by common SNPs in ABCG5 and ABCG8 genes.

Methods and Results: Weight loss was achieved in 35 hypercholesterolemic women through alterations in dietary and physical activity energy balance. Cholesterol kinetics was measured using stable isotope techniques. Mean weight loss was 11.7±2.5 kg ($P<0.001$). Heterozygous D19H individuals had lower ($P=0.028$, and $P=0.007$, respectively), initial TC and LDL-C levels, and reduced ($P=0.008$) post-weight loss LDL-C values than homozygous wild type individuals. Carriers of at least one C54Y variant had higher ($P=0.047$) post-weight loss LDL-C values compared with homozygous wild type individuals. Homozygous Q604E variant individuals had opposite ($P<0.05$) changes in cholesterol absorption and synthesis in contrast to heterozygous and homozygous carriers. Heterozygous C54Y carriers had smaller ($P=0.047$) reductions in FSR compared to homozygous mutant carriers. Subjects with at least one C54Y variant had higher ($P=0.042$) post-weight loss FSR in relation to homozygous wild types.

Conclusion: SNPs in ABCG5 and ABCG8 were found to regulate cholesterol concentration and metabolism after weight loss. Understanding the effect of these SNPs on cholesterol risk factors will help establish genetic screening tools to determine optimal lipid lowering treatment routes for these patients.

Condensed Abstract: We examined whether weight related changes in cholesterol metabolism were affected by SNPs in ABCG5/G8. D19H, C54Y, and Q604E SNPs affected cholesterol levels and metabolism. Understanding effects of these SNPs on cholesterol risk factors may have therapeutic implications in determining optimal lipid lowering treatment routes for these patients.

Keywords: cholesterol metabolism, weight loss, women, ABCG5, ABCG8

6.2 Introduction

In North America, if the prevalence of obesity continues to increase, children will have a lower life expectancy than their parents (Olshansky et al., 2005). The shorter life expectancy associated with obesity may be attributed to the multitude of comorbidities associated with the disease. A common comorbidity of obesity is cardiovascular disease (CVD), often resulting from a chronic underlying dyslipidemia. Obesity associated dyslipidemia is likely caused by perturbed cholesterol metabolism, commonly found in these individuals (Di Buono et al., 1999a; Simonen et al., 2000; Stahlberg et al., 1997). It has been well established that weight loss ameliorates dyslipidemia in obese individuals (Dattilo et al., 1992b). Weight loss has been associated with changes in cholesterol metabolism, including decreases in cholesterol synthesis which are not compensated for by corresponding increases in cholesterol absorption (Di Buono et al., 1999a; Simonen et al., 2000; Simonen et al., 2002a). On the other hand, the extent by which individuals are able to decrease cholesterol levels through weight loss may be genetically predetermined (Aberle et al., 2005).

Cholesterol homeostasis is maintained through the balance of cholesterol absorption, biosynthesis, and turnover. ATP-Binding Cassette (ABC) G5 (MIM 605459) and ABCG8 (MIM 605460) transporters have been identified as affecters of cholesterol metabolism (Berge et al., 2000). The location of these transporters in apical membranes of hepatocytes, and enterocytes in the proximal small intestine in humans make ABCG5 and ABCG8 ideally situated to regulate cholesterol kinetics (Klett et al., 2004). Indeed, expression of these transporters from 13 exon genes serves to reduce net cholesterol absorption by promoting efflux of cholesterol from the enterocyte into the intestinal lumen. This in turn results in an increased hepatic cholesterol synthesis (Klett et al., 2004). Genetic mutations in DNA that encodes these proteins may affect the efficacy with which ABCG5 and ABCG8 function, thereby affecting the response of cholesterol lowering therapies (Kajinami et al., 2004a; Kajinami et al., 2004b).

Recently, several single nucleotide polymorphisms (SNP) have been identified on introns and exons of ABCG5 and ABCG8. More specifically, these SNPs include Q604E (RS6720173), I18427 (RS4148189), I7892 (RS4131229), and M216

(RS3806471) on ABCG5 and C54Y (RS4148211), D19H (RS11887534), T400K (RS4148217), and I14222 (RS6709904) on ABCG8.

Though dyslipidemia and perturbed cholesterol metabolism is common in overweight individuals, no study has examined the effect that SNPs in ABCG5 and ABCG8 may have in cholesterol lowering and metabolism after weight loss. Understanding the role that these SNPs may play in affecting dyslipidemia and cholesterol metabolism will help establish genetic screening tools to determine optimal lipid lowering treatment routes for these patients. Therefore, the objective of this study was to examine whether changes in cholesterol lowering and metabolism after weight loss were affected by common SNPs in ABCG5 and ABCG8 genes.

6.3 Methods

Subjects

Newspaper advertisements and word of mouth was used to recruit women from the Montreal area to participate in a 24-week weight loss study. To be selected in the study women were required to be 35-60 years of age with body mass indices of 28-39 kg/m^2. Subjects who used oral anti-hypertensive agents, thyroid hormones, and hormone replacement therapy were also included, regardless of menopausal status, provided they were stable and continued on the same dose throughout the duration of the study. Potential subjects were screened for fasting plasma low density lipoprotein cholesterol (LDL-C) concentrations of 3.4-6.7 mmol/L and triacylglycerol (TAG) concentrations >1.5 mmol/L. Exclusion criteria included treatment with oral hyperlipidemic therapy less than six months prior to the start of the study, any history of chronic illness, and previous history of eating disorders. All selected subjects were given an outline of the study protocol, and were required to sign a consent form before starting the study. The Faculty of Medicine Ethics Review Board at McGill University, Montreal, QC and the Human Investigation Review Committee of Tufts University, Boston, MA, USA approved the experimental protocol for ethics.

Protocol

Experimental design

Subjects served as their own controls in a 24-week longitudinal study design. During the 24 weeks, participants were required to undergo three consecutive dietary periods:

a two week pre-loss stabilization period, a 20 week weight loss period, and a two week post-loss stabilization period.

During the two-week stabilization periods, subjects were required to maintain a stable weight, as well as usual food and exercise habits. Changes in lipid levels were assessed as the difference between days 15 of each stabilization period. Analysis of cholesterol kinetics was measured with the use of stable isotopes between days 11 and 15 in each phase. In measuring cholesterol absorption and turnover, baseline fasting blood samples were collected on day 11 of each stabilization phase before subjects received an intravenous injection of 15 mg [25, 26, 26, 26, 27, 27, 27-D_7]cholesterol and a 75 mg oral dose of [3,4-^{13}C]cholesterol (>99 atom percent excess for both; CDN Isotopes, Montreal). Cholesterol fractional absorption was then determined by comparing the ratio of ingested [^{13}C]cholesterol to intravenous [D_7]cholesterol enrichment in red blood cell (RBC) on days 11, 12, and 13. Cholesterol turnover was taken as the rate of decay in enrichment of D_7 in RBC cholesterol between day 11 hour 12, and day 14. Cholesterol biosynthesis was measured on day 14 and 15 using the deuterium incorporation method, which involved ingestion of 0.7 g D_2O/kg estimated body water (99.8 atom percent excess; CDN Isotopes, Montreal). Body water was estimated at 60% of total body weight.

Weight loss protocol

Weight loss was accomplished in a free-living environment by decreasing dietary energy intake by 20% and increasing energy expenditure by 10% through physical activity. The diet was taught via an exchange system and consisted of 50-60% of energy from carbohydrates, 20% of energy from protein, and <30% of energy from fat. To help participants achieve a caloric deficit of 10% by energy expenditure, semi-private group sessions with a personal trainer were held at the beginning and midway through the weight loss period. Throughout the weight loss period, compliance was determined at weekly weigh-ins, where subjects wore the same clothes. Participant motivation was encouraged using an award point system and visual graphs on which they were able to monitor weekly weight loss.

Endpoint analyses

After phlebotomy, blood samples were promptly centrifuged at 1500 rpm for 15 minutes to separate RBC and plasma and immediately stored at -20°C until analyzed. Analysis of TC, and high density lipoprotein cholesterol (HDL-C) concentrations is described elsewhere (McNamara et al., 1987b). LDL-C concentration was measured using the dextran/magnesium sulfate method, as previously described (Rifai et al., 1998; Wang et al., 2001b).

Cholesterol absorption

A detailed description of cholesterol absorption determination by dual isotope ratio methodology may be found elsewhere (Vanstone et al., 2002b). Briefly, lipids from RBC were extracted using a modified Folch method, isolated and combusted to produce CO_2, enriched with ^{13}C and water, enriched with D_7 (Folch et al., 1957). The CO_2 and water generated by combustion were separated using vacuum distillation. D_7-enriched water was further reduced with zinc reagent (Biogeochemical Laboratories, Indiana University, Bloomington, IN) to produce D_7-labelled hydrogen gas. Enrichment of free cholesterol by D_7 and ^{13}C were measured by differential IRMS (VG Isomass 903D, and SIRA 12;Isomass, Cheshire, United Kingdom, respectively). The cholesterol absorption was calculated for days 12 and 13 by the following equation (Bosner et al., 1993):

$$\text{Cholesterol Absorption (\%)} = \frac{\Delta\,^{13}C \times 7/46 \times D_7\text{-cholesterol IV dose (mg)} \times 0.0122}{\Delta D_7 \times 2/27 \times {}^{13}C\text{-cholesterol oral dose (mg)} \times 0.000155} \times 100$$

where Δ for ^{13}C and D_7 is the difference between samples at 48 and 72 hours and the baseline abundance (t=0) in parts per thousand relative to PDB and SMOW standards, respectively. The factors 7/46 and 2/27 reflect the ratio of labelled atoms/mg dose and the constants 0.0112 and 0.000155 are conversion factors of the part per thousand units into atom percent excess for the PDB and SMOW scales, respectively.
CAC at 48 h and 72 h were then averaged to determine overall cholesterol absorption.

Cholesterol biosynthesis

The deuterium incorporation method was used to assess cholesterol biosynthesis, as previously described (Vanstone et al., 2002b). Briefly, erythrocyte cholesterol was extracted and water from the extracted cholesterol was isolated, at which point

enrichment was determined using differential IRMS (VG Micromass 903D) for days 14 and 15 of each stabilization period, as described above (Folch et al., 1957; Jones et al., 1993a). In addition, deuterium enrichment of plasma water was measured.

The following equation, which corrects for cholesterol deuterium:protium ratio, was used to determine fractional synthesis rate (FSR) over 24 hours (Jones et al., 1993a):

$$FSR(\%/d) = (\Delta_{cholesterol}/\Delta_{plasma}) \times 0.478 * 100,$$

where Δ refers to deuterium enrichment above baseline level over 24 hours in parts per thousand relative to a SMOW standard. The factor 0.478 reflects to ratio of labelled H atoms replaced by deuterium during in vivo biosynthesis (Jones et al., 1993a).

Cholesterol turnover

Turnover of plasma free unesterified cholesterol is defined as the difference between the rate of influx from synthesis and dietary absorption, and the rate of efflux from esterification, excretion or transfer from other body pools (Goodman et al., 1973; Nestel et al., 1973; Vanstone et al., 2002b). Thus, the rate of erythrocyte free cholesterol turnover was assessed as the rate of decay of injected D_7-cholesterol over hours 12 to 72.

Assessment of ABCG5 and G8 SNPs

Leukocyte DNA was extracted from 5 to 10 mL of whole blood as described by Miller et al. (1988). SNPs Q604E, I18429, I7892, and M216 on ABCG5, and C54Y, D19H, I14222, and T400K on ABCG8 were determined using PCR-based TaqMan allele discrimination assays (Applied Biosystems (ABI), Foster City, CA) (McGuigan et al., 2002). The primers used for PCR have been described previously (Hubacek et al., 2001; Kajinami et al., 2004a). A GeneAmp PCR System 9700 thermal-cycler (ABI, Foster City, CA) was used for PCR analysis. A 7900HT sequence detection system (ABI, Foster City, CA) was used to carry out PCR analysis. Reactions were subject to 50°C for 2 min, 95°C for 10 min, and 40 cycles, each of 95°C for 15 s and 60°C for 1 min.

Statistics

All data are expressed as means±SD. Student's paired t-tests were used to determine statistically significant changes in lipid and cholesterol metabolism. The Wilcoxon signed rank test was applied where appropriate. To test whether there was a difference in lipid and cholesterol kinetic changes within an SNP, one-way analysis of variance (ANOVA) with post hoc Scheffe analysis was applied. The Kruskal-Wallis test was used where appropriate. Data from homozygous mutant carriers and heterozygous individuals were then regrouped and tested for associations. Homozygous mutant groups with less than three individuals were automatically collapsed into the heterozygous group. A Student's t-test for unpaired groups or a Mann-Whitney U test was then applied to the regrouped data, depending on whether a normal distribution was present. All values were defined to be statistically significant at $P<0.05$. Data were analysed using SPSS for Windows (version 12.0.0; SPSS Inc., Chicago, IL).

6.4 Results

Forty-two subjects were accepted into the study with 35 completing the study. Participants withdrew due to inability to fulfill study requirements (n=2), and difficulty adhering to the weight loss protocol and scheduled study visits (n=5). The 35 participants who completed the study had an average age of 49.4±6.7 years, BMI was 31.4±2.8 kg/m^2, and body weight was 81.4±9.5 kg. Overall, participants lost an average of 11.7±2.5 kg (14.5±3.1% of original body weight) ($P<0.001$).

Changes in lipid and cholesterol metabolism

TC and LDL-C levels decreased post weight loss ($P<0.001$) by 0.62±0.65 mmol/L, and 0.32±0.51 mmol/L ($P=0.001$), respectively, and HDL-C increased ($P=0.03$) by 0.08±0.21 mmol/L. In response to weight loss, FSR fell ($P=0.003$) by 3.86±9.33%/d. No changes were seen in cholesterol absorption (3.31±19.4%; $P= .32$) or rates of cholesterol turnover (-0.17±9.56%; $P=0.92$).

SNP distribution and frequency

Genotype distribution and frequency of SNPs of ABCG5 and ABCG8 are shown in **Table 6-1**. All genotypes were distributed according to the Hardy-Weinberg equilibrium.

Effects of genotypes on lipid levels

The D19H SNP on ABCG8 was associated with pre-weight loss TC values. Heterozygous individuals had lower (P=0.028) initial TC levels (5.49±0.85 mmol/L) than those who were homozygous for the wild type (6.28±0.90 mmol/L) (**Figure 6-1**). Subjects who were heterozygous for the D19H SNP also had lower pre- and post-weight loss LDL-C values (**Figure 6-2**). Initial LDL-C concentrations for heterozygous participants were 3.25±0.50 mmol/L compared to 3.94±0.65 mmol/L (P=0.007) in those who were homozygous wild type. Similarly, post-weight loss LDL-C values in heterozygous subjects were 3.05±0.47 mmol/L, which was lower (P=0.008) than in those who were homozygous for the wild type (3.57±0.48 mmol/L).

When those who were homozygous for the variant allele were grouped with participants who were heterozygous, the C54Y SNP on ABCG8 was found to be associated with post-weight loss LDL-C values (P=0.047) (**Figure 6-3**). More specifically, LDL-C in individuals who were homozygous wild type (3.25±0.45 mmol/L) was lesser than that of subjects who were carriers of the variant allele (3.60±0.54 mmol/L).

There were also trends seen for initial LDL-C in the I14222 SNP on ABCG8, where subjects who were homozygous for the wild type (3.90±0.65 mmol/L) tended to have higher levels (P=0.058) of LDL-C before weight loss than subjects who were heterozygous (3.42±0.66 mmol/L). Similarly, a trend was also seen for the T400K SNP on ABCG8, where decreases in LDL-C levels in participants who were homozygous for the wild type tended (P=0.061) to be smaller (-0.20±0.48 mmol/L) than in participants who were heterozygous (-0.53±0.52 mmol/L).

No SNPs on ABCG5 were found to be associated with initial, end or changes in lipid values.

Effects of genotypes on cholesterol metabolism

Changes in cholesterol absorption were related to Q604E SNP on ABCG5 (see **Table 6-2**). More specifically, individuals who were heterozygous (7.33±15.6%) and homozygous wild type (5.94±18.3%) carriers had larger increases than those who were homozygous for the variant (-30.8±1.90%) (P=0.017 and P=0.010, respectively).

Individuals who were homozygous for the Q604E SNP also had higher initial cholesterol absorption (86.5±13.3%) compared to heterozygous (57.2±11.8%), and homozygous wild type (59.7±18.5%) subjects (P=0.026 and P=0.039, respectively). Changes in cholesterol FSR were also seen where those who were homozygous for the mutation had increases (1.69±10.0%) after weight loss and subjects who were heterozygous decreased in FSR after weight loss (-7.39±9.36%) (P=0.041). When individuals who were heterozygous were grouped with individuals that were homozygous for the Q604E SNP variant, no significant differences were identified in indicators of cholesterol metabolism.

A trend existed in the I7892 SNP on ABCG5 where initial FSR values tended to be higher in participants who had at least one mutation (12.4±9.03%) than in those who were homozygous for the wild type SNP (7.75±5.08%) (P=0.058).

The C54Y SNP on ABCG8 was also associated with changes in FSR. More specifically, heterozygous carriers had lesser reductions in FSR (-0.17±7.60%) compared to participants who were homozygous for the mutation (-8.09±10.3%) (P=0.047) (refer to **Table 6-2**). Combining heterozygous and homozygous variant individuals resulted in lower post-weight loss FSR (5.18±4.88%) in homozygous wild type subjects in relation to subjects who carried at least one variant (7.76±4.90%) (P=0.042).

No further differences in cholesterol metabolism were found to be associated with SNPs in ABCG5 or ABCG8.

6.5 Discussion

This study is the first to show that changes in cholesterol metabolism after weight loss are associated with SNPs in ABCG5 and ABCG8. The results indicate that cholesterol levels pre- and post- weight loss were related to SNPs in ABCG8, but not ABCG5. More specifically, the D19H SNP on ABCG8 was found to be associated to lower pre-weight loss TC and LDL-C, as well as decreased post-weight loss LDL-C concentrations. Another SNP that was associated with lesser post-weight loss LDL-C levels was the C54Y SNP on ABCG8. This study also found that the homozygous carriers of the Q604E SNP on ABCG5 experienced larger decreases in cholesterol

absorption and increased FSR after weight loss. Individuals homozygous for the C54Y SNP on ABCG8 exhibited lower post weight-loss FSR.

ABCG5 and ABCG8 are two half transporters which likely function as heterodimers to promote cholesterol and non-cholesterol sterol efflux at the level of the intestine and to pump sterols into bile at the level of the liver (Hazard et al., 2006). Point replacement of an amino acid residue by another of different charge and structure may result in changes to physiological protein function. Indeed, the nonsynonymous cSNPs examined in this trial affect highly conserved amino acid residues, suggesting a possible alteration of the function of these transporters through these SNPs (Hazard et al., 2006). Understanding how the roles of SNPs in ABCG5 and ABCG8 in cholesterol kinetics can be altered may be important in defining optimal routes of treatment of dyslipidemia.

The finding that individuals heterozygous for the mutation encoding histidine in the D19H SNP had lower initial TC and LDL-C levels and lesser post-weight loss LDL-C than individuals who were homozygous for the wild type is consistent with the outcome of previous studies (Gylling et al., 2004b; Kajinami et al., 2004a; Kajinami et al., 2004b). A non-intervention study by Gylling et al. (2004b) involving 263 mild to moderately hypercholesterolemic subjects found that TC and LDL-C levels were higher in homozygous wild type carriers compared to carriers who had at least one mutant variant. Another intervention trial, which examined the potential effect of genetics on lipid lowering by 10 mg of atorvastatin, showed that individuals who had at least one variant allele had lower initial TC and lower post treatment TC and LDL-C (Kajinami et al., 2004a). Though the overall change in LDL-C was not different between D19H SNPs in the present trial, the percent decrease of LDL-C in the aforementioned study was larger in those that carried the H19 variant compared to wild type (Kajinami et al., 2004a). The lack of association between the D19H SNP and change in TC or LDL-C in the present study may be due to the small sample size that was used compared to other trials.

Some studies showed no differences in plasma cholesterol levels depending on the C54Y polymorphism (Gylling et al., 2004b; Kajinami et al., 2004b). However, a study in a Czech population, in which lower lipid levels were observed after reducing

the consumption of animal fat over eight years, showed that in female carriers of the C54Y variant on ABCG8 encoding tyrosine, decreases in TC and LDL-C were larger (Hubacek et al., 2001). The difference in initial and final TC and LDL-C between genotypes was not discernible. In contrast, the present study demonstrated that females who were homozygous for the cysteine allele had lower final LDL-C levels than those who had at least one tyrosine allele. There were no associations however, between the C54Y SNP and changes in TC or LDL-C. Thus, further investigation is required to determine whether nutrient-gene interactions may modulate the impact of C54Y SNP on TC and LDL-C concentrations.

In the present study, the data indicate no association between cholesterol concentrations and Q604E or T400K SNPs. Though one study by Weggemans et al. (2002) found a connection between Q604E and TC, where those who were of homozygous wild type had lower TC than carriers of at least one mutant allele, other studies did not find the same association (Gylling et al., 2004b; Plat et al., 2005). No relationships were observed between T400K and blood cholesterol levels in any trials that examined baseline values (Gylling et al., 2004b; Hubacek et al., 2001; Plat et al., 2005). The atorvastatin intervention did not discern variations in changes in blood cholesterol levels and the Q604E and T400K SNPs. A relationship was seen in the experiment by Hubacek et al. (2001), where male participants homozygous for the threonine allele had a larger decrease in TC and LDL-C concentrations over eight years in contrast to individuals who had at least one K400 mutation. However, this relationship was not found in female participants, suggesting that sex may affect the impact of the K400 mutation on cholesterol concentrations.

A study in mice indicated that ABCG5 and ABCG8 expression may be upregulated via the intestinal estrogen receptor α pathway (Duan et al., 2006). However, evidence shows that there may be inconsistencies between the murine promoter sequences and those in humans (Hazard et al., 2006). Thus, regulation of the murine ABCG5 and ABCG8 may be different. Whether there is a gender difference in the regulation of ABCG5 and ABCG8 in humans and the role of SNPs in this scenario is yet to be determined.

Changes in cholesterol metabolism have never been examined in the context of ABCG5 and ABCG8 SNPs. Only studies that investigated the effect of these SNPs on baseline cholesterol absorption exist. In contrast with our results showing no relation between initial cholesterol kinetic levels and D19H, one study ascertained a connection between the D19H SNP, where the H19 allele was associated with higher cholesterol absorption (Gylling et al., 2004b). Another study did not detect any differences between cholesterol absorption and ABCG5 and ABCG8 SNPs (Miwa et al., 2005). One explanation for the deviations in the findings was that the subject population varied between studies with one including only Japanese participants (Miwa et al., 2005) and the other including men and women (Gylling et al., 2004b). A novel finding of this trial is that change in cholesterol absorption and synthesis after weight loss, measured by stable isotopes, were affected by the Q604E and C54Y SNPs. There were only three subjects that were homozygous carriers for the E604 allele, because the estimated population frequency of the allele is only 3.5%. It would be difficult to obtain a large number of homozygous individuals, and despite their small number, those who were homozygous for the variant were examined as a group in the present study to see if there may be any differences in cholesterol metabolism among these individuals. Participants who were homozygous for the glutamine allele had decreases in cholesterol absorption and increases in cholesterol synthesis compared to carriers of at least one wild type allele, who had increases in cholesterol absorption and decreases in cholesterol synthesis. These results suggest that the Q604E SNP has an affect on cholesterol metabolism. The use of stable isotopes for determination of cholesterol kinetics in this study is unique and allows for the direct quantification of associated parameters. However, the time and cost related to using stable isotopes does not allow this technique to be utilized with large sample sizes. Thus, further studies are required to confirm this conclusion.

Homozygous carriers of the mutation that results in the transcription of tyrosine in the C54Y SNP were observed to have larger reductions in FSR in response to weight loss than those who were heterozygous for the mutation. Additionally, participants with at least one variant had smaller post-weight loss FSR than homozygous wild type subjects. Since individuals who had at least one variant also had lower post-weight loss LDL-C concentrations than homozygous wild type participants, it appears that

lower cholesterol FSR in the carriers of the C54Y mutation was responsible for the lower post-weight loss circulating concentrations of LDL-C.

This study was the first to examine the potential of ABCG5 and ABCG8 SNPs in regulating changes of lipid concentration and metabolism after weight loss. In doing so, it was observed that the D19H and C54Y SNP may be connected to lipid concentrations pre- and post-weight loss, and the Q604E and C54Y was associated with changes in cholesterol metabolism. The regulation of lipid levels and metabolism by SNPs in ABCG5 and ABCG8 make them a target in determining optimal lipid lowering treatment routes for hyperlipidemic patients undergoing weight loss.

6.6 Acknowledgements

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6.7 Figure Legends

Figure 6-1. Initial and final TC according to the D19H SNP. *Significant at P = 0.028 between groups.

Figure 6-2. Initial and final LDL-C according to the D19H SNP. Significant at *P = 0.007; **P = 0.008 between groups.

Figure 6-3. Initial and final LDL-C according to the C54Y SNP. *Significant at P = 0.047 between groups.

Table 6-1. Genotype distribution and frequency of SNPs of ABCG5 and ABCG8 in studied population.

SNP:		Homozygous Wild		Heterozygous		Homozygous Variant	
ABCG5		(n)	(%)	(n)	(%)	(n)	(%)
	Q604E	19	54.3	13	37.1	3	8.6
	I18429	22	62.9	13	37.1	0	0
	I7892	15	42.9	13	37.1	7	20.0
	M216	18	51.4	10	28.6	7	20.0
ABCG8							
	C54Y	16	45.7	12	34.3	7	20.0
	D19H	26	74.3	9	25.7	0	0
	I14222	25	71.4	10	28.6	0	0
	T400K	22	62.9	11	31.4	2	5.7

Table 6-2. Cholesterol metabolism and change according to exon SNPs in ABCG5 and ABCG8.

	Cholesterol Absorption (%)			Cholesterol Biosynthesis (%)		
	Initial	Final	Change	Initial	Final	Change
Q604E	59.7±18.5*	65.7±13.7	5.94±18.3‡	8.48±6.76	6.16±5.66	-2.32±8.86
QQ						
QE	57.2±11.8†	64.5±18.0	7.33±15.6§	13.9±9.05	6.48±3.95	-7.39±9.36*
EE	86.5±13.3*,†	55.7±13.5	-30.8±1.90‡,§	7.91±4.91	9.60±5.09	1.69±10.0*
QE/EE	62.6±16.6	62.8±17.2	0.18±20.7	12.8±8.63	7.07±4.18	-5.69±9.84
C54Y	64.9±15.4	67.5±16.4	2.67±22.0	9.96±8.83	5.18±4.88*	-4.78±9.62
CC						
CY	54.2±13.5	65.4±12.2	11.2±12.9	8.95±5.34	8.79±5.35	-0.17±7.60*
YY	64.1±25.6	55.3±15.6	-8.80±17.9	14.1±9.07	5.99±3.74	-8.09±10.3*
CY/YY	57.9±18.8	61.7±14.0	3.85±17.5	10.8±7.16	7.76±4.90*	-3.08±9.28
D19H	58.8±15.9	61.8±13.3	3.00±17.2	10.1±6.68	6.54±5.28	-3.55±8.28
DD						
DH	67.5±21.2	71.7±18.8	4.19±25.9	11.4±11.0	6.68±4.35	-4.76±12.4
T400K	64.4±19.4	63.6±17.2	-0.76±20.2	10.4±8.91	6.91±5.51	-3.53±10.9
TT						
TK/KK	55.4±12.2	65.6±11.6	10.2±16.5	10.4±5.99	6.01±4.12	-4.42±6.29

*,†Significant difference between groups $P<0.05$. ‡,§Significant difference between groups $P<0.01$.

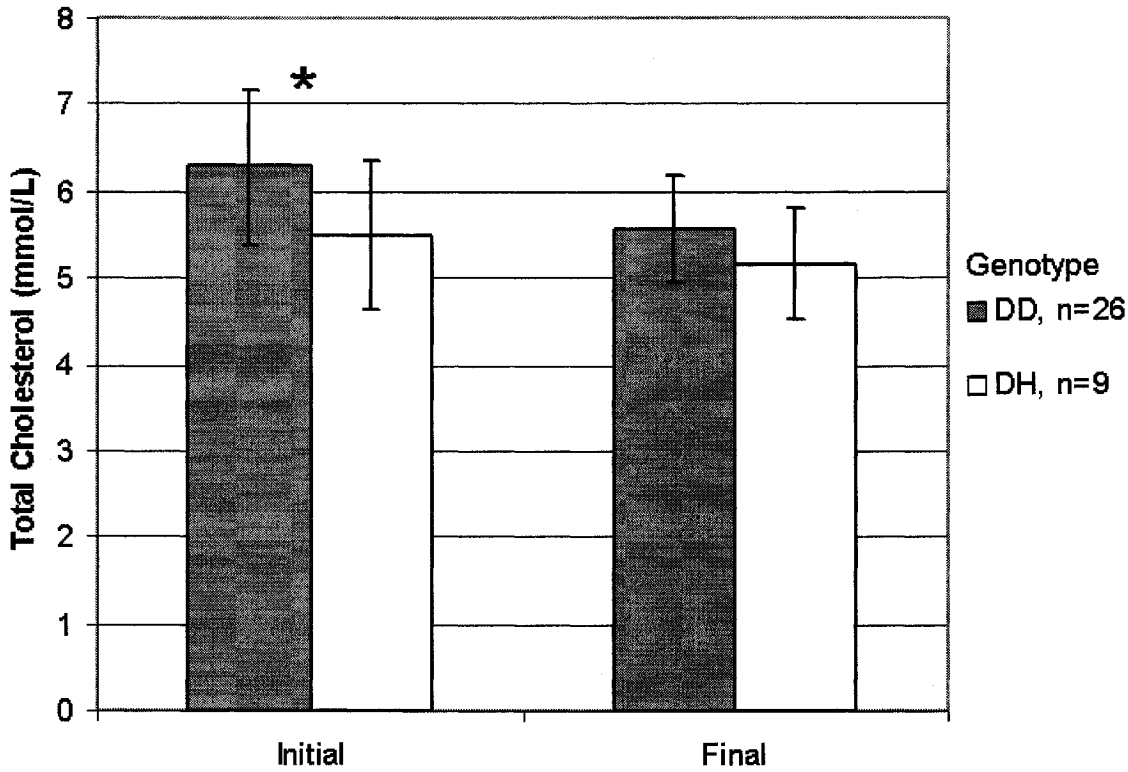

Figure 6-1. Initial and final TC according to the D19H SNP. *Significant at P = 0.028 between groups.

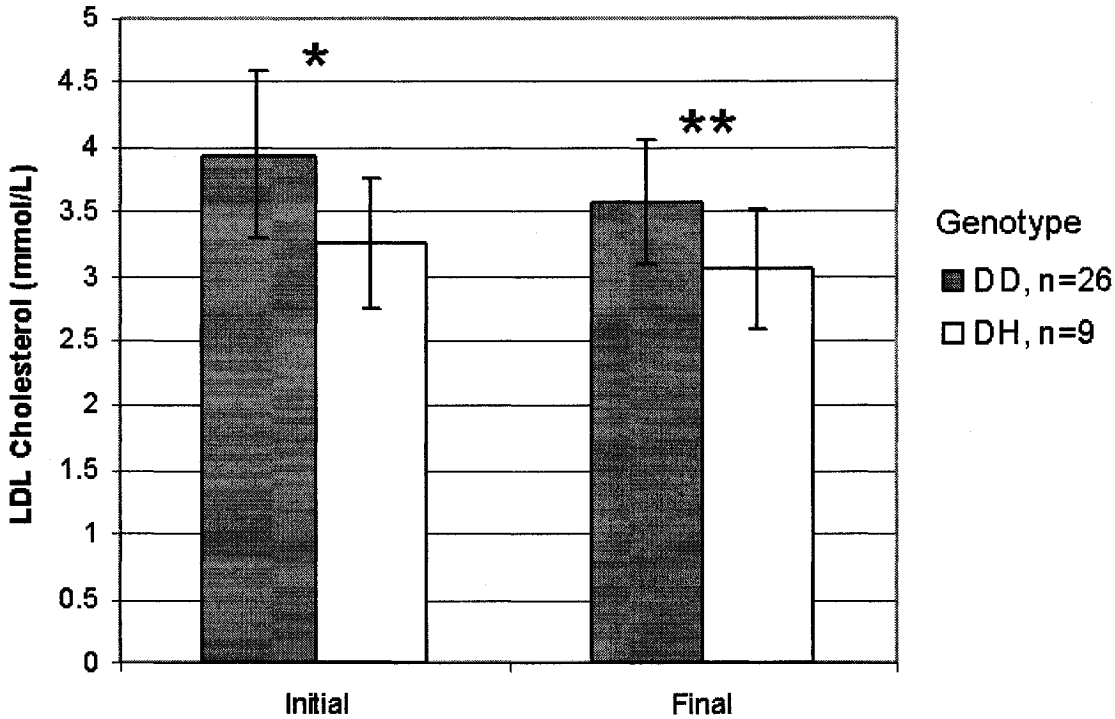

Figure 6-2. Initial and final LDL-C according to the D19H SNP. Significant at *P = 0.007; **P = 0.008 between groups.

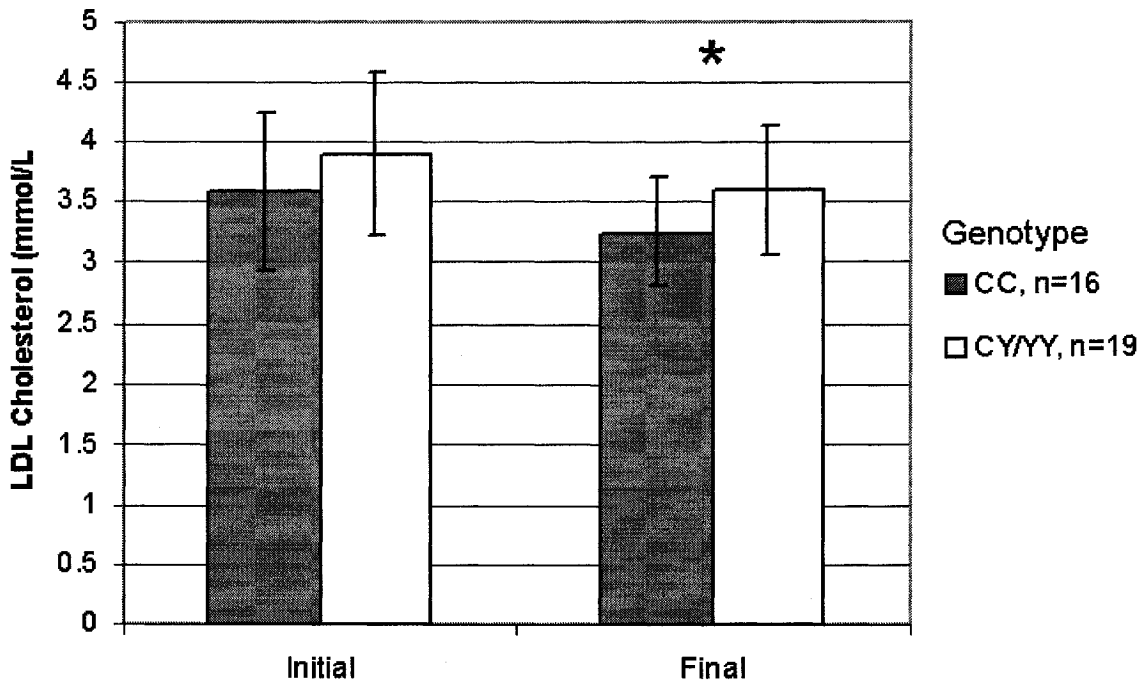

Figure 6-3. Initial and final LDL-C according to the C54Y SNP. *Significant at P = 0.047 between groups.

DISCUSSION & CONCLUSION

Overweight and obesity exerts a toll on the Canadian health care system costing an estimated $4.3 billion in direct and indirect costs (Katzmarzyk et al., 2004). The World Health Organization predicts the impact of the obesity will have an equal impact on health as the smoking epidemic (Statistics Canada, 2002). It is well established that these individuals are at greater risk for various comobidities including sleep apnea, infertility, dyslipidemia, hypertension, type 2 diabetes mellitus (DM), infertility in women and CVD (Must et al., 1999). There is no question as to the dire impact of obesity on health; many uncertainties remain in determining the best course of treatment of its associated comorbidities. The key to unlocking the effective routes of treatment is to understand the mechanism behind obesity-related metabolic changes.

The main objective of this thesis was to characterize changes in cholesterol metabolism as a result of moderate weight loss in overweight, hyperlipidemic women. This thesis demonstrates a successful method for moderate weight loss through self-selected diet and exercise. As a result, we were able to conduct an original study that examined the effects of significant weight loss on various aspects of cholesterol kinetics. The knowledge of hyperlipidemia may be important in determining weight loss success since baseline lipid values were shown to predict total weight loss. In conducting the research, the lack of an association found between insulin, leptin, adiponectin, and ghrelin indicates that though these hormones are all involved in regulating energy balance, they may contribute to weight loss independently of each other. Moderate weight loss in hyperlipidemic overweight and obese females also resulted in cardioprotective changes in cholesterol levels, despite minimal effects on LDL particle size. Since absorption and turnover did not change, amelioration of lipid levels is likely due to decreases in cholesterol synthesis. Additionally, the research conducted provides evidence that weight-related variations in cholesterol absorption, synthesis, and turnover are affected by alterations in regional body composition and SNPs in ABCG5 and ABCG8.

One of the key treatments in alleviating obesity related comorbidities is weight loss. This is the case with CVD where weight loss has been shown to result in cardioprotective

changes in blood lipid concentrations (Dattilo et al., 1992b). Despite the established benefits of weight loss, the kinetics and regulation of cholesterol levels is not known. The characterization of cholesterol trafficking was investigated in this thesis through the inclusion of women.

In observing favourable changes in cholesterol levels, we set out to determine the kinetics behind these changes. Stable isotope ratio techniques were used to examine cholesterol absorption and turnover, and deuterium incorporation was used to determine synthesis rates. This thesis was the first to apply these techniques to the examination of cholesterol kinetics with respect to weight loss. Other studies that examined the effects of weight loss on cholesterol metabolism in people with type 2 diabetes used serum sterols as indicators of absorption and synthesis. There is still concern in using sterols as indicators of cholesterol absorption in situations where dietary plant sterol intake may be altered (Matthan et al., 2004). Additionally, the dual isotope ratio method allows for the quantification of absorption, synthesis, and turnover, while serum sterols indicate changes relative to the levels that were initially present. Use of the dual isotope method provides an accurate determination of changes in cholesterol kinetics in response to weight loss. Significant weight loss in this thesis resulted in decreases in cholesterol synthesis without any changes in absorption or turnover. Though not directly examined, it is postulated that increased mobilization of adipose tissue stores of cholesterol due to weight loss may provide negative feedback to HMG CoA reductase resulting in decreased rates of synthesis and consequently, lower concentrations of blood cholesterol (Dattilo et al., 1992b). The previously observed presence of enlarged livers and elevated HMG CoA reductase suggest that any changes in absorption may be secondary to higher synthesis rates. Differences in cholesterol absorption after weight loss may not have been seen in this study due to high inter subject variability, or other extraneous factors such as variations in body composition changes or in SNPs of ABCG5 and ABCG8. Indeed, the data indicate that variations in cholesterol absorption were positively related to changes in SM and that changes in cholesterol turnover were negatively related to differences in VAT. Additionally, the data provide evidence that the Q604E SNP in ABCG5 and the D19H SNP in ABCG8 were related to alterations in cholesterol kinetics. These

observations suggest that development of a genetic screening tool that includes SNPs of ABCG5 and ABCG8 may be beneficial in developing a more aggressive treatment approach to dyslipidemia in overweight individuals undergoing weight loss. In looking at the overall picture of cholesterol kinetics, these findings suggest that more aggressive treatment of dyslipidemia in individuals undergoing weight loss might include agents that inhibit absorption. Future studies that examine whether incorporation of absorption inhibitors with weight loss would further reduce cholesterol levels need to be conducted to confirm this hypothesis.

The investigation of weight related changes in cholesterol trafficking in women adds a unique aspect to this study, since most studies in this area have focussed on men. The tendency toward central distribution of adipose tissue in men and lower body distribution in women may indicate a potential for gender-related differences in cholesterol kinetics since central adipose tissue is thought to be more metabolically active. As well, the concentrations of hormones such as adiponectin have been shown to be higher in women compared to men (Nishizawa et al., 2002). As a result of these differences, variations may exist in how weight loss affects cholesterol trafficking in women. Unfortunately, the number of females included in the study did not provide enough power to diversify the parameters based on menopausal status. Therefore, future studies may aim to further part out discrepancies between pre and postmenopausal women.

This thesis did not compare the effects of weight loss on various parameters to a constant weight control group. A meta-analysis by Datillo and Kris-Etherton (1992b) showed that changes in lipids by weight loss were not dependent on a control group, therefore, exclusion of a control group in this thesis was justified.

A unique aspect of this research was the attempt to isolate effects of weight loss and minimize the effect of diet and exercise treatments on measured parameters through the inclusion of two-week pre- and post- weight loss stabilization periods. Two-weeks were chosen as the duration of the stabilization periods because previous studies indicate that changes in lipid levels occur within two weeks after commencing a new dietary period

(Hodson et al., 2002). Several other groups have also incorporated washout and run-in periods lasting two weeks in duration in experiments that examine changes in cholesterol levels (Binkoski et al., 2005; McCombs et al., 1994; Sacks et al., 1981). A longer period was not used because it was suspected that, since subjects had to return to pre-weight loss diet and physical activity habits, lower compliance might occur during post loss stabilization due to fear of weight regain. Repeated measures ANOVA on cholesterol levels indicated that subjects were in a steady state. Weights taken during stabilization remained within 1 kg of the average weight during the period. Therefore, stability was achieved for cholesterol levels and body weight throughout both these periods.

The findings from this research add to our current knowledge of obesity-related dyslipidemia by elucidating how significant moderate weight loss in overweight and obese hyperlipidemic women affects cholesterol levels and metabolism, and characterizing factors that may influence these changes. Observations that weight related decreases in blood lipid levels may be attributed to reductions in cholesterol synthesis suggest that more aggressive treatment of dyslipidemia in overweight and obese women should include therapies that target cholesterol absorption such as plant sterols and ezetimibe. Use of therapies that target cholesterol absorption might also be considered with weight loss protocols that aim to decrease AT while maintaining or increases SM, since increases in SM were associated with increases in absorption. This research project also found that SNPs in ABCG5 and ABCG8, specifically the D19H, C54Y, and Q604E polymorphisms, may be associated with weight related response of cholesterol levels and metabolism. Knowing how such polymorphisms affect cholesterol kinetics will help in the development of genetic screening tools that will result in more individualized treatment approaches to lower blood lipid risk factors of CVD. Thus, understanding the mechanism associated with cholesterol trafficking in obesity will help in the development of more effective treatment approaches for dyslipidemia in these individuals.

REFERENCES

Aberle, J., Evans, D., Beil, F. U., & Seedorf, U. (2005). A polymorphism in the apolipoprotein A5 gene is associated with weight loss after short-term diet. *Clin Genet, 68*(2), 152-154.

Allison, D. B., Zannolli, R., Faith, M. S., Heo, M., Pietrobelli, A., VanItallie, T. B., Pi-Sunyer, F. X., & Heymsfield, S. B. (1999). Weight loss increases and fat loss decreases all-cause mortality rate: results from two independent cohort studies. *Int J Obes Relat Metab Disord, 23*(6), 603-611.

Altmann, S. W., Davis, H. R., Jr., Yao, X., Laverty, M., Compton, D. S., Zhu, L. J., Crona, J. H., Caplen, M. A., Hoos, L. M., Tetzloff, G., Priestley, T., Burnett, D. A., Strader, C. D., & Graziano, M. P. (2002). The identification of intestinal scavenger receptor class B, type I (SR-BI) by expression cloning and its role in cholesterol absorption. *Biochimica et Biophysica Acta, 1580*(1), 77-93.

Altmann, S. W., Davis, H. R., Jr., Zhu, L. J., Yao, X., Hoos, L. M., Tetzloff, G., Iyer, S. P., Maguire, M., Golovko, A., Zeng, M., Wang, L., Murgolo, N., & Graziano, M. P. (2004). Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science, 303*(5661), 1201-1204.

The American Dietetic Association and American Diabetes Association. Exchange Lists for Meal Planning. (1995).). Chicago, Ill: American Dietetic Association.

Andersen, R. E., Franckowiak, S. C., Bartlett, S. J., & Fontaine, K. R. (2002). Physiologic changes after diet combined with structured aerobic exercise or lifestyle activity. *Metabolism, 51*(12), 1528-1533.

Andersen, R. E., Wadden, T. A., Bartlett, S. J., Vogt, R. A., & Weinstock, R. S. (1995). Relation of weight loss to changes in serum lipids and lipoproteins in obese women. *Am J Clin Nutr, 62*(2), 350-357.

Andersen, R. E., Wadden, T. A., Bartlett, S. J., Zemel, B., Verde, T. J., & Franckowiak, S. C. (1999). Effects of lifestyle activity vs structured aerobic exercise in obese women: a randomized trial. *Jama, 281*(4), 335-340.

Anderson, A. S. (2000). How to implement dietary changes to prevent the development of metabolic syndrome. *Br J Nutr, 83 Suppl 1*, S165-168.

Anderson, J. W., & Konz, E. C. (2001). Obesity and disease management: effects of weight loss on comorbid conditions. *Obes Res, 9 Suppl 4*, 326S-334S.

Angel, A., & Bray, G. A. (1979). Synthesis of fatty acids and cholesterol by liver, adipose tissue and intestinal mucosa from obese and control patients. *Eur J Clin Invest, 9*(5), 355-362.

Angelin, B., Backman, L., Einarsson, K., Eriksson, L., & Ewerth, S. (1982). Hepatic cholesterol metabolism in obesity: activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J Lipid Res, 23*(5), 770-773.

Archer, W. R., Lamarche, B., St-Pierre, A. C., Mauger, J. F., Deriaz, O., Landry, N., Corneau, L., Despres, J. P., Bergeron, J., Couture, P., & Bergeron, N. (2003). High carbohydrate and high monounsaturated fatty acid diets similarly affect LDL electrophoretic characteristics in men who are losing weight. *J Nutr, 133*(10), 3124-3129.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T., & Matsuzawa, Y. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun, 257*(1), 79-83.

Austin, M. A., Breslow, J. L., Hennekens, C. H., Buring, J. E., Willett, W. C., & Krauss, R. M. (1988). Low-density lipoprotein subclass patterns and risk of myocardial infarction. *Jama, 260*(13), 1917-1921.

Baratta, R., Amato, S., Degano, C., Farina, M. G., Patane, G., Vigneri, R., & Frittitta, L. (2004). Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. *J Clin Endocrinol Metab, 89*(6), 2665-2671.

Beard, C. M., Barnard, R. J., Robbins, D. C., Ordovas, J. M., & Schaefer, E. J. (1996). Effects of diet and exercise on qualitative and quantitative measures of LDL and its susceptibility to oxidation. *Arterioscler Thromb Vasc Biol, 16*(2), 201-207.

Berg, A. H., Combs, T. P., Du, X., Brownlee, M., & Scherer, P. E. (2001). The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med, 7*(8), 947-953.

Berg, A. H., Combs, T. P., & Scherer, P. E. (2002). ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab, 13*(2), 84-89.

Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R., & Hobbs, H. H. (2000). Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science, 290*(5497), 1771-1775.

Berge, K. E., von Bergmann, K., Lutjohann, D., Guerra, R., Grundy, S. M., Hobbs, H. H., & Cohen, J. C. (2002). Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *Journal of Lipid Research, 43*(3), 486-494.

Binkoski, A. E., Kris-Etherton, P. M., Wilson, T. A., Mountain, M. L., & Nicolosi, R. J. (2005). Balance of unsaturated fatty acids is important to a cholesterol-lowering diet: comparison of mid-oleic sunflower oil and olive oil on cardiovascular disease risk factors. *J Am Diet Assoc, 105*(7), 1080-1086.

Bjornheden, T., Babyi, A., Bondjers, G., & Wiklund, O. (1996). Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis, 123*(1-2), 43-56.

Boomsma, D. I., Princen, H. M., Frants, R. R., Gevers Leuven, J. A., & Kempen, H. J. (2003). Genetic analysis of indicators of cholesterol synthesis and absorption: lathosterol and phytosterols in Dutch twins and their parents. *Twin Research, 6*(4), 307-314.

Bosner, M. S., Lange, L. G., Stenson, W. F., & Ostlund, R. E., Jr. (1999a). Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J Lipid Res, 40*(2), 302-308.

---. (1999b). Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *Journal of Lipid Research, 40*(2), 302-308.

Bosner, M. S., Ostlund, R. E., Jr., Osofisan, O., Grosklos, J., Fritschle, C., & Lange, L. G. (1993). Assessment of percent cholesterol absorption in humans with stable isotopes. *J Lipid Res, 34*(6), 1047-1053.

Brook, R. D., Bard, R. L., Glazewski, L., Kehrer, C., Bodary, P. F., Eitzman, D. L., & Rajagopalan, S. (2004). Effect of short-term weight loss on the metabolic syndrome and conduit vascular endothelial function in overweight adults. *Am J Cardiol, 93*(8), 1012-1016.

Buhman, K. K., Accad, M., Novak, S., Choi, R. S., Wong, J. S., Hamilton, R. L., Turley, S., & Farese, R. V., Jr. (2000). Resistance to diet-induced hypercholesterolemia and gallstone formation in ACAT2-deficient mice. *Nature Medicine, 6*(12), 1341-1347.

Canadian Diabetes Association. (1999). *Good Health Eating Guide Resource.*

Carter, C. P., Howles, P. N., & Hui, D. Y. (1997). Genetic variation in cholesterol absorption efficiency among inbred strains of mice. *Journal of Nutrition, 127*(7), 1344-1348.

Cella, L. K., Van Cauter, E., & Schoeller, D. A. (1995). Effect of meal timing on diurnal rhythm of human cholesterol synthesis. *American Journal of Physiology, 269*(5 Pt 1), E878-883.

Chiang, J. Y. (2004). Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms. *Journal of Hepatology, 40*(3), 539-551.

Cleland R, G. D., Hubbard V, Kahn LK, Stern JS, Wadden TA, Weinsier R, Yanovski S. (1997). *Commercial Weight Loss Products and Programs What Consumers Stand To Gain and Lose*, Washington, DC.

Cleroux, J., Feldman, R. D., & Petrella, R. J. (1999). Lifestyle modifications to prevent and control hypertension. 4. Recommendations on physical exercise training. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Centre for Disease Control at Health Canada, Heart and Stroke Foundation of Canada. *Cmaj, 160*(9 Suppl), S21-28.

Cohen, J. C., Pertsemlidis, A., Fahmi, S., Esmail, S., Vega, G. L., Grundy, S. M., & Hobbs, H. H. (2006). Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proceedings of the National Academy of Sciences of the United States of America, 103*(6), 1810-1815.

Connor, W. E., Hodges, R. E., & Bleiler, R. E. (1961). Effect of dietary cholesterol upon serum lipids in man. *J Lab Clin Med, 57*, 331-342.

Connor, W. E., Stone, D. B., & Hodges, R. E. (1964). The Interrelated Effects of Dietary Cholesterol and Fat Upon Human Serum Lipid Levels. *J Clin Invest, 43*, 1691-1696.

Contois, J., McNamara, J. R., Lammi-Keefe, C., Wilson, P. W., Massov, T., & Schaefer, E. J. (1996a). Reference intervals for plasma apolipoprotein A-1 determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem, 42*(4), 507-514.

Contois, J. H., McNamara, J. R., Lammi-Keefe, C. J., Wilson, P. W., Massov, T., & Schaefer, E. J. (1996b). Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem, 42*(4), 515-523.

Cui, Y., Blumenthal, R. S., Flaws, J. A., Whiteman, M. K., Langenberg, P., Bachorik, P. S., & Bush, T. L. (2001). Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. *Arch Intern Med, 161*(11), 1413-1419.

Cummings, S., Parham, E. S., & Strain, G. W. (2002). Position of the American Dietetic Association: weight management. *J Am Diet Assoc, 102*(8), 1145-1155.

Dattilo, A. M., & Kris-Etherton, P. M. (1992a). Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *American Journal of Clinical Nutrition, 56*(2), 320-328.

---. (1992b). Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am J Clin Nutr, 56*(2), 320-328.

Davies, J. P., Scott, C., Oishi, K., Liapis, A., & Ioannou, Y. A. (2005). Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. *J Biol Chem, 280*(13), 12710-12720.

Daviglus, M. L., Liu, K., Pirzada, A., Yan, L. L., Garside, D. B., Feinglass, J., Guralnik, J. M., Greenland, P., & Stamler, J. (2003). Favorable cardiovascular risk profile in middle age and health-related quality of life in older age. *Arch Intern Med, 163*(20), 2460-2468.

Davis, H. R., Jr., Zhu, L. J., Hoos, L. M., Tetzloff, G., Maguire, M., Liu, J., Yao, X., Iyer, S. P., Lam, M. H., Lund, E. G., Detmers, P. A., Graziano, M. P., & Altmann, S. W. (2004). Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *Journal of Biological Chemistry, 279*(32), 33586-33592.

DeFronzo, R. A., & Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care, 14*(3), 173-194.

Desroches, S., & Lamarche, B. (2004). Diet and low-density lipoprotein particle size. *Curr Atheroscler Rep, 6*(6), 453-460.

Di Buono, M., Hannah, J. S., Katzel, L. I., & Jones, P. J. (1999a). Weight loss due to energy restriction suppresses cholesterol biosynthesis in overweight, mildly hypercholesterolemic men. *J Nutr, 129*(8), 1545-1548.

---. (1999b). Weight loss due to energy restriction suppresses cholesterol biosynthesis in overweight, mildly hypercholesterolemic men. *Journal of Nutrition, 129*(8), 1545-1548.

Dietschy, J. M., & Siperstein, M. D. (1967). Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *Journal of Lipid Research, 8*(2), 97-104.

Dietschy, J. M., Turley, S. D., & Spady, D. K. (1993). Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res, 34*(10), 1637-1659.

Diez, J. J., & Iglesias, P. (2003). The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol, 148*(3), 293-300.

Donnelly, J. E., Jacobsen, D. J., Heelan, K. S., Seip, R., & Smith, S. (2000). The effects of 18 months of intermittent vs. continuous exercise on aerobic capacity, body weight and composition, and metabolic fitness in previously sedentary, moderately obese females. *Int J Obes Relat Metab Disord, 24*(5), 566-572.

Donnelly, J. E., & Smith, B. K. (2005). Is exercise effective for weight loss with ad libitum diet? Energy balance, compensation, and gender differences. *Exerc Sport Sci Rev, 33*(4), 169-174.

Duan, L. P., Wang, H. H., Ohashi, A., & Wang, D. Q. (2006). Role of intestinal sterol transporters Abcg5, Abcg8, and Npc111 in cholesterol absorption in mice: gender and age effects. *Am J Physiol Gastrointest Liver Physiol, 290*(2), G269-276.

Edwards, P. A., Muroya, H., & Gould, R. G. (1972). In vivo demonstration of the circadian thythm of cholesterol biosynthesis in the liver and intestine of the rat. *Journal of Lipid Research, 13*(3), 396-401.

Elosua, R., Molina, L., Fito, M., Arquer, A., Sanchez-Quesada, J. L., Covas, M. I., Ordonez-Llanos, J., & Marrugat, J. (2003). Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis, 167*(2), 327-334.

Esposito, K., Pontillo, A., Di Palo, C., Giugliano, G., Masella, M., Marfella, R., & Giugliano, D. (2003). Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *Jama, 289*(14), 1799-1804.

Evans, E. M., Saunders, M. J., Spano, M. A., Arngrimsson, S. A., Lewis, R. D., & Cureton, K. J. (1999). Effects of diet and exercise on the density and composition of the fat-free mass in obese women. *Med Sci Sports Exerc, 31*(12), 1778-1787.

Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). (2001). *Journal of the American Medical Association, 285*(19), 2486-2497.

Farnsworth, E., Luscombe, N. D., Noakes, M., Wittert, G., Argyiou, E., & Clifton, P. M. (2003). Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr, 78*(1), 31-39.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem, 226*(1), 497-509.

Frankenfield, D., Roth-Yousey, L., & Compher, C. (2005). Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: a systematic review. *J Am Diet Assoc, 105*(5), 775-789.

Franz, M. J., Bantle, J. P., Beebe, C. A., Brunzell, J. D., Chiasson, J. L., Garg, A., Holzmeister, L. A., Hoogwerf, B., Mayer-Davis, E., Mooradian, A. D., Purnell, J. Q., & Wheeler, M. (2004). Nutrition principles and recommendations in diabetes. *Diabetes Care, 27 Suppl 1*, S36-46.

Galeano, N. F., Al-Haideri, M., Keyserman, F., Rumsey, S. C., & Deckelbaum, R. J. (1998). Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *J Lipid Res, 39*(6), 1263-1273.

Garg, A. (2004). Acquired and inherited lipodystrophies. *N Engl J Med, 350*(12), 1220-1234.

Garg, A., & Misra, A. (2004). Lipodystrophies: rare disorders causing metabolic syndrome. *Endocrinol Metab Clin North Am, 33*(2), 305-331.

Gerhard, G. T., Ahmann, A., Meeuws, K., McMurry, M. P., Duell, P. B., & Connor, W. E. (2004). Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. *Am J Clin Nutr, 80*(3), 668-673.

Goodman, D. S., Noble, R. P., & Dell, R. B. (1973). Three-pool model of the long-term turnover of plasma cholesterol in man. *J Lipid Res, 14*(2), 178-188.

Gremaud, G., Piguet, C., Baumgartner, M., Pouteau, E., Decarli, B., Berger, A., & Fay, L. B. (2001a). Simultaneous assessment of cholesterol absorption and synthesis in humans using on-line gas chromatography/ combustion and gas chromatography/pyrolysis/isotope-ratio mass spectrometry. *Rapid Communications in Mass Spectrometry, 15*(14), 1207-1213.

---. (2001b). Simultaneous assessment of cholesterol absorption and synthesis in humans using on-line gas chromatography/ combustion and gas chromatography/pyrolysis/isotope-ratio mass spectrometry. *Rapid Commun Mass Spectrom, 15*(14), 1207-1213.

Griffin, M., Frazer, A., Johnson, A., Collins, P., Owens, D., & Tomkin, G. H. (1998). Cellular cholesterol synthesis--the relationship to post-prandial glucose and insulin following weight loss. *Atherosclerosis, 138*(2), 313-318.

Grundy, S. M., & Ahrens, E. H., Jr. (1969a). Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *Journal of Lipid Research, 10*(1), 91-107.

Grundy, S. M., Ahrens, E. H., Jr., & Davignon, J. (1969b). The interaction of cholesterol absorption and cholesterol synthesis in man. *J Lipid Res, 10*(3), 304-315.

Gylling, H., Hallikainen, M., Pihlajamaki, J., Agren, J., Laakso, M., Rajaratnam, R. A., Rauramaa, R., & Miettinen, T. A. (2004a). Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *Journal of Lipid Research, 45*(9), 1660-1665.

---. (2004b). Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *J Lipid Res, 45*(9), 1660-1665.

Gylling, H., Kontula, K., & Miettinen, T. A. (1995). Cholesterol absorption and metabolism and LDL kinetics in healthy men with different apoprotein E phenotypes and apoprotein B Xba I and LDL receptor Pvu II genotypes. *Arteriosclerosis, Thrombosis and Vascular Biology, 15*(2), 208-213.

Gylling, H., & Miettinen, T. A. (2002a). Baseline intestinal absorption and synthesis of cholesterol regulate its response to hypolipidaemic treatments in coronary patients. *Atherosclerosis, 160*(2), 477-481.

---. (2002b). Inheritance of cholesterol metabolism of probands with high or low cholesterol absorption. *Journal of Lipid Research, 43*(9), 1472-1476.

Gylling, H., Radhakrishnan, R., & Miettinen, T. A. (1997). Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine: women and dietary sitostanol. *Circulation, 96*(12), 4226-4231.

Halaas, J. L., Gajiwala, K. S., Maffei, M., Cohen, S. L., Chait, B. T., Rabinowitz, D., Lallone, R. L., Burley, S. K., & Friedman, J. M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. *Science, 269*(5223), 543-546.

Halle, M., Berg, A., Garwers, U., Baumstark, M. W., Knisel, W., Grathwohl, D., Konig, D., & Keul, J. (1999). Influence of 4 weeks' intervention by exercise and diet on low-density lipoprotein subfractions in obese men with type 2 diabetes. *Metabolism, 48*(5), 641-644.

Hamprecht, B., Nussler, C., & Lynen, F. (1969). Rhythmic changes of hydroxymethylglutaryl coenzyme a reductase activity in livers of fed and fasted rats. *FEBS Letters, 4*(2), 117-121.

Hansen, D., Astrup, A., Toubro, S., Finer, N., Kopelman, P., Hilsted, J., Rossner, S., Saris, W., Van Gaal, L., James, W., & Goulder For The, S. S. G. M. (2001). Predictors of weight loss and maintenance during 2 years of treatment by sibutramine in obesity. Results from the European multi-centre STORM trial. Sibutramine Trial of Obesity Reduction and Maintenance. *Int J Obes Relat Metab Disord, 25*(4), 496-501.

Hansen, T. K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J. S., & Jorgensen, J. O. (2002). Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol (Oxf), 56*(2), 203-206.

Harris, J. A., Benedict, F. G. (1919). A biometric study of the basal metabolism in man., Publication No. 279.

Hazard, S. E., & Patel, S. B. (2006). Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. *Pflugers Arch*, 1-8.

Health Canada. (1992). *Canada's Food Guide to Healthy Eating.*

Hedley, A. A., Ogden, C. L., Johnson, C. L., Carroll, M. D., Curtin, L. R., & Flegal, K. M. (2004). Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *Jama, 291*(23), 2847-2850.

Hegele, R. A., Guy, J., Ban, M. R., & Wang, J. (2005). NPC1L1 haplotype is associated with inter-individual variation in plasma low-density lipoprotein response to ezetimibe. *Lipids Health Dis, 4*, 16.

Ho, K. J. (1975). Effect of cholesterol feeding on circadian rhythm of hepatic and intestinal cholesterol biosynthesis in hamsters. *Proceedings of the Society for Experimental Biology and Medicine, 150*(2), 271-277.

---. (1979). Circadian rhythm of cholesterol biosynthesis: dietary regulation in the liver and small intestine of hamsters. *International Journal of Chronobiology, 6*(1), 39-50.

Hoag, S., Marshall, J. A., Jones, R. H., & Hamman, R. F. (1995). High fasting insulin levels associated with lower rates of weight gain in persons with normal glucose

tolerance: the San Luis Valley Diabetes Study. *Int J Obes Relat Metab Disord, 19*(3), 175-180.

Hodson, L., Skeaff, C. M., & McKenzie, J. E. (2002). Maximal response to a plasma cholesterol-lowering diet is achieved within two weeks. *Nutr Metab Cardiovasc Dis, 12*(5), 291-295.

Hopkins, P. N. (1992). Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. *Am J Clin Nutr, 55*(6), 1060-1070.

Howard, B. V., & Howard, W. J. (1994). Dyslipidemia in non-insulin-dependent diabetes mellitus. *Endocr Rev, 15*(3), 263-274.

Hubacek, J. A., Berge, K. E., Cohen, J. C., & Hobbs, H. H. (2001). Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. *Hum Mutat, 18*(4), 359-360.

Ishida, H., Yamashita, C., Kuruta, Y., Yoshida, Y., & Noshiro, M. (2000). Insulin is a dominant suppressor of sterol 12 alpha-hydroxylase P450 (CYP8B) expression in rat liver: possible role of insulin in circadian rhythm of CYP8B. *Journal of Biochemistry (Tokyo), 127*(1), 57-64.

Jakulj, L., Trip, M. D., Sudhop, T., von Bergmann, K., Kastelein, J. J., & Vissers, M. N. (2005). Inhibition of cholesterol absorption by the combination of dietary plant sterols and ezetimibe: effects on plasma lipid levels. *Journal of Lipid Research, 46*(12), 2692-2698.

James, D. E., & Piper, R. C. (1994). Insulin resistance, diabetes, and the insulin-regulated trafficking of GLUT-4. *J Cell Biol, 126*(5), 1123-1126.

Janssen, I., Fortier, A., Hudson, R., & Ross, R. (2002). Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care, 25*(3), 431-438.

Janssen, I., & Ross, R. (1999). Effects of sex on the change in visceral, subcutaneous adipose tissue and skeletal muscle in response to weight loss. *Int J Obes Relat Metab Disord, 23*(10), 1035-1046.

Jeffery, R. W., Wing, R. R., Sherwood, N. E., & Tate, D. F. (2003). Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr, 78*(4), 684-689.

Jeu, L., & Cheng, J. W. (2003). Pharmacology and therapeutics of ezetimibe (SCH 58235), a cholesterol-absorption inhibitor. *Clinical Therapeutics, 25*(9), 2352-2387.

Jolley, C. D., Dietschy, J. M., & Turley, S. D. (1999). Genetic differences in cholesterol absorption in 129/Sv and C57BL/6 mice: effect on cholesterol responsiveness. *American Journal of Physiology, 276*(5 Pt 1), G1117-1124.

Jones, P. J., Leitch, C. A., Li, Z. C., & Connor, W. E. (1993a). Human cholesterol synthesis measurement using deuterated water. Theoretical and procedural considerations. *Arterioscler Thromb, 13*(2), 247-253.

Jones, P. J., Main, B. F., & Frohlich, J. J. (1993b). Response of cholesterol synthesis to cholesterol feeding in men with different apolipoprotein E genotypes. *Metabolism, 42*(8), 1065-1071.

Jones, P. J., Pappu, A. S., Hatcher, L., Li, Z. C., Illingworth, D. R., & Connor, W. E. (1996). Dietary cholesterol feeding suppresses human cholesterol synthesis measured by deuterium incorporation and urinary mevalonic acid levels. *Arterioscler Thromb Vasc Biol, 16*(10), 1222-1228.

Jones, P. J., Raeini-Sarjaz, M., Ntanios, F. Y., Vanstone, C. A., Feng, J. Y., & Parsons, W. E. (2000a). Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *Journal of Lipid Research, 41*(5), 697-705.

---. (2000b). Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *J Lipid Res, 41*(5), 697-705.

Jones, P. J., & Schoeller, D. A. (1990). Evidence for diurnal periodicity in human cholesterol synthesis. *Journal of Lipid Research, 31*(4), 667-673.

Jurevics, H., Hostettler, J., Barrett, C., Morell, P., & Toews, A. D. (2000). Diurnal and dietary-induced changes in cholesterol synthesis correlate with levels of mRNA for HMG-CoA reductase. *Journal of Lipid Research, 41*(7), 1048-1054.

Kajinami, K., Brousseau, M. E., Nartsupha, C., Ordovas, J. M., & Schaefer, E. J. (2004a). ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. *J Lipid Res, 45*(4), 653-656.

Kajinami, K., Brousseau, M. E., Ordovas, J. M., & Schaefer, E. J. (2004b). Interactions between common genetic polymorphisms in ABCG5/G8 and CYP7A1 on LDL cholesterol-lowering response to atorvastatin. *Atherosclerosis, 175*(2), 287-293.

Kamel, E. G., McNeill, G., & Van Wijk, M. C. (2000). Change in intra-abdominal adipose tissue volume during weight loss in obese men and women: correlation between magnetic resonance imaging and anthropometric measurements. *Int J Obes Relat Metab Disord, 24*(5), 607-613.

Kang, H. S., Gutin, B., Barbeau, P., Owens, S., Lemmon, C. R., Allison, J., Litaker, M. S., & Le, N. A. (2002). Physical training improves insulin resistance syndrome markers in obese adolescents. *Med Sci Sports Exerc, 34*(12), 1920-1927.

Katan, M. B., Grundy, S. M., Jones, P., Law, M., Miettinen, T., & Paoletti, R. (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc, 78*(8), 965-978.

Katch F, M. W. e. (1993). *Nutrition, weight control, and exercise.* Philadelphia: Lea and Febiger.

Katzel, L. I., Coon, P. J., Rogus, E., Krauss, R. M., & Goldberg, A. P. (1995). Persistence of low HDL-C levels after weight reduction in older men with small LDL particles. *Arterioscler Thromb Vasc Biol, 15*(3), 299-305.

Katzmarzyk, P. T., & Janssen, I. (2004). The economic costs associated with physical inactivity and obesity in Canada: an update. *Can J Appl Physiol, 29*(1), 90-115.

Kazumi, T., Kawaguchi, A., Hirano, T., & Yoshino, G. (2004). Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. *Metabolism, 53*(5), 589-593.

Kempen, H. J., Glatz, J. F., Gevers Leuven, J. A., van der Voort, H. A., & Katan, M. B. (1988). Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *Journal of Lipid Research, 29*(9), 1149-1155.

Kesaniemi, Y. A., Ehnholm, C., & Miettinen, T. A. (1987a). Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *Journal of Clinical Investigation, 80*(2), 578-581.

Kesaniemi, Y. A., & Miettinen, T. A. (1987b). Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur J Clin Invest, 17*(5), 391-395.

Kestin, M., Clifton, P. M., Rouse, I. L., & Nestel, P. J. (1989). Effect of dietary cholesterol in normolipidemic subjects is not modified by nature and amount of dietary fat. *Am J Clin Nutr, 50*(3), 528-532.

Keys, A. (1984). Serum cholesterol response to dietary cholesterol. *Am J Clin Nutr, 40*(2), 351-359.

Keys, A., Anderson, J. T., Mickelsen, O., Adelson, S. F., & Fidanza, F. (1956). Diet and serum cholesterol in man; lack of effect of dietary cholesterol. *J Nutr, 59*(1), 39-56.

Klett, E. L., Lee, M. H., Adams, D. B., Chavin, K. D., & Patel, S. B. (2004). Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine. *BMC Gastroenterol, 4*, 21.

Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Jr., Kris-Etherton, P., Goldberg, I. J., Kotchen, T. A., Lichtenstein, A. H., Mitch, W. E., Mullis, R., Robinson, K., Wylie-Rosett, J., St Jeor, S., Suttie, J., Tribble, D. L., & Bazzarre, T. L. (2000). AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation, 102*(18), 2284-2299.

Kummerow, F. A., Kim, Y., Hull, J., Pollard, J., Ilinov, P., Drossiev, D. L., & Valek, J. (1977). The influence of egg consumption on the serum cholesterol level in human subjects. *Am J Clin Nutr, 30*(5), 664-673.

Kwiterovich, P. O., Jr. (2000). The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. *Am J Cardiol, 86*(12A), 5L-10L.

Lambert, G., Amar, M. J., Guo, G., Brewer, H. B., Jr., Gonzalez, F. J., & Sinal, C. J. (2003). The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *Journal of Biological Chemistry, 278*(4), 2563-2570.

Lapidus, L., Bengtsson, C., Larsson, B., Pennert, K., Rybo, E., & Sjostrom, L. (1984). Distribution of adipose tissue and risk of cardiovascular disease and death: a 12

year follow up of participants in the population study of women in Gothenburg, Sweden. *Br Med J (Clin Res Ed), 289*(6454), 1257-1261.

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I., & Friedman, J. M. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature, 379*(6566), 632-635.

Lee, M. H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A. K., Salen, G., Dean, M., & Patel, S. B. (2001). Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nature Genetics, 27*(1), 79-83.

Leenen, R., van der Kooy, K., Droop, A., Seidell, J. C., Deurenberg, P., Weststrate, J. A., & Hautvast, J. G. (1993). Visceral fat loss measured by magnetic resonance imaging in relation to changes in serum lipid levels of obese men and women. *Arterioscler Thromb, 13*(4), 487-494.

Lennernas, H., & Fager, G. (1997). Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. *Clin Pharmacokinet, 32*(5), 403-425.

Liscum, L. (2002). Biochemistry of Lipids, Lipoproteins and Membranes. In D. Vance, Vance, JE (Ed.) (4th ed., pp. 409-431). Amsterdam, The Netherlands: Elsevier.

Lofgren, I., Zern, T., Herron, K., West, K., Sharman, M. J., Volek, J. S., Shachter, N. S., Koo, S. I., & Fernandez, M. L. (2005a). Weight loss associated with reduced intake of carbohydrate reduces the atherogenicity of LDL in premenopausal women. *Metabolism, 54*(9), 1133-1141.

Lofgren, I. E., Herron, K. L., West, K. L., Zern, T. L., Brownbill, R. A., Ilich, J. Z., Koo, S. I., & Fernandez, M. L. (2005b). Weight loss favorably modifies anthropometrics and reverses the metabolic syndrome in premenopausal women. *J Am Coll Nutr, 24*(6), 486-493.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., & et al. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med, 1*(11), 1155-1161.

Mardones, P., Quinones, V., Amigo, L., Moreno, M., Miquel, J. F., Schwarz, M., Miettinen, H. E., Trigatti, B., Krieger, M., VanPatten, S., Cohen, D. E., & Rigotti, A. (2001). Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. *Journal of Lipid Research, 42*(2), 170-180.

Markovic, T. P., Campbell, L. V., Balasubramanian, S., Jenkins, A. B., Fleury, A. C., Simons, L. A., & Chisholm, D. J. (1998). Beneficial effect on average lipid levels from energy restriction and fat loss in obese individuals with or without type 2 diabetes. *Diabetes Care, 21*(5), 695-700.

Martin, A. D., Daniel, M. Z., Drinkwater, D. T., & Clarys, J. P. (1994). Adipose tissue density, estimated adipose lipid fraction and whole body adiposity in male cadavers. *Int J Obes Relat Metab Disord, 18*(2), 79-83.

Matthan, N. R., & Lichtenstein, A. H. (2004). Approaches to measuring cholesterol absorption in humans. *Atherosclerosis, 174*(2), 197-205.

Mazzone, T., Foster, D., & Chait, A. (1984). In vivo stimulation of low-density lipoprotein degradation by insulin. *Diabetes, 33*(4), 333-338.

McCombs, R. J., Marcadis, D. E., Ellis, J., & Weinberg, R. B. (1994). Attenuated hypercholesterolemic response to a high-cholesterol diet in subjects heterozygous for the apolipoprotein A-IV-2 allele. *N Engl J Med, 331*(11), 706-710.

McGuigan, F. E., & Ralston, S. H. (2002). Single nucleotide polymorphism detection: allelic discrimination using TaqMan. *Psychiatr Genet, 12*(3), 133-136.

McLaughlin, T., Abbasi, F., Kim, H. S., Lamendola, C., Schaaf, P., & Reaven, G. (2001). Relationship between insulin resistance, weight loss, and coronary heart disease risk in healthy, obese women. *Metabolism, 50*(7), 795-800.

McNamara, D. J., Kolb, R., Parker, T. S., Batwin, H., Samuel, P., Brown, C. D., & Ahrens, E. H., Jr. (1987a). Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quantity. *J Clin Invest, 79*(6), 1729-1739.

McNamara, J. R., & Schaefer, E. J. (1987b). Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin Chim Acta, 166*(1), 1-8.

Mel'nikov, S. M., Seijen ten Hoorn, J. W., & Eijkelenboom, A. P. (2004). Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: an in vitro study. *Chemistry and Physics of Lipids, 127*(2), 121-141.

Miettinen, T. A. (1982). Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins. *Journal of Lipid Research, 23*(3), 466-473.

Miettinen, T. A., & Gylling, H. (2000a). Cholesterol absorption efficiency and sterol metabolism in obesity. *Atherosclerosis, 153*(1), 241-248.

---. (2002). Ineffective decrease of serum cholesterol by simvastatin in a subgroup of hypercholesterolemic coronary patients. *Atherosclerosis, 164*(1), 147-152.

---. (2003a). Cholesterol synthesis and absorption in coronary patients with lipid triad and isolated high LDL cholesterol in a 4S subgroup. *Atherosclerosis, 168*(2), 343-349.

---. (2003b). Synthesis and absorption markers of cholesterol in serum and lipoproteins during a large dose of statin treatment. *European Journal of Clinical Investigation, 33*(11), 976-982.

Miettinen, T. A., Gylling, H., Lindbohm, N., Miettinen, T. E., Rajaratnam, R. A., & Relas, H. (2003c). Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. *J Lab Clin Med, 141*(2), 131-137.

---. (2003d). Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. *The Journal of Laboratory and Clinical Medicine, 141*(2), 131-137.

Miettinen, T. A., Gylling, H., Vanhanen, H., & Ollus, A. (1992). Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arteriosclerosis and Thrombosis, 12*(9), 1044-1052.

Miettinen, T. A., & Kesaniemi, Y. A. (1989). Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. *Am J Clin Nutr, 49*(4), 629-635.

Miettinen, T. A., Strandberg, T. E., & Gylling, H. (2000b). Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. *Arteriosclerosis, Thrombosis and Vascular Biology, 20*(5), 1340-1346.

Miettinen, T. A., Tilvis, R. S., & Kesaniemi, Y. A. (1990). Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *American Journal of Epidemiology, 131*(1), 20-31.

Miller, E. R., 3rd, Erlinger, T. P., Young, D. R., Jehn, M., Charleston, J., Rhodes, D., Wasan, S. K., & Appel, L. J. (2002). Results of the Diet, Exercise, and Weight Loss Intervention Trial (DEW-IT). *Hypertension, 40*(5), 612-618.

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res, 16*(3), 1215.

Miller, W. C., Koceja, D. M., & Hamilton, E. J. (1997). A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int J Obes Relat Metab Disord, 21*(10), 941-947.

Miller, W. M., Nori-Janosz, K. E., Lillystone, M., Yanez, J., & McCullough, P. A. (2005). Obesity and lipids. *Curr Cardiol Rep, 7*(6), 465-470.

Miwa, K., Inazu, A., Kobayashi, J., Higashikata, T., Nohara, A., Kawashiri, M., Katsuda, S., Takata, M., Koizumi, J., & Mabuchi, H. (2005). ATP-binding cassette transporter G8 M429V polymorphism as a novel genetic marker of higher cholesterol absorption in hypercholesterolaemic Japanese subjects. *Clin Sci (Lond), 109*(2), 183-188.

Morgan, C. R., & Lazarow, A. (1962). Immunoassay of insulin using a two-antibody system. *Proc Soc Exp Biol Med, 110*, 29-32.

Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G., & Dietz, W. H. (1999). The disease burden associated with overweight and obesity. *Jama, 282*(16), 1523-1529.

Nestel, P. J., Schreibman, P. H., & Ahrens, E. H., Jr. (1973). Cholesterol metabolism in human obesity. *J Clin Invest, 52*(10), 2389-2397.

Nieman, D. C., Brock, D. W., Butterworth, D., Utter, A. C., & Nieman, C. C. (2002). Reducing diet and/or exercise training decreases the lipid and lipoprotein risk factors of moderately obese women. *J Am Coll Nutr, 21*(4), 344-350.

Nishizawa, H., Shimomura, I., Kishida, K., Maeda, N., Kuriyama, H., Nagaretani, H., Matsuda, M., Kondo, H., Furuyama, N., Kihara, S., Nakamura, T., Tochino, Y.,

Funahashi, T., & Matsuzawa, Y. (2002). Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes, 51*(9), 2734-2741.

Noakes, M., Foster, P. R., Keogh, J. B., & Clifton, P. M. (2004). Meal replacements are as effective as structured weight-loss diets for treating obesity in adults with features of metabolic syndrome. *J Nutr, 134*(8), 1894-1899.

Noakes, M., Keogh, J. B., Foster, P. R., & Clifton, P. M. (2005). Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *Am J Clin Nutr, 81*(6), 1298-1306.

Nordmann, A. J., Nordmann, A., Briel, M., Keller, U., Yancy, W. S., Jr., Brehm, B. J., & Bucher, H. C. (2006). Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med, 166*(3), 285-293.

O'Neill, F. H., Patel, D. D., Knight, B. L., Neuwirth, C. K., Bourbon, M., Soutar, A. K., Taylor, G. W., Thompson, G. R., & Naoumova, R. P. (2001). Determinants of variable response to statin treatment in patients with refractory familial hypercholesterolemia. *Arteriosclerosis, Thrombosis and Vascular Biology, 21*(5), 832-837.

Olshansky, S. J., Passaro, D. J., Hershow, R. C., Layden, J., Carnes, B. A., Brody, J., Hayflick, L., Butler, R. N., Allison, D. B., & Ludwig, D. S. (2005). A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med, 352*(11), 1138-1145.

Ordovas, J. M., & Tai, E. S. (2002). The babel of the ABCs: novel transporters involved in the regulation of sterol absorption and excretion. *Nutrition Reviews, 60*(1), 30-33.

Ostlund, R. E., Jr., Yang, J. W., Klein, S., & Gingerich, R. (1996). Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab, 81*(11), 3909-3913.

Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Yamashita, S., Funahashi, T., &

Matsuzawa, Y. (1999). Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation, 100*(25), 2473-2476.

Ouchi, N., Kihara, S., Arita, Y., Nishida, M., Matsuyama, A., Okamoto, Y., Ishigami, M., Kuriyama, H., Kishida, K., Nishizawa, H., Hotta, K., Muraguchi, M., Ohmoto, Y., Yamashita, S., Funahashi, T., & Matsuzawa, Y. (2001). Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation, 103*(8), 1057-1063.

Pappu, A. S., & Illingworth, D. R. (1994). Diurnal variations in the plasma concentrations of mevalonic acid in patients with abetalipoproteinaemia. *European Journal of Clinical Investigation, 24*(10), 698-702.

Park, H. S., & Lee, K. (2005). Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. *Diabet Med, 22*(3), 266-272.

Parker, T. S., McNamara, D. J., Brown, C., Garrigan, O., Kolb, R., Batwin, H., & Ahrens, E. H., Jr. (1982). Mevalonic acid in human plasma: relationship of concentration and circadian rhythm to cholesterol synthesis rates in man. *Proceedings of the National Academy of Sciences of the United States of America, 79*(9), 3037-3041.

Peeters, A., Barendregt, J. J., Willekens, F., Mackenbach, J. P., Al Mamun, A., & Bonneux, L. (2003). Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Ann Intern Med, 138*(1), 24-32.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., & Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science, 269*(5223), 540-543.

Pfohl, M., Schreiber, I., Liebich, H. M., Haring, H. U., & Hoffmeister, H. M. (1999). Upregulation of cholesterol synthesis after acute myocardial infarction--is cholesterol a positive acute phase reactant? *Atherosclerosis, 142*(2), 389-393.

Pi-Sunyer, F. X. (2004). The epidemiology of central fat distribution in relation to disease. *Nutr Rev, 62*(7 Pt 2), S120-126.

Pischon, T., Girman, C. J., Hotamisligil, G. S., Rifai, N., Hu, F. B., & Rimm, E. B. (2004). Plasma adiponectin levels and risk of myocardial infarction in men. *Jama, 291*(14), 1730-1737.

Plat, J., Bragt, M. C., & Mensink, R. P. (2005). Common sequence variations in ABCG8 are related to plant sterol metabolism in healthy volunteers. *J Lipid Res, 46*(1), 68-75.

Purnell, J. Q., Kahn, S. E., Albers, J. J., Nevin, D. N., Brunzell, J. D., & Schwartz, R. S. (2000). Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. *J Clin Endocrinol Metab, 85*(3), 977-982.

Purnell, J. Q., Weigle, D. S., Breen, P., & Cummings, D. E. (2003). Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *J Clin Endocrinol Metab, 88*(12), 5747-5752.

Qader, S. S., Lundquist, I., Ekelund, M., Hakanson, R., & Salehi, A. (2005). Ghrelin activates neuronal constitutive nitric oxide synthase in pancreatic islet cells while inhibiting insulin release and stimulating glucagon release. *Regul Pept, 128*(1), 51-56.

Raeini-Sarjaz, M., Vanstone, C. A., Papamandjaris, A. A., Wykes, L. J., & Jones, P. J. (2001). Comparison of the effect of dietary fat restriction with that of energy restriction on human lipid metabolism. *Am J Clin Nutr, 73*(2), 262-267.

Ravussin, E., Tschop, M., Morales, S., Bouchard, C., & Heiman, M. L. (2001). Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *J Clin Endocrinol Metab, 86*(9), 4547-4551.

Ray, K. K., & Cannon, C. P. (2004). Intensive statin therapy in acute coronary syndromes: clinical benefits and vascular biology. *Curr Opin Lipidol, 15*(6), 637-643.

Rebuffe-Scrive, M., Anderson, B., Olbe, L., & Bjorntorp, P. (1990). Metabolism of adipose tissue in intraabdominal depots in severely obese men and women. *Metabolism, 39*(10), 1021-1025.

Riemens, S. C., Van Tol, A., Stulp, B. K., & Dullaart, R. P. (1999). Influence of insulin sensitivity and the TaqIB cholesteryl ester transfer protein gene polymorphism on

plasma lecithin:cholesterol acyltransferase and lipid transfer protein activities and their response to hyperinsulinemia in non-diabetic men. *J Lipid Res, 40*(8), 1467-1474.

Rifai, N., Iannotti, E., DeAngelis, K., & Law, T. (1998). Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg2+ method. *Clin Chem, 44*(6 Pt 1), 1242-1250.

Rizzo, M., & Berneis, K. (2006a). Low-density lipoprotein size and cardiovascular risk assessment. *Qjm, 99*(1), 1-14.

---. (2006b). Should we measure routinely the LDL peak particle size? *Int J Cardiol, 107*(2), 166-170.

Roberts, S. L., McMurry, M. P., & Connor, W. E. (1981). Does egg feeding (i.e., dietary cholesterol) affect plasma cholesterol levels in humans? The results of a double-blind study. *Am J Clin Nutr, 34*(10), 2092-2099.

Ross, R. (1996). Magnetic resonance imaging provides new insights into the characterization of adipose and lean tissue distribution. *Can J Physiol Pharmacol, 74*(6), 778-785.

Ross, R., Pedwell, H., & Rissanen, J. (1995). Effects of energy restriction and exercise on skeletal muscle and adipose tissue in women as measured by magnetic resonance imaging. *Am J Clin Nutr, 61*(6), 1179-1185.

Ross, R., Rissanen, J., Pedwell, H., Clifford, J., & Shragge, P. (1996). Influence of diet and exercise on skeletal muscle and visceral adipose tissue in men. *J Appl Physiol, 81*(6), 2445-2455.

Ryan, A. S., Nicklas, B. J., Berman, D. M., & Elahi, D. (2003). Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. *Int J Obes Relat Metab Disord, 27*(9), 1066-1071.

Sacks, F. M., & Campos, H. (2003). Clinical review 163: Cardiovascular endocrinology: Low-density lipoprotein size and cardiovascular disease: a reappraisal. *J Clin Endocrinol Metab, 88*(10), 4525-4532.

Sacks, F. M., Donner, A., Castelli, W. P., Gronemeyer, J., Pletka, P., Margolius, H. S., Landsberg, L., & Kass, E. H. (1981). Effect of ingestion of meat on plasma cholesterol of vegetarians. *Jama, 246*(6), 640-644.

Salehi, A., Dornonville de la Cour, C., Hakanson, R., & Lundquist, I. (2004). Effects of ghrelin on insulin and glucagon secretion: a study of isolated pancreatic islets and intact mice. *Regul Pept, 118*(3), 143-150.

Santosa S, Demonty, I., Lichtenstein, A.H., Cianflone, K., Jones, P.J.H. (2006). An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr, in press.*

Scaglione, R., Argano, C., Di Chiara, T., & Licata, G. (2004). Obesity and cardiovascular risk: the new public health problem of worldwide proportions. *Expert Rev Cardiovasc Ther, 2*(2), 203-212.

Schwarz, M., Davis, D. L., Vick, B. R., & Russell, D. W. (2001). Genetic analysis of intestinal cholesterol absorption in inbred mice. *Journal of Lipid Research, 42*(11), 1801-1811.

Sehayek, E., Yu, H. J., von Bergmann, K., Lutjohann, D., Stoffel, M., Duncan, E. M., Garcia-Naveda, L., Salit, J., Blundell, M. L., Friedman, J. M., & Breslow, J. L. (2004). Phytosterolemia on the island of Kosrae: founder effect for a novel ABCG8 mutation results in high carrier rate and increased plasma plant sterol levels. *Journal of Lipid Research, 45*(9), 1608-1613.

Shimano, H., Shimomura, I., Hammer, R. E., Herz, J., Goldstein, J. L., Brown, M. S., & Horton, J. D. (1997). Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *Journal of Clinical Investigation, 100*(8), 2115-2124.

Simonen, P., Gylling, H., Howard, A. N., & Miettinen, T. A. (2000). Introducing a new component of the metabolic syndrome: low cholesterol absorption. *Am J Clin Nutr, 72*(1), 82-88.

Simonen, P., Gylling, H., & Miettinen, T. A. (2002a). Acute effects of weight reduction on cholesterol metabolism in obese type 2 diabetes. *Clin Chim Acta, 316*(1-2), 55-61.

---. (2002b). Acute effects of weight reduction on cholesterol metabolism in obese type 2 diabetes. *Clinica Chimica Acta, 316*(1-2), 55-61.

Smith, J. L., Roach, P. D., Wittenberg, L. N., Riottot, M., Pillay, S. P., Nestel, P. J., & Nathanson, L. K. (2000). Effects of simvastatin on hepatic cholesterol metabolism, bile lithogenicity and bile acid hydrophobicity in patients with gallstones. *Journal of Gastroenterology and Hepatology, 15*(8), 871-879.

Spady, D. K., & Dietschy, J. M. (1983). Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *Journal of Lipid Research, 24*(3), 303-315.

Spranger, J., Kroke, A., Mohlig, M., Bergmann, M. M., Ristow, M., Boeing, H., & Pfeiffer, A. F. (2003). Adiponectin and protection against type 2 diabetes mellitus. *Lancet, 361*(9353), 226-228.

St-Pierre, A. C., Ruel, I. L., Cantin, B., Dagenais, G. R., Bernard, P. M., Despres, J. P., & Lamarche, B. (2001). Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. *Circulation, 104*(19), 2295-2299.

Stahlberg, D., Rudling, M., Angelin, B., Bjorkhem, I., Forsell, P., Nilsell, K., & Einarsson, K. (1997). Hepatic cholesterol metabolism in human obesity. *Hepatology, 25*(6), 1447-1450.

Stampfer, M. J., Krauss, R. M., Ma, J., Blanche, P. J., Holl, L. G., Sacks, F. M., & Hennekens, C. H. (1996). A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *Jama, 276*(11), 882-888.

Statistics Canada. Canadian Community Health Survey: A first look 2000/01. (2002). Retrieved March 19, 2006, 2006, from http://www.statcan.ca/Daily/English/020508/d020508a.htm

Stefanick, M. L., Mackey, S., Sheehan, M., Ellsworth, N., Haskell, W. L., & Wood, P. D. (1998). Effects of diet and exercise in men and postmenopausal women with low levels of HDL cholesterol and high levels of LDL cholesterol. *N Engl J Med, 339*(1), 12-20.

Sudhop, T., Lutjohann, D., Kodal, A., Igel, M., Tribble, D. L., Shah, S., Perevozskaya, I., & von Bergmann, K. (2002). Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation, 106*(15), 1943-1948.

Swinburn, B. A., Nyomba, B. L., Saad, M. F., Zurlo, F., Raz, I., Knowler, W. C., Lillioja, S., Bogardus, C., & Ravussin, E. (1991). Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest, 88*(1), 168-173.

Tanaka, K., Okura, T., Shigematsu, R., Nakata, Y., Lee, D. J., Wee, S. W., & Yamabuki, K. (2004). Target value of intraabdominal fat area for improving coronary heart disease risk factors. *Obes Res, 12*(4), 695-703.

Taskinen, M. R. (1990). Hyperlipidaemia in diabetes. *Baillieres Clin Endocrinol Metab, 4*(4), 743-775.

Tilvis, R. S., & Miettinen, T. A. (1986). Serum plant sterols and their relation to cholesterol absorption. *American Journal of Clinical Nutrition, 43*(1), 92-97.

Tjepkema, M. (2004). *Measured obesity adult obesity in Canada. Measured height and weight* (No. 1). Ottawa: Statistics Canada.

Tschop, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., & Heiman, M. L. (2001). Circulating ghrelin levels are decreased in human obesity. *Diabetes, 50*(4), 707-709.

Unger, R. H. (2005). Hyperleptinemia: protecting the heart from lipid overload. *Hypertension, 45*(6), 1031-1034.

Vanstone, C. A., Raeini-Sarjaz, M., Parsons, W. E., & Jones, P. J. (2002a). Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. *American Journal of Clinical Nutrition, 76*(6), 1272-1278.

---. (2002b). Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. *Am J Clin Nutr, 76*(6), 1272-1278.

Varady, K. A., St-Pierre, A. C., Lamarche, B., & Jones, P. J. (2005). Effect of plant sterols and endurance training on LDL particle size and distribution in previously sedentary hypercholesterolemic adults. *Eur J Clin Nutr, 59*(4), 518-525.

Vlahcevic, Z. R., Eggertsen, G., Bjorkhem, I., Hylemon, P. B., Redford, K., & Pandak, W. M. (2000). Regulation of sterol 12alpha-hydroxylase and cholic acid biosynthesis in the rat. *Gastroenterology, 118*(3), 599-607.

Volek, J. S., Gomez, A. L., Love, D. M., Weyers, A. M., Hesslink, R., Jr., Wise, J. A., & Kraemer, W. J. (2002). Effects of an 8-week weight-loss program on cardiovascular disease risk factors and regional body composition. *Eur J Clin Nutr, 56*(7), 585-592.

Wang, D. Q., Paigen, B., & Carey, M. C. (2001a). Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice. *Journal of Lipid Research, 42*(11), 1820-1830.

Wang, H. H., Afdhal, N. H., Gendler, S. J., & Wang, D. Q. (2004a). Lack of the intestinal Muc1 mucin impairs cholesterol uptake and absorption but not fatty acid uptake in Muc1-/- mice. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 287*(3), G547-554.

Wang, T. Y., Haddad, M., & Wang, T. S. (2001b). Low triglyceride levels affect calculation of low-density lipoprotein cholesterol values. *Arch Pathol Lab Med, 125*(3), 404-405.

Wang, Y., Jones, P. J., Ausman, L. M., & Lichtenstein, A. H. (2004b). Soy protein reduces triglyceride levels and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects. *Atherosclerosis, 173*(2), 269-275.

Weggemans, R. M., Zock, P. L., Tai, E. S., Ordovas, J. M., Molhuizen, H. O., & Katan, M. B. (2002). ATP binding cassette G5 C1950G polymorphism may affect blood cholesterol concentrations in humans. *Clin Genet, 62*(3), 226-229.

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. E., & Tataranni, P. A. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab, 86*(5), 1930-1935.

Williams, P. T., Krauss, R. M., Vranizan, K. M., & Wood, P. D. (1990). Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation, 81*(4), 1293-1304.

Wilson, J. D., & Lindsey, C. A., Jr. (1965). Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man. *Journal of Clinical Investigation, 44*(11), 1805-1814.

Wilson, P. W., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., & Kannel, W. B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation, 97*(18), 1837-1847.

Wing, R. R., & Jeffery, R. W. (1995). Effect of modest weight loss on changes in cardiovascular risk factors: are there differences between men and women or between weight loss and maintenance? *Int J Obes Relat Metab Disord, 19*(1), 67-73.

Wing, R. R., Venditti, E., Jakicic, J. M., Polley, B. A., & Lang, W. (1998). Lifestyle intervention in overweight individuals with a family history of diabetes. *Diabetes Care, 21*(3), 350-359.

Wood, R. J., Volek, J. S., Liu, Y., Shachter, N. S., Contois, J. H., & Fernandez, M. L. (2006). Carbohydrate restriction alters lipoprotein metabolism by modifying VLDL, LDL, and HDL subfraction distribution and size in overweight men. *J Nutr, 136*(2), 384-389.

Woollett, L. A., Buckley, D. D., Yao, L., Jones, P. J., Granholm, N. A., Tolley, E. A., & Heubi, J. E. (2003). Effect of ursodeoxycholic acid on cholesterol absorption and metabolism in humans. *Journal of Lipid Research, 44*(5), 935-942.

Woollett, L. A., Buckley, D. D., Yao, L., Jones, P. J., Granholm, N. A., Tolley, E. A., Tso, P., & Heubi, J. E. (2004). Cholic acid supplementation enhances cholesterol absorption in humans. *Gastroenterology, 126*(3), 724-731.

World Health Organization. Global strategy on diet, physical activity, and health: obesity and overweight. (2003). Retrieved March 19, 2006, 2006, from http://www.who.int/hpr/NPH/docs/gs_obesity.pdf

Wu, J. T., & Kral, J. G. (2004). Ghrelin: integrative neuroendocrine peptide in health and disease. *Ann Surg, 239*(4), 464-474.

Xydakis, A. M., Case, C. C., Jones, P. H., Hoogeveen, R. C., Liu, M. Y., Smith, E. O., Nelson, K. W., & Ballantyne, C. M. (2004). Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid

weight loss through caloric restriction. *J Clin Endocrinol Metab, 89*(6), 2697-2703.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P., & Kadowaki, T. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med, 7*(8), 941-946.

Yancy, W. S., Jr., Olsen, M. K., Guyton, J. R., Bakst, R. P., & Westman, E. C. (2004). A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med, 140*(10), 769-777.

Zahorska-Markiewicz, B., Mizia-Stec, K., Olszanecka-Glinianowicz, M., & Janowska, J. (2004). Effect of weight reduction on serum ghrelin and TNFalpha concentrations in obese women. *Eur J Intern Med, 15*(3), 172-175.

APPENDIX

SUBJECT CONSENT FORM OF RESEARCH PROTOCOL

Impact of Weight Loss on Lipid Kinetics in Overweight, Hyperlipidemic Women

Subject name : _____ **Protocol number :** _____

School of Dietetics and Human Nutrition, Macdonald Campus, McGill University
Principal Investigator: Dr. Peter Jones
Phone: (514) 398-7547, 398-7527
Contact Physician: Dr. William Parsons
Phone: (514) 694-4869

1. NATURE AND DURATION OF PROCEDURE

The current study objectives are to assess the effects of 5-months of weight loss, achieved through combined dietary food restriction and exercise activity, and genetic patterning for cholesterol metabolism on blood lipid profiles and movement of these lipids between compartments within the body.

As part of a pre-screening visit, a fasting blood sample of 20 mL (4 teaspoons) will be taken for the laboratory to confirm the absence of health abnormalities. The sample will also be used to measure your blood lipid levels, which will indicate the level of various fats in your blood. Following qualification for the study, you will be invited back for further screening where a fasting blood sample of 20 mL will be obtained to measure your genetic status for a marker that may be related to the responsiveness of your blood lipids to diet. As well, a complete blood count will be performed. An electrocardiogram may be done at the discretion of the physician in charge. Prior to beginning the study, you will be examined by a physician to ensure that your are in good health and some questions about your medical history will be asked.

You will also need to ensure that you (1) have not consumed any lipid lowering drugs, including fish oils, fibers, or laxatives within the last 6 months, and (2) have not developed thyroid disease, diabetes mellitus, kidney disease or liver disease within the past 3 months.

Once you start the clinical trial, you will be asked to consume only the diet as instructed by the study coordinators for a period of roughly 5 months. At the start and termination of experimental period, your dietary food consumption will allow you to keep a constant weight for two periods of two weeks each. Between these two weight stabilization periods you will adhere to a weight loss diet for 20 weeks, where it is anticipated that you will lose up to 15 kilograms (33 pounds) of body. For the weight loss diet period you will receive information on how to adjust your home diet to provide less calories. In addition to this weight loss diet, it is expected that you will commence an exercise program where you will conduct regular physical activity (e.g. ride a stationary bike, climb the stair master, walk, etc.) 3 times per week. To establish how much energy to provide to you during the different phases of the study, you will have your metabolic rate measured using a canopy placed over your head for 30 minutes, during which time the rate of use of oxygen will be monitored. At that point, we will also ask you about your dietary habits. During the five-week weight loss period, you

will be asked to come at the Research Unit once a week to be weighted and once a month to have a blood draw.

Also, at the end of each period of stable weight (ie at close to the beginning and end of the overall study) your lipid levels and parameters of whole body lipid metabolism will be determined through the collection of blood samples and provision of some tagged materials. All of these tags are non-radioactive and pose no toxicity hazard. As well we will measure the amount of fat in your body using magnetic resonance imaging. For this procedure, you will need to lie in a horizontal position for about 40 minutes while a series of magnetic scans are taken. There is a risk of discomfort while in the magnetic resonance imaging machine, most likely in the form of claustrophobia. A separate consent from the hospital will be required for this procedure.

To measure lipid metabolism, four days before the end of each of these 2 week controlled dietary phases, you will receive an injection containing a small amount of tagged cholesterol. At the same time, you will also be requested to consume a small quantity of a similarly tagged cholesterol. The movement of these tagged materials will permit assessment of how much cholesterol your body absorbs from the diet. On the last day of study, you will also be requested to consume three tablespoons of water tagged with deuterium (hydrogen containing an extra neutron, $_2H^1$) in order to establish the amount of cholesterol being produced inside your body. Blood samples (20 mL) will be collected daily on the mornings of each of the last four (4) days of the study, as well as the morning of completion of the study, in order to examine the movement of these tags across body compartments. A further blood sample (20 mL) will be obtained 12 h after giving the tagged cholesterol 4 days before the end of the study. In addition to blood samples, fecal samples will be required on days 11, 12, 13 and 14 of the third period to measure the amount of tagged and untagged cholesterol, in order to determine cholesterol absorption.

The total amount of blood drawn during each of the two phases of the study will be 250 mL (about 1 cup). The total blood volume, including the screening sample, required for this trial will be 510 mL (a little more than 2 cups). No more than 300 mL (about 1 ¼ cups) will be drawn in any eight-(8) week period.

2. POTENTIAL RISKS AND/OR BENEFITS

The weight loss regimen can be expected to have no negative side effects on health, based on many previous animal and human experiments. The magnetic resonance imaging procedure is routine and without hazard, as long as you have no metal containing items surgically placed within your body cavity. Bruising and pain is associated with venipuncture and injection of the tagged cholesterol. Volunteers may run the risk of becoming anemic, but this will be tested at the end of each diet phase, and anemic volunteers will receive iron supplementation (ferrous gluconate, 325 mg orally, once a day, for 2 weeks). In case you feel any discomfort during the experimental trial, a physician, Dr. Parsons, will be available to contact at any time. Dr. Parsons can be reached at (514)

694-4869. If any health abnormalities are identified in the clinical tests conducted during the experiment, you will be informed of such by the doctor associated with the study.

CONSENT OF THE SUBJECT

The substance of the project and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and procedures involved. I am aware that no guarantee or assurance has been given by anyone as to results to be obtained. Confidentiality of records concerning my involvement in this project will be maintained in an appropriated manner. Samples will not be utilized for any additional analyses, nor stored for any prolonged period, nor shared with any other group, other than is indicated in the protocol, without my specific consent.

I, _____, have read the above description with one of the

investigators , _____. I fully understand the procedures, advantages and disadvantages of the study, which have been explained to me. I understand that, in compensation for the inconvenience of the study schedule, I will receive $1,000. I will also receive access to my test results when they become available. If I decide to withdraw before completion or should the study be terminated early, I will receive an appropriate pro-rated fraction of this amount.

I acknowledge receiving a copy of this consent form and all appropriate attachments and agree to be contacted by a member of the Research Ethics Committee.

_____ _____

Investigator Signature of Subject

_____

Witness

_____ _____

Date Time

Gmail by Google BETA

Sylvia Santosa <sylviasantosa@gmail.com>

Ethics certificate for thesis submission

5 messages

Sylvia Santosa <sylviasantosa@gmail.com> **Tue, May 9, 2006 at 12:10 PM**
To: vanessa.fabris@mcgill.ca

Dear Vanessa,
My name is Sylvia Santosa (student# 110247748) and I am a Ph.D. student with the School of Dietetics and Human Nutrition. I am in the process of preparing my thesis for submission and the issue of inclusion of an ethics certificate arose. I have the letter of ethics approval from the IRB and I also have the subsequent approved renewal forms. Unfortunately, I have been unable to obtain the actual certificate and the IRB does not have a copy, nor are they able to issue a replacement. Ethics approval was issued by the Faculty of Medicine IRB and they have record of ethics approval. Is the letter and/or renewals sufficient in lieu of the certificate? Please let me know what I can include. Thank you for your attention to this matter.
Sincerely,
Sylvia

--

Sylvia Santosa, BASc
PhD Candidate
School of Dietetics and Human Nutrition
McGill University, Macdonald Campus
21 111 Lakeshore Road
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Vanessa D Fabris, Miss <vanessa.fabris@mcgill.ca> **Wed, May 10, 2006 at 9:21 AM**
To: Sylvia Santosa <sylviasantosa@gmail.com>

Dear Sylvia,

I discussed your situation with our Thesis Adminstrator and he suggested that you inform us (via email) as to why the certificate itself will not be provided with the thesis copies and other forms, so I can put the explanation in your file. Nothing complicated. Just reiterate what you wrote below and mention the reason why your supervisor can't provide you with it (you mentioned that he's left the province and all of his documents are in boxes.)

I hope this helps and wil be waiting for your email. You can reply to this one.

Best,

Vanessa Fabris
Thesis Coordinator

Graduate & Postdoctoral Studies Office
Registration, Records & Theses
James Administration Building, Room 400

Tel.: 398-3990. ext. 094220
Fax: 398-6283
Email: vanessa.fabris@mcgill.ca

From: Sylvia Santosa [mailto:sylviasantosa@gmail.com]
Sent: Tuesday, May 09, 2006 12:11 PM
To: Vanessa D Fabris, Miss
Subject: Ethics certificate for thesis submission

[Quoted text hidden]

Sylvia Santosa <sylviasantosa@gmail.com> **Wed, May 10, 2006 at 9:28 AM**
To: "Vanessa D Fabris, Miss" <vanessa.fabris@mcgill.ca>

Dear Vanessa,
My name is Sylvia Santosa (student# 110247748) and I am a Ph.D. student with the School of Dietetics and Human Nutrition. I am in the process of preparing my thesis for submission and the issue of inclusion of an ethics certificate arose. I have the letter of ethics approval from the IRB and I also have the subsequent approved renewal forms. Unfortunately, I have been unable to obtain the actual certificate and the IRB does not have a copy, nor are they able to issue a replacement. I have not been able to obtain one from my supervisor because he has relocated to Winnepeg and as a consequence, all files are in boxes there. Thus, it would be extremely difficult to find. Ethics approval was issued by the Faculty of Medicine IRB and they have record of ethics approval. I can include the letter and/or renewal forms in my thesis. Please let me know whether this is sufficient. Thank you for your attention to this matter.
Sincerely,
Sylvia
[Quoted text hidden]

Sylvia Santosa <sylviasantosa@gmail.com> **Wed, May 10, 2006 at 9:29 AM**
To: "Vanessa D Fabris, Miss" <vanessa.fabris@mcgill.ca>

Dear Vanessa,
I just sent you the e-mail. Is that what you meant? Let me know if you need anything further and thank you for looking into this.
Sylvia

On 5/10/06, **Vanessa D Fabris, Miss** <vanessa.fabris@mcgill.ca> wrote:
[Quoted text hidden]

[Quoted text hidden]

Vanessa D Fabris, Miss <vanessa.fabris@mcgill.ca> **Wed, May 10, 2006 at 9:36 AM**
To: Sylvia Santosa <sylviasantosa@gmail.com>

Hi Sylvia,

Received your email and it will do fine. I'll include it in your thesis file and everything should go smoothly.

Best,

Vanessa Fabris
Thesis Coordinator
Graduate & Postdoctoral Studies Office
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James Administration Building, Room 400

Tel.: 398-3990, ext. 094220
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From: Sylvia Santosa [mailto:sylviasantosa@gmail.com]
Sent: Wednesday, May 10, 2006 9:30 AM
To: Vanessa D Fabris, Miss
Subject: Re: Ethics certificate for thesis submission

[Quoted text hidden]

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September 30, 2002

Dr. Peter J. H. Jones
Professor of Nutrition
School of Dietetics and Human Nutrition
21,111 Lakeshore
Ste-Anne-De-Bellevue, Quebec H9X 3V9

Dear Dr. Jones,

We have received correspondence in support of the research proposal A00-M81-02A entitled "Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women" which received full Board approval by the Institutional Review Board, Faculty of Medicine at its meeting of September 9, 2002.

The responses and revisions were found to be acceptable and we are pleased to inform you that final approval for the revised protocol (September 25, 2002), English and French recruitment ad (September 27, 2002) and revised consent form (September 23, 2002) was provided on September 30, 2002, valid until **September 2003**. You will find the certification document (executed) enclosed.

A review of all research involving human subjects is required on an annual basis in accord with the date of initial approval. Should any modification to the study or unanticipated development occur prior to the next review, please advise IRB promptly.

We ask you to note that it is the responsibility of the investigator to deposit a copy of the approved research protocol and consent form with the Research Ethics Board of each hospital where subject recruitment or data collection will take place.

Yours sincerely,

Neil MacDonald, M.D.,
Chair,
Institutional Review Board

cc: A09-M81-02A

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler
Montréal, QC, H3G 1Y6

Fax/Télécopieur: (514) 398-3595

October 7, 2003

Dr. Peter J. H. Jones
Professor of Nutrition
School of Dietetics and Human Nutrition
21,111 Lakeshore
Ste-Anne-de-Bellevue, Quebec H9X 3V9

Dear Dr. Jones:

We are writing in response to your request for continuing review by the Institutional Review Board of the study A09-M81-02A entitled **"Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women".**

The progress report was reviewed and we are pleased to inform you that full board re-approval for the study was provided on ***October 6, 2003***, valid until ***September 2004***. The certification of annual review has been enclosed.

We ask that you take note of the investigator's responsibility to assure that the current protocol, study amendments and consent document are deposited on an annual basis with the Research Ethics Boards of each hospital where patient enrollment or data collection is carried out.

Should further study revision or an unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

Neil MacDonald

Neil MacDonald, M.D.
Chair
Institutional Review Board

cc: A09-M81-02A

Principal Investigator: PETER JH JONES Department/Institution: DHN

IRB Review Number: A09-M91-02A Study Number (if any): _____ Review Interval: annual

Title of Research Study: IMPACT OF WEIGHT LOSS ON LIPID KINETICS IN OVERWEIGHT, HYPERLIPIDEMIC WOMEN

Date of initial IRB approval: Sept. 30/02 Date of previous continuing review (if applicable): _____

INTERIM REPORT (PLEASE CHECK OR SPECIFY)

Current Status of Study:

Active Study: X On Hold: _____ Closed to Enrolment: X

Interim Analysis: X Final Analysis: _____ Study Not Activated*: _____

*If the study has not become active at McGill, please provide correspondence to explain; enclosed: _____

McGill hospital(s) where study is being conducted and has received approval of local Research Ethics Board(s) (if applicable):

JGH: ☐ MUHC/MCH: ☐ MUHC/MGH: ☐ MUHC/MNH-MNI: ☐

MUHC/RVH: ☐ SMH: ☐ Douglas ☐ Other: ☒ MARY EMILY CLINICAL NUTRITION RESEARCH UNIT

McGill hospital(s) where study has not received approval of local Research Ethics Board(s) (if applicable): N/A

If study sponsorship or financial support has changed, please provide correspondence to explain; enclosed: N/A

Number of subjects to be enrolled by the McGill PI: ____ Number of subjects enrolled by the McGill PI to date: 43

Number of subjects enrolled by the McGill PI since last review: 43

Have any of these subjects withdrawn from the study?: Y - 8

Has the study been revised since the last review?: N/A Have the study revisions been approved by the IRB?: N/A

Has the consent form been revised since the last review?: N Date of the current consent form: _____

Are there new data since the last review that could influence a subject's willingness to provide continuing consent?: N

Have there been any serious adverse experiences (SAEs)?: N

Have all serious adverse experiences (SAEs) and safety reports relevant to the study been reported to the IRB?: N/A

SIGNATURES:

Principal Investigator: _____ Date: Sept 10 03

IRB Chair: Neil Mackenzie Date: October 6, 2003

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
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Fax/Télécopieur: (514) 398-3595

September 14, 2004

Dr. Peter J. H. Jones
Professor of Nutrition
School of Dietetics and Human Nutrition
21,111 Lakeshore
Ste-Anne-de-Bellevue, Quebec H9X 3V9

Dear Dr. Jones:

We are writing in response to your request for continuing review by the Institutional Review Board of the study A09-M81-02A entitled **"Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women".**

The progress report was reviewed and we are pleased to inform you that full board re-approval for the study was provided on ***September 13, 2004***, valid until ***September 12, 2005***. The certification of annual review has been enclosed.

We ask that you take note of the investigator's responsibility to assure that the current protocol, study amendments and consent document are deposited on an annual basis with the Research Ethics Boards of each hospital where patient enrollment or data collection is carried out.

Should further study revision or an unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

C. Celeste Johnston

Celeste Johnston, DEd, RN
Co-Chair
Institutional Review Board

cc: A09-M81-02A

McGill Faculty of Medicine
Institutional Review Board
-Continuing Review-

Principal Investigator: Peter JH Jones Department/Institution: Dietetics and Nutrition

IRB Review Number: A09-M81-02A Study Number (if any): _____ Review Interval: Annual

Title of Research Study: Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women

Date of initial IRB approval: September 2002 Date of previous continuing review (if applicable): September 2003

INTERIM REPORT (PLEASE CHECK OR SPECIFY)

Current Status of Study:

Active Study: _____ On Hold: _____ Closed to Enrolment: X

Interim Analysis: X Final Analysis: _____ Study Not Activated*: _____

*If the study has not become active at McGill, please provide correspondence to explain: enclosed: _____

McGill hospital(s) where study is being conducted and has received approval of local Research Ethics Board(s) (if applicable):

JGH: ☐ MUHC/MCH: ☐ MUHC/MGH: ☐ MUHC/MNH-MNI: ☐
MUHC/RVH: ☐ SMH: ☐ Douglas ☐ Other: ☒ Mary Emily Clinical Nutrition Research Unit

McGill hospital(s) where study has not received approval of local Research Ethics Board(s) (if applicable): _____

If study sponsorship or financial support has changed, please provide correspondence to explain: enclosed: _____

Number of subjects to be enrolled by the McGill PI: 42 Number of subjects enrolled by the McGill PI to date: 42

Number of subjects enrolled by the McGill PI since last review: 0

Have any of these subjects withdrawn from the study?: 7

Has the study been revised since the last review?: N Have the study revisions been approved by the IRB?: N/A

Has the consent form been revised since the last review?: N Date of the current consent form: _____

Are there new data since the last review that could influence a subject's willingness to provide continuing consent?: N

Have there been any serious adverse experiences (SAEs)?: N

Have all serious adverse experiences (SAEs) and safety reports relevant to the study been reported to the IRB?: Y

SIGNATURES:

Principal Investigator: _____ Date: Aug 24 '04

IRB Chair: C. Clarke Johnston Date: 13 SEP 2004

McGill

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3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

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3655, Promenade Sir William Osler
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Fax/Télécopieur: (514) 398-3595

September 13, 2005

Dr. Peter J. H. Jones
Professor of Nutrition
School of Dietetics and Human Nutrition
21,111 Lakeshore
Ste-Anne-de-Bellevue, Quebec H9X 3V9

Dear Dr. Jones:

We are writing in response to your request for continuing review by the Institutional Review Board of the study A09-M81-02A entitled **"Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women"**.

The progress report was reviewed and we are pleased to inform you that full board re-approval for the study was provided on ***September 12, 2005***, valid until ***September 11, 2006***. The certification of annual review has been enclosed.

We ask that you take note of the investigator's responsibility to assure that the current protocol, study amendments and consent document are deposited on an annual basis with the Research Ethics Boards of each hospital where patient enrollment or data collection is carried out.

Should further study revision or an unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

Celeste Johnston, DEd, RN
Co-Chair
Institutional Review Board

cc: A09-M81-02A

McGill Faculty of Medicine
Institutional Review Board
-Continuing Review-

Principal Investigator: Peter JH Jones Department/Institution: Dietetics and Nutrition

IRB Review Number: A09-M51-02A Study Number (if any): _____ Review Interval: Annual

Title of Research Study: Impact of Weight Loss on Lipid Kinetics in Overweight Hyperlipidemic Women

Date of initial IRB approval: September 2002 Date of previous continuing review (if applicable): September 2004

INTERIM REPORT (PLEASE CHECK OR SPECIFY)

Current Status of Study:

Active Study: _____ On Hold: _____ Closed to Enrolment: X

Interim Analysis: X Final Analysis: _____ Study Not Activated*: _____

*If the study has not become active at McGill, please provide correspondence to explain; enclosed: _____

McGill hospital(s) where study is being conducted and has received approval of local Research Ethics Board(s) (if applicable):

JGH: ☐ MUHC/MCH: ☐ MUHC/MGH: ☐ MUHC/MNH-MNI: ☐
MUHC/RVH: ☐ SMH: ☐ Douglas ☐ Other: ☐ _____

McGill hospital(s) where study has not received approval of local Research Ethics Board(s) (if applicable): _____

If study sponsorship or financial support has changed, please provide correspondence to explain; enclosed: _____

Number of subjects to be enrolled by the McGill PI: 42 Number of subjects enrolled by the McGill PI to date: 42

Number of subjects enrolled by the McGill PI since last review: 0

Have any of these subjects withdrawn from the study?: 7

Has the study been revised since the last review?: N Have the study revisions been approved by the IRB?: NA

Has the consent form been revised since the last review?: N Date of the current consent form: _____

Are there new data since the last review that could influence a subject's willingness to provide continuing consent?: N

Have there been any serious adverse experiences (SAEs)?: N

Have all serious adverse experiences (SAEs) and safety reports relevant to the study been reported to the IRB?: Y

SIGNATURES:

Principal Investigator: _____ Date: Aug 29 '05

IRB Chair: _____ Date: 12 SEP 2005

Att. Sylvia Santosa

May 23, 2006

To Suhad AbuMweis:

The purpose of this letter is to confirm that Suhad AbuMweis, co-author of the below mentioned manuscript, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

Manuscripts to be included:

1. Physiological and therapeutic factors affecting cholesterol metabolism: Does a reciprocal relationship between cholesterol absorption and synthesis really exist?

The candidate's role in writing this manuscript was in writing the sections on how body weight affects cholesterol metabolism and therapeutic factors affecting absorption and synthesis. The candidate also coordinated the revision of the manuscript between coauthors.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.

Suhad AbuMweis
Suhad May 29, 2006

Suhad AbuMweis

May 8, 2006

To Dr. Isabelle Demonty:

The purpose of this letter is to confirm that Isabelle Demonty, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

Manuscripts to be included:

- Santosa S, Demonty I, Lichtenstein AH, Ciardone K, Jones PJH. An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr.* (accepted pending revision)
- Varady KA, Lamarche B, Santosa S, Demonty I, Jones PJH. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women. *Metabolism.* (accepted pending revision)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. An effective self-directed protocol for moderate weight loss through diet and exercise in women. (Submitted to *Can J Diet Pract Res*)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. Changes in cholesterol metabolism and body composition as a result of moderate weight loss through diet and exercise in women. (Submitted to *Int J Obes*)
- Santosa S, Demonty I, Lichtenstein AH, Ordovas JM, Jones PJH. ABCG5 and ABCG8 single nucleotide polymorphisms contribute to the responsiveness of cholesterol kinetics to weight loss. (Submitted to *Arterioscler Thromb Vasc Biol*)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The candidate was fully responsible for the analysis of samples for cholesterol kinetics and body composition, and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.

Isabelle Demonty

Isabelle Demonty

May 2, 2006

To Amélie Charest:

The purpose of this letter is to confirm that Amélie Charest, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

Manuscripts to be included:

- Varady KA, Lamarche B, Santosa S, Demonty I, Charest A, Jones PJH. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women. *Metabolism*. (accepted pending revision)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The manuscript was written with guidance and feedback from the candidate.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.

Amélie Charest

Amélie Charest

May 2, 2006

To Dr. Katherine Cianflone:

The purpose of this letter is to confirm that Katherine Cianflone, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

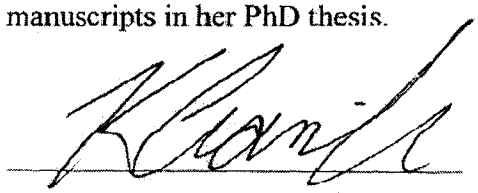
Manuscripts to be included:

- Santosa S, Demonty I, Lichtenstein AH, Cianflone K, Jones PJH. An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr*. (accepted pending revision)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The candidate was fully responsible for the analysis of samples for cholesterol kinetics and body composition, and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.



Katherine Cianflone

May 31, 2006

To Whom It May Concern:

The purpose of this letter is to confirm that Peter JH Jones, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

Manuscripts to be included:

- Santosa S, Varady KA, AbumWeis S, Jones PJH. Physiological and therapeutic factors affecting cholesterol metabolism: Does a reciprocal relationship between cholesterol absorption and synthesis really exist?
- Santosa S, Demonty I, Lichtenstein AH, Cianflone K, Jones PJH. An investigation of hormone and lipid associations after weight loss in women.
- Varady KA, Lamarche B, Santosa S, Demonty I, Charest, A, Jones PJH. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women.
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. An effective self-directed protocol for moderate weight loss through diet and exercise in women.
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. Changes in cholesterol metabolism and body composition as a result of moderate weight loss through diet and exercise in women.
- Santosa S, Demonty I, Lichtenstein AH, Ordovas JM, Jones PJH. ABCG5 and ABCG8 single nucleotide polymorphisms contribute to the responsiveness of cholesterol kinetics to weight loss.

The candidate's role in writing the absorption/synthesis manuscript was in writing the sections on how body weight affects cholesterol metabolism and therapeutic factors affecting absorption and synthesis. The candidate also coordinated the revision of the manuscript between coauthors. The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The candidate was fully responsible for the analysis of samples for cholesterol kinetics and body composition, and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors with the exception of the LDL particle size manuscript that was written under the guidance of the candidate.

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.

Peter Jones

May 2, 2006

To Dr. Jose Ordovas:

The purpose of this letter is to confirm that Jose Ordovas, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

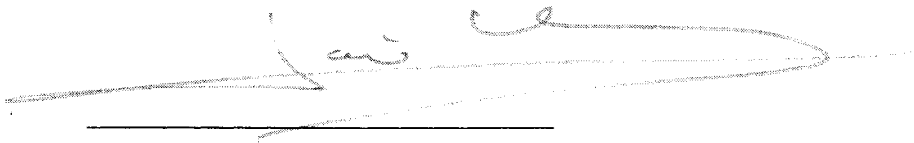
Manuscripts to be included:

- Santosa S, Demonty I, Lichtenstein AH, Ordovas JM, Jones PJH. ABCG5 and ABCG8 single nucleotide polymorphisms contribute to the responsiveness of cholesterol kinetics to weight loss. (Submitted to *Arterioscler Thromb Vasc Biol*)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The candidate was fully responsible for the analysis of samples for cholesterol kinetics and body composition, and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.



Jose Ordovas

May 2, 2006

To Dr. Alice Lichtenstein:

The purpose of this letter is to confirm that Alice H. Licherenstein, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

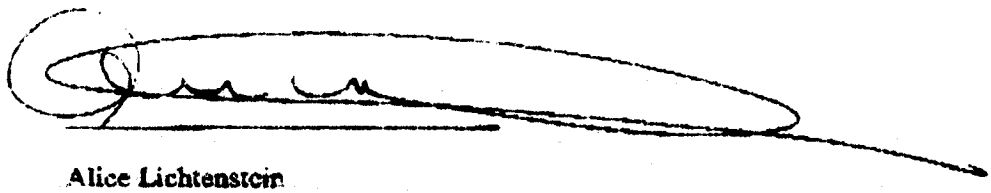
Manuscripts to be included:

- Santosa S, Demonty I, Lichtenstein AH, Cianflone K, Jones PJH. An investigation of hormone and lipid associations after weight loss in women. *J Am Coll Nutr*. (accepted pending revision)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. An effective self-directed protocol for moderate weight loss through diet and exercise in women. (Submitted to *Can J Diet Pract Res*)
- Santosa S, Demonty I, Lichtenstein AH, Jones PJH. Changes in cholesterol metabolism and body composition as a result of moderate weight loss through diet and exercise in women. (Submitted to *Int J Obes*)
- Santosa S, Demonty I, Lichtenstein AH, Ordovas JM, Jones PJH. ABCG5 and ABCG8 single nucleotide polymorphisms contribute to the responsiveness of cholesterol kinetics to weight loss. (Submitted to *Arterioscler Thromb Vasc Biol*)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The candidate was fully responsible for the analysis of samples for cholesterol kinetics and body composition, and statistical analysis of data. The candidate wrote the manuscript with guidance and feedback of the co-authors.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.



Alice Lichtenstein

May 2, 2006

To Benoit Lamarche:

The purpose of this letter is to confirm that Benoit Lamarche, co-author of the below mentioned manuscripts, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

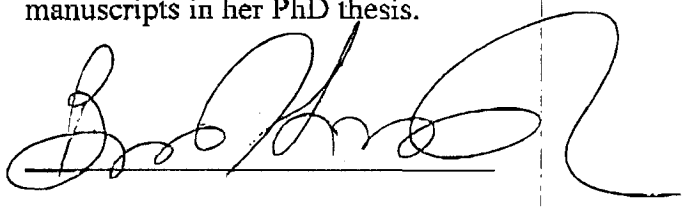
Manuscripts to be included:

- Varady KA, Lamarche B, Santosa S, Demonty I, Charest A, Jones PJH. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women. *Metabolism.* (accepted pending revision)

The candidate's role in these studies included sharing responsibility for the screening and recruitment of subjects from the Montreal area, conducting the clinical trial, and sample collection. The manuscript was written with guidance and feedback from the candidate.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.



Benoit Lamarche

May 23, 2006

To Krista Varady:

The purpose of this letter is to confirm that Krista A. Varady, co-author of the below mentioned manuscript, agrees to the inclusion of these manuscripts in the thesis of Sylvia Santosa, PhD candidate.

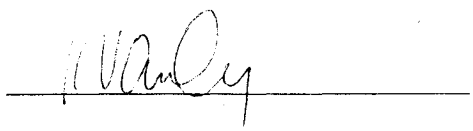
Manuscripts to be included:

1. Physiological and therapeutic factors affecting cholesterol metabolism: Does a reciprocal relationship between cholesterol absorption and synthesis really exist?
2. Effect of weight loss resulting from a combined low fat diet/exercise regimen on LDL particle size and distribution in obese women

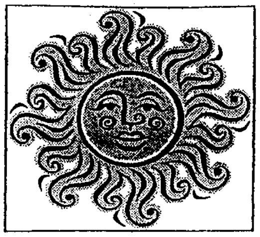
The candidate's role in writing the absorption/synthesis manuscript was in writing the sections on how body weight affects cholesterol metabolism and therapeutic factors affecting absorption and synthesis. The candidate also coordinated the revision of the manuscript between coauthors. The LDL particle size manuscript was put together under the guidance of the candidate.

Sylvia Santosa

I, the co-author, agree that the candidate, Sylvia Santosa, include the above mentioned manuscripts in her PhD thesis.

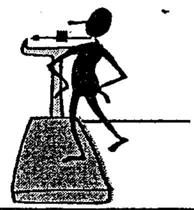


Krista Varady



Journal alimentaire

Note:



*Nom:*________________________________

*Date:*________________________________

Temps	*Quantité*	*Aliments et quantités d'ingrédients*

Durée	*tensité*	*rcices*

1400 calories
Fruits: ☐☐☐
Légumes: ☐☐☐
Pain: ☐☐☐☐☐☐☐
Lait: ☐☐☐☐
Gras: ☐☐☐
Prot.: ☐☐☐☐☐☐
Eau: ☐☐☐☐☐☐☐☐

Groupes d'aliments

le groupe des protéines (viandes et substituts)

importance dans l'alimentation: Les aliments de ce groupe sont pour la plupart une excellente source de vitamines du groupe B, de fer et de minéraux essentiels. Ils sont riches en protéines et généralement pauvres en glucides complexes (exception faite des pois et fèves). Une portion de ce groupe contient approximativement 65 calories, 7 g de protéines et 4 g de gras.

les meilleurs choix

1 on (30g) de poulet ou dinde (sans la peau)
1 on (30 g) de veau (sauf escalopes)
1 on (30 g) de bœuf maigre
(ronde, surlonge, filet, steak de flanc)
1 on (30 g) de porc maigre
(jambon frais, filet)
1 on (30g) de poisson (frais ou congelé)
$\frac{1}{4}$ tasse de thon en conserve dans l'eau
1 on (30 g) de fromage léger (moins de 20% M.G.)
$\frac{1}{2}$ tasse de fromage cottage 1% M.G.
1 œuf entier ou 3 blancs d'œufs
1 c. a soupe de beurre d'arachide *(de temps en temps)*

à éviter autant que possible (plus riches en gras et en sel)

coupes de bœuf grasses
(« *ribs* » ou côtes, corned beef, jambon)
coupes de porc grasses
(« *spareribs* », porc haché, saucisses)
agneau haché
poisson frit
saucisses et charcuteries
(ex : saucisses italiennes, saucisses de Francfort, merguez, salami, saucisson de Bologne, etc.)
fromages réguliers en M.G.
(ex : cheddar, suisse, bleu, etc.)

Groupes d'aliments

le groupe des glucides complexes
(céréales et grains, pâtes, légumes riches en amidon)

importance dans l'alimentation: Les aliments de ce groupe sont pour la plupart une excellente source de fibres, de vitamines du groupe B et de fer. Ils sont habituellement pauvres en gras et en cholestérol. Une portion de ce groupe contient approximativement 80 calories, 3 g de protéines et des traces de gras.

les meilleurs choix

- ¾ tasse de céréales à déjeuner a grains entiers non sucrées
- 1/3 tasse de céréales de son concentrées (ex : All Bran)
- ½ tasse de céréales de son en flocons (ex : Bran Flakes)
- ¾ tasse de gruau ou céréales chaudes
- 1 tranche de pain de blé entier
- ½ bagel, muffin anglais, pain à hamburger ou à hot-dog
- ½ pain pita de 6 po de diamètre
- 1 tortilla de 6 po de diamètre
- 1 tasse de croutons faibles en gras
- 3 tasses de maïs éclaté (popcorn) sans gras ajouté
- 5 toasts melba
- 3 galettes de riz soufflé nature, sans sucre ajouté
- ½ tasse de nouilles ou pâtes alimentaires
- ½ tasse de riz, de bulghur, cuits
- ½ tasse de maïs
- ½ tasse de pois verts, congelés ou en conserve
- 1/3 tasse de légumineuses cuites
  (pois chiches, haricots rouges, haricots blancs, feves de Lima)
- 1 petite pomme de terre cuite (1 on ou 30 g)
- ½ tasse de purée de pommes de terre faible en gras

à éviter autant que possible (plus riches en gras, en sel et/ou en sucre)

- Croissants
- Muffins commerciaux, beignes, biscuits
- Céréales du genre granola
- Pâtes et riz frits
- Pommes de terre frites
- Chips
- Chips de maïs
- Popcorn pour le micro-ondes
- Céréales à déjeuner pré-sucrées
- Coquilles a tacos
- Gaufres
- Crêpes
- Craquelins au fromage, au beurre

Groupes d'aliments

le groupe des fruits et légumes

Importance dans l'alimentation: Les aliments de ce groupe sont pour la plupart une excellente source de fibres, de vitamines et de minéraux essentiels. Ils sont une bonne source de vitamine A et de vitamine C. Ils contiennent aussi beaucoup de glucides, mais contiennent habituellement peu de gras et de sel. Une portion de fruits contient approximativement 60 calories et 15 g de glucides. Une portion de légumes contient environ 25 calories, 5 g de glucides et 2 g de protéines.

les meilleurs choix

FRUITS

1 fruit frais de grosseur moyenne
(ex : pomme, nectarine, orange, poire, pêche, gros kiwi...)
2 prunes
½ banane, pamplemousse, mangue
1 tasse de cubes de cantaloup, de papayes, de framboises
1 ¼ tasse de melon d'eau
¾ tasse d'ananas frais
1/3 tasse d'ananas en conserve
½ tasse de compote de pomme non sucrée
½ tasse d'abricots, de pêches, de poires ou de fruits mélangés en conserve
¾ tasse de bleuets frais
½ tasse de jus de pomme, pamplemousse, orange ou ananas
1/3 tasse de jus de canneberges, raisins, ou pruneaux

LEGUMES

1 tasse de légumes crus
½ tasse de légumes cuits
½ tasse de jus de légumes
exemples : asperges, choux de Bruxelles, brocoli, chou, carottes, chou fleur, aubergine, poivrons verts, betteraves, poireaux, rutabagas, épinards, zuchinis, etc.
1 tomate
N.B. Ce groupe ne comprend pas les légumes riches en amidon

à éviter autant que possible (plus riches en gras, en sel ou en sucre)

Boissons aux fruits (coktails, limonades, punchs)	Avocat
Fruits séchés	Olives
Noix de coco	Légumes frits

Groupes d'aliments

le groupe du lait et des produits laitiers

ice dans l'alimentation: Les aliments de ce groupe sont pour la plupart une e source de calcium, de phosphore, de riboflavine, de vitamine A et de vitamine D. ennent aussi beaucoup de protéines et de glucides. Une portion de ce groupe approximativement 90 calories, 12 g de glucides et 8 g de protéines.

les meilleurs choix

- 1 tasse de lait écremé ou à 1% M.G.
- 1 tasse (8 on) de yogourt nature non gras
- ½ tasse de lait évaporé non gras

éviter autant que possible (plus riches en gras et/ou en sucre)

- lait entier (3.25% M.G.)
- yogourt sucré
- lait entier évaporé
- lait condensé sucré
- lait glacé
- yogourt glacé
- crème glacée

Groupes d'aliments

le groupe des gras

importance dans l'alimentation: Les aliments de ce groupe sont pour la plupart une excellente source de vitamines A et E. Ils contiennent des acides gras essentiels. Une petite quantité de gras est donc nécessaire chaque jour. Une portion de ce groupe contient approximativement 45 calories et 5 g de gras.

les meilleurs choix (diminuent le mauvais cholestérol)

GRAS MONOINSATURES OU POLYINSATURES

1 c. a ~~soupe~~ de margarine molle
1 c. a ~~soupe~~ de mayonnaise, régulière ou réduite en calories
1 c. a ~~soupe~~ d'huile végétale
(les huiles d'olive et de canola sont les meilleures, mais les huiles de maïs, soya, tournesol, sésame et arachide sont aussi très bonnes)
1 c. a soupe de vinaigrette, régulière ou légère
2 c. a soupe de « salad dressing » de type mayonnaise
1 c. a soupe de fromage à la crème léger

à éviter autant que possible (augmentent le mauvais cholestérol)

GRAS SATURES

beurre	fromage à la crème régulier
bacon	huile de coco
lard	noix de coco rapée
shortening d'huile végétale	lait condensé sucré
bouillon de viande non dégraissé	lait glacé
crème a café, de table	yogourt glacé
crème a fouetter	crème glacée
crème sûre régulière	

Groupes d'aliments

le groupe des « free foods »

efinition: Les aliments de ce groupe contiennent moins de 20 calories par portion. Ils euvent être inclus dans le régime alimentaire amaigrissant, avec modération toutefois.

Boissons

ouillons pauvres en sodium et en gras
oissons gazeuses diètes
au gazeuse
afé, thé
c. a soupe de poudre de cacao non sucré

Fruits

tasse de canneberges, non sucrées
tasse de rhubarbe, non sucrée

Légumes crus

1 TASSE

hou	champignons
hou chinois	germes de haricots
éleri	radis
oncombre	zucchinis
iments forts	persil
échalotes	

Légumes verts feuillus

ndives	laitue iceberg
escarole	laitue frisée
aiture romaine	épinards

Autres

nonstick vegetable Pan spray »

Condiments

1 c. a table de ketchup
1 c. a table de moutarde
1 c. a table de sauce barbecue
1 c. a table de sauce à taco
1 c. a table de vinaigre
2 c. a table de vinaigrette sans huile

Assaisonnements

épices	herbes
graines de céleri	citron
ciboulette	lime
curry ou cari	jus de citron
sauce de piment fort	ail
poudre d'oignon	fenouil

essences
vanille, amande, citron, menthe, etc.
sauce Worcestershire
sauce soya réduite en sodium

Substituts de sucre

aspartame (pas plus de 4 sachets/j)
saccharine

Calculating Exchanges For Ambiguous Foods

Sometimes we run into foods (such as meal replacements) that don't fit into any one particular category.

Here is an easy way to calculate
how these foods fit into your exchange system.

1. Start with Carbohydrates.
   Take the amount of carbohydrates (g) and divide by 15 g.

   This is the number of Starch Exchanges the food has.

2. Calculate the Protein Exchanges.
   Take the number of Starch Exchanges and multiply it by 3 g.
   Subtract this number from the total amount of protein (g).
   Divide by 7 g.

   This is the number of Protein Exchanges the food has.

3. Calculate the Fat Exchanges.
   Take the number of Starch Exchanges and multiply it by 1 g.
   Take the number of Protein Exchanges and multiply it by 4 g.
   Subtract this number from the total amount of fat (g).
   Divide by 5 g.

   This is the number of Fat Exchanges the food has.

Note that sometimes the numbers don't work out evenly.
Nothing is perfect.
Just use your best judgement!

A Sample Calculation To Get You Started...

A food that you want to eat has:

30 g Carbohydrates
13 g Protein
10 g Fat

How does it fit into your exchange system?

1. Start with Carbohydrates.

   Take the amount of carbohydrates (g) and divide by 15 g.

   This food has 30 g of Carbohydrate so... 30 g ÷ 15 g = 2

   Therefore, this food has **2 Starch Exchanges.**

2. Calculate the Protein Exchanges.

   Take the number of Starch Exchanges and multiply it by 3 g.

   2 Starch Exchanges x 3 g = 6 g

   Subtract this number from the total amount of protein (g).

   This food has 13 g Protein so...13 g - 6 g = 7 g

   Divide by 7 g.

   7 g ÷ 7 g = 1

   Therefore, this food has **1 Protein Exchange.**

3. Calculate the Fat Exchanges.

   Take the number of Starch Exchanges and multiply it by 1 g.

   2 Starch Exchanges x 1 g = 2 g

   Take the number of Protein Exchanges and multiply it by 4 g.

   1 Protein Exchange x 4 g = 4 g

   Subtract this number from the total amount of fat (g).

   This food has 10 g Fat so...10 g - 1 g - 4 g = 5 g

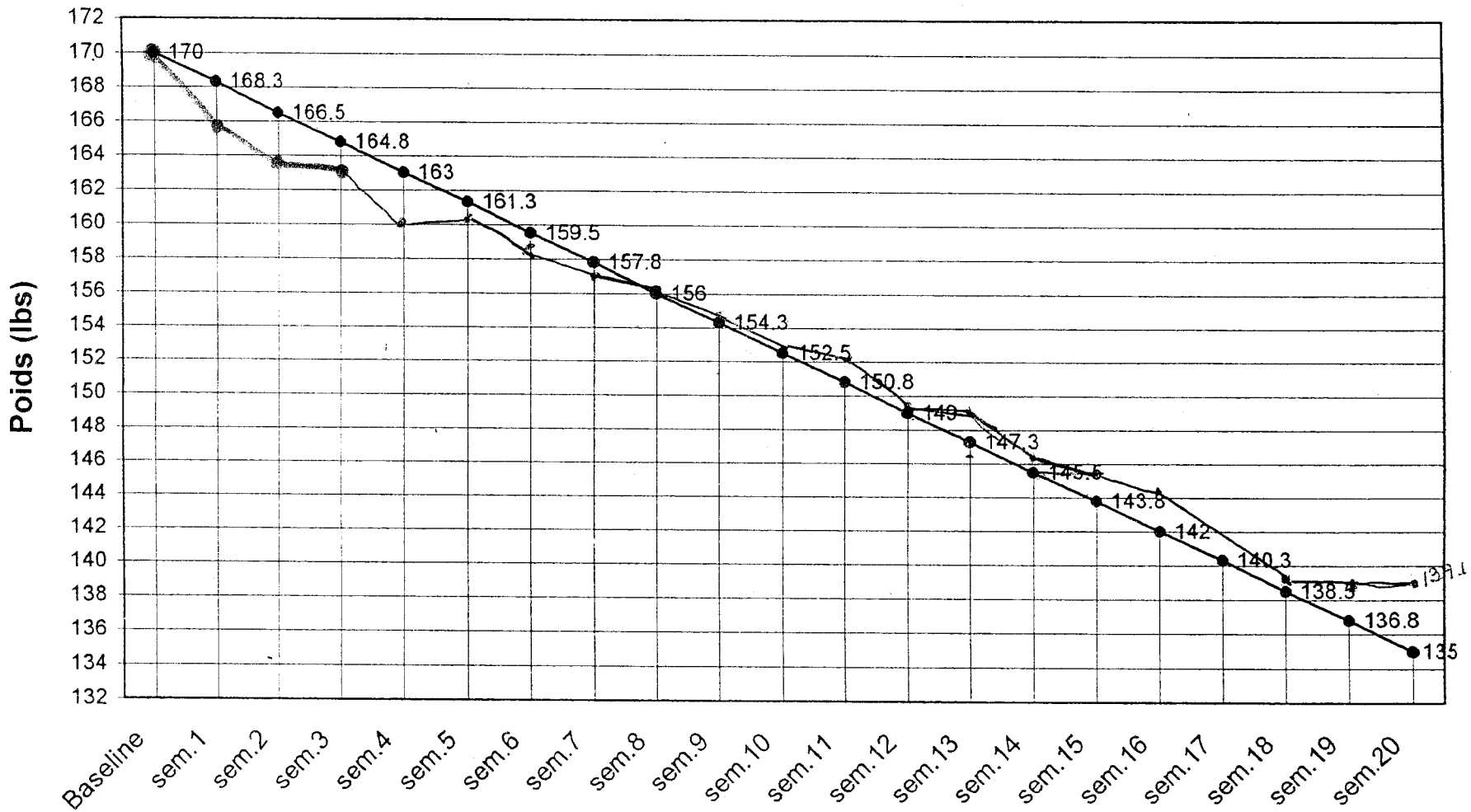
   Divide by 5 g.

   5 g ÷ 5 g = 1

   Therefore, this food has **1 Fat Exchange.**

Total Exchanges for this food is...2 Starches, 1 Protein, 1 Fat.
N.B. They don't always work out so evenly.
You can round for an estimate.

Courbe de perte de poids ideale



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Effect of weight loss resulting from a combined low-fat diet/exercise regimen on low-density lipoprotein particle size and distribution in obese women

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Abstract

Weight loss resulting from diet interventions has been shown to favorably affect low-density lipoprotein (LDL) particle size and distribution, and, hence, decrease cardiovascular disease risk. However, the effect of a dietary weight loss strategy when combined with exercise, on LDL electrophoretic characteristics, has yet to be tested. This study examined the effect of a weight loss intervention that combined a low-fat diet with moderate endurance training, on LDL particle size and distribution in obese women. Thirty obese, hypercholesterolemic women participated in a controlled longitudinal weight loss trial, which consisted of (1) a 2-week pre-stabilization phase, (2) a 20-week weight loss phase, and (3) a 2-week post-stabilization phase. Weight reduction resulted from a low-fat diet (<30% fat, 50%-60% carbohydrate, 20% protein) combined with an endurance training program (>40 minutes moderate training, 3 times per week). Mean weight loss was 14.8% ($P < .01$) of initial body weight. Total, LDL cholesterol, and triacylglycerol concentrations decreased ($P < .01$) by 8.9%, 7.5%, and 27.1%, respectively, whereas high-density lipoprotein cholesterol concentrations increased ($P < .01$) by 9.9%. No significant differences were noted for LDL peak or integrated particle size. The relative proportion of small, medium, and large particles was not significantly different posttreatment. Estimated cholesterol concentrations in large- and medium-sized LDL particles decreased ($P < .05$) by 15.3% and 5.9%, respectively, as a result of weight loss. No effect was noted for estimated cholesterol concentrations in small size LDL particles. In conclusion, these findings suggest that weight loss, resulting from a low-fat diet/exercise program, has only a minimal effect on LDL particle size and distribution.

1. Introduction

Results from several recent epidemiologic and clinical intervention trials indicate that obesity is a major independent risk factor for cardiovascular disease [1]. Accordingly, weight loss has been shown to favorably affect several indicators of cardiovascular risk, such as plasma lipid [2,3], homocysteine [4], and C-reactive protein [5] concentrations. In addition to these commonly investigated parameters, weight loss has also been shown to beneficially modulate low-density lipoprotein (LDL) size and distribution [6-10]. More specifically, Archer et al [6] demonstrated that a low-fat/high-carbohydrate diet reduced the average body weight of overweight men by 2%, while causing reductions in the cholesterol content of small- and medium-sized LDL particles. Similarly, Markovic et al [7] found that after consuming a low-fat diet for 28 days, mildly obese patients lost an average of 6.2 kg and increased the proportion of large LDL particles in plasma. Furthermore, in a study by Katzel et al [8], it was shown that an average loss of 10 kg of body weight, induced by a low-fat diet, resulted in a significant increase in LDL peak particle diameter. Although these data indicate that diet-induced weight loss positively alters LDL electrophoretic characteristics, the effect of a low-fat diet when placed in combination with exercise, another commonly implemented weight loss strategy, on LDL particle diameter, has yet to be tested. In addition, the

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effect of substantial weight loss, that is, loss of more than 10% of initial body weight, on LDL size and distribution still needs further clarification.

Thus, the objective of the present study was to examine the effect of a 24-week weight loss intervention that combined a low-fat diet with moderate endurance training, on LDL particle size and distribution in obese, hypercholesterolemic women. In addition, the extent to which substantial changes in body weight modulated LDL electrophoretic was also examined.

2. Subjects and methods

2.1. Subjects

Subjects were recruited from the greater Montreal area by means of newspaper advertisements. After a preliminary questionnaire, blood screening, and physical examination, 42 subjects were deemed eligible to partake in the trial. Key inclusion criteria were as follows: nonpregnant women; age of 35 to 60 years; body mass index (BMI) between 28 and 37 kg/m^2; LDL cholesterol (LDL-C) concentrations of greater than 4.5 mmol/L; triacylglycerol concentrations of greater than 1.5 mmol/L; free of cardiovascular disease; free of gastrointestinal, renal, pulmonary, hepatic, or biliary disease; free of cancer; no history of disordered eating; alcohol intake of less than 2 drinks per day; use of fiber or stimulant laxatives of less than 2 doses per week; not taking lipid-lowering medications for the past 6 months; and less than 16736 kJ/wk (4000 kcal/wk) expended by endurance training. The protocol was approved by the human ethical review committee of the Faculty of Medicine at McGill University (Montreal, Quebec, Canada). Before the commencement of the study, all volunteers gave their written informed consent to participate in the trial.

2.2. Experimental design

A 24-week longitudinal design was implemented as a means of testing the study objectives. Each subject acted as her own control, and therefore, no control group was required [11]. The trial consisted of 3 consecutive dietary periods: (1) a 2-week pre-loss stabilization phase, (2) a 20-week weight loss phase, and (3) a 2-week post-loss stabilization phase. During the first and third phases, subjects were required to maintain a stable weight and were instructed to continue with their usual food habits. In contrast, during the second phase, subjects were required to reduce their energy intake by 20% and increase their energy expenditure by 10%. As a result, target weight loss was approximately 0.75 kg/wk. Premenopausal women started each of the 3 phases at identical points during their menstrual cycles.

2.3. Dietary protocol

Each subject attended individual dietary counseling sessions with a registered dietician or nutritionist on a bi-weekly basis to decrease energy intake by 20% throughout the weight loss phase. During these sessions, volunteers were taught how to attain daily energy goals using an exchange system that provided 50% to 60% of energy from carbohydrates, 20% of energy from protein, and less than 30% of energy from fat. Teaching aids, which outlined the energy contents of commonly ingested food items, as well as sample menus and recipes, were distributed to the volunteers.

2.4. Exercise protocol

As a means of increasing energy expenditure by 10% throughout the 20-week weight loss phase, subjects were instructed to perform moderate aerobic training 3 times per week, for a minimum of 40 minutes. During the trial, each subject met with a personal trainer and was taught proper training techniques and routines. The exercise was performed independently at home or at the Mary-Emily Clinical Nutrition Research Unit.

2.5. Weight loss assessment and compliance

Compliance with both the dietary and exercise protocol was determined by way of weigh-ins that took place once per week throughout the trial. Continuous, regular weight loss throughout the second phase of the study was encouraged by means of a point system as well as visual graphs, which plotted weekly weight changes.

2.6. Blood collection protocol

Twelve-hour fasting blood samples were collected on the mornings of days 0, 13, 14, 15 (phase 1), and 167, 168, and 169 (phase 3) of the trial. Blood was centrifuged for 15 minutes at 520g and 4°C to separate plasma from red blood cells, and was stored at −20°C until analyzed.

2.7. Analyses

2.7.1. Plasma lipid profile determination

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triacylglycerol concentrations were measured in duplicate by automated methods through a Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN) using enzymatic or immunoturbidometric reagents [12]. Low-density lipoprotein cholesterol was determined directly by the dextran/magnesium sulfate method to separate it from HDL-C (N-geneous LDL-C assay, Equal Diagnostics, Exton, PA) [13,14].

2.8. Low-density lipoprotein particle size determination

Low-density lipoprotein particle diameter analysis was performed on whole plasma using nondenaturing 2% to 16% polyacrylamide gradient gel electrophoresis [15]. Plasma samples (3.5 μL) were mixed with a sampling buffer containing 20% sucrose and 0.25% bromophenol blue in a 1:1 (vol/vol) ratio. The 6 samples, representing days 13, 14, 15, 167, 168, and 169, for each individual subject were placed on one unique gel. After a 15-minute

prerun, electrophoresis was performed at 150 V for 3 hours. Gels were then stained for 1 hour with Sudan black (0.07%) and stored in a 0.81% acetic acid/4% methanol solution until analysis. The Imagemaster 1-D Prime computer software (Amersham Pharmacia Biotech, Piscataway, NJ) was used to analyze the gels. Mean LDL particle size was computed by integrating the relative contribution of each LDL particle subfraction within a sample and corresponding to the weighted mean of all LDL subfractions. Integrated LDL particle size was calculated as the sum of LDL subspecies' diameter multiplied by its relative proportion. The relative proportion of LDL having a diameter of less than 25.5 nm (termed $LDL\%_{<255\ Å}$) was obtained by computing the relative area of the densitometric scan of less than 25.5 nm. The absolute concentration of cholesterol within the LDL subfraction characterized by a diameter of less than 25.5 nm (termed $LDL\text{-}C_{<25.5\ nm}$) was crudely estimated by multiplying total plasma LDL-C concentrations by $LDL\%_{<25.5\ nm}$ as previously described [15]. A similar approach was used to assess the relative and absolute concentrations of cholesterol in the LDL subfractions with a diameter of more than 26 nm ($LDL\%_{>26\ nm}$ and $LDL\text{-}C_{>26\ nm}$, respectively) and in those with a diameter between 255 and 26 nm ($LDL\%_{25.5\text{-}26\ nm}$ and $LDL\text{-}C_{25.5\text{-}26\ nm}$, respectively).

2.9. Statistics

Results are presented as means ± SEM. A paired t test was implemented as a means of comparing baseline and posttreatment weight, lipid, LDL particle size, and distribution values. Pearson correlation coefficients were calculated to test for associations between treatment-induced changes in body weight, plasma lipid concentrations, and LDL electrophoretic characteristics. A level of statistical significance at $P < .05$ was used in all analyses. Data were analyzed using SAS software (version 8.0; SAS Institute, Cary, NC).

3. Results

3.1. Subject baseline characteristics and compliance

Forty-two subjects commenced the study, with 35 completing the entire 24-week trial. The 7 subjects who did not complete the trial dropped out because of an inability to comply with the study protocol. In addition, data from 5 subjects were not analyzed because of plasma sample loss. Therefore, after accounting for these loses, a total of 30 complete subject data sets were used for LDL particle size and distribution analysis. On day 0 of the trial, the mean age, body weight, and BMI of the 30 participants was 48.9 ± 1.24 years, 82.1 ± 1.74 kg, and 31.5 ± 0.52 kg/m^2, respectively. During the weekly meetings with the registered dietician/nutritionist, compliance with the energy-reduced, low-fat diet was reported to be adequate, as demonstrated by proper use of the checklist exchange system. Also during these meetings, reported increases in energy expenditure during the weight loss phase of the study were shown to be satisfactory, as the subjects reported increasing their physical activity levels by a minimum of 120 minutes per week.

3.2. Body weight and plasma lipid profiles

Changes in body weight, BMI, and plasma lipid profiles over the course of the 24-week trial are presented in Table 1. The mean rate of weight loss over the 20-week weight loss phase was 0.59 kg/wk (Fig. 1). From the beginning to the end of the trial, the 30 volunteers experienced a significant ($P < .01$) mean reduction in body weight of 12.0 ± 0.41 kg. When expressed as the difference between day 15 and day 169 values, subjects were shown to have decreased their overall body weight by 14.8%. Body mass index decreased significantly ($P < .01$) from 31.5 to 26.9 kg/m^2 from baseline to posttreatment.

As reported previously [16], plasma lipid parameters were significantly altered as a result of weight loss. Total cholesterol and LDL-C concentrations decreased significantly ($P < .01$) by 8.9% and 7.5%, respectively, after the intervention period. In addition, HDL-C concentrations increased ($P < .01$) by 9.9%, whereas triacylglycerol concentrations decreased by 27.1% from baseline to the end of the trial.

3.3. Low-density lipoprotein peak particle size, integrated size, and distribution

Low-density lipoprotein peak particle size, integrated size, and distribution over the course of the trial are presented in Table 2. No differences were noted with respect to LDL peak particle size from the beginning to the end of the trial. Similarly, no significant differences were noted for LDL integrated size when baseline values were compared with posttreatment values. Furthermore, the relative distributions of cholesterol among small (<25.5 nm), medium (25.5-26 nm), and large (>26 nm) LDL particles showed no significant differences from baseline to posttreatment. However, with respect to the absolute distribution of the different LDL particle subfractions, the estimated

Table 1
Body weight, BMI, and plasma lipid concentrations at baseline and posttreatment

n = 30	Baseline	Posttreatment	% Change	P^a
	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Body weight (kg)	82.14 ± 1.74	70.09 ± 1.71	−14.80 ± 0.52	<.01
BMI	31.53 ± 0.52	26.90 ± 0.52	−14.80 ± 0.52	<.01
Total cholesterol (mmol/L)	6.05 ± 0.16	5.46 ± 0.11	−8.94 ± 1.78	<.01
LDL-C (mmol/L)	3.77 ± 0.12	3.44 ± 0.09	−7.54 ± 2.21	<.01
HDL-C (mmol/L)	1.17 ± 0.04	1.27 ± 0.05	9.92 ± 3.01	<.01
Triacylglycerol (mmol/L)	1.80 ± 0.13	1.25 ± 0.08	−27.14 ± 3.59	<.01

[a] P value within group: Student paired t test comparing day 15 values to day 169 values.

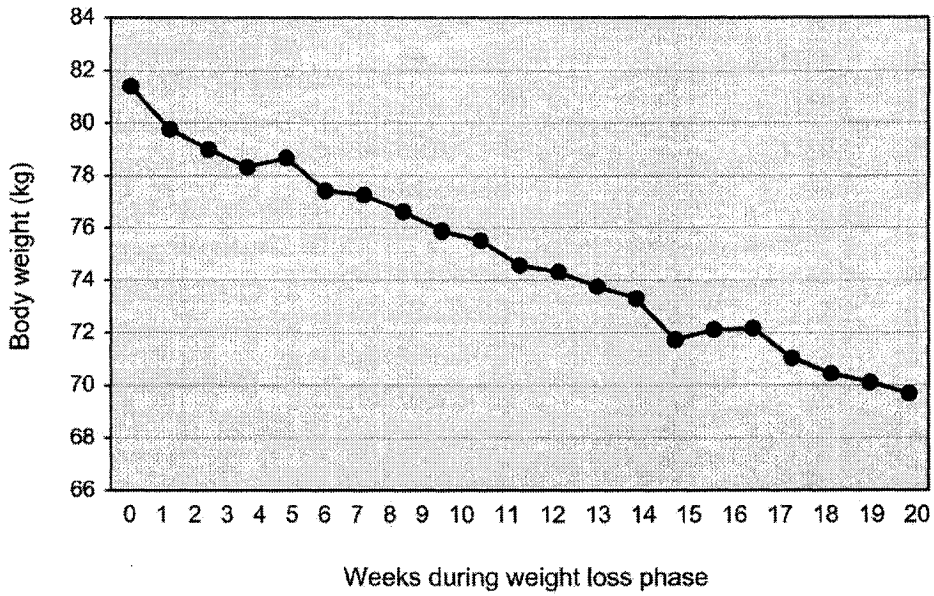

Fig. 1. Body weight reduction during the 20-week weight loss phase. The data presented are the mean body weight values at each week of the trial. The mean rate of weight loss over the 20-week weight loss phase was 0.59 kg/wk. Posttreatment body weight values were shown to be significantly different ($P < .01$) when compared with baseline (Student paired t test).

cholesterol concentration in large-sized LDL particles was shown to decrease significantly ($P < .05$) by 15.3% (0.80-0.68 mmol/L) from the beginning to the end of the trial. In addition, the estimated cholesterol concentration in medium-sized LDL particles was shown to decrease ($P < .05$) by 5.9% (1.39-1.31 mmol/L) posttreatment. No significant changes were noted for the estimated cholesterol concentration in small LDL particles.

3.4. Correlation between body weight and LDL particle size

Body weight has been shown to correlate with variations in LDL particle size and distribution. For this reason, analyses were performed to see if the substantial decrease in body weight resulting from the diet/exercise intervention had an effect on LDL electrophoretic characteristics. Correlational analysis revealed that the greater change in body weight over the course of the trial was associated with increased LDL peak particle size posttreatment ($r = -0.14$, $P = .03$).

3.5. Correlation between triacylglycerol concentrations and LDL particle size

Posttreatment triacylglycerol concentrations were not significantly correlated with any of the posttreatment LDL particle size or distribution parameters measured. In addition, posttreatment triacylglycerol concentrations showed no significant associations with either percent body weight change or posttreatment body weight.

4. Discussion

The results of the present study indicate that a substantial amount of weight loss, that is, loss of approximately 15% of initial body weight, resulting from a low-fat diet combined

Table 2
Low-density lipoprotein peak particle size, integrated size, and distribution at baseline and after a 24-week diet/exercise weight loss intervention

n = 30	Baseline[a]	Posttreatment[b]	% Change[c]	P[d]
	Mean ± SEM	Mean ± SEM	Mean ± SEM	
LDL peak particle size (nm)	25.626 ± .041	25.623 ± .026	−0.01 ± 0.12	.913
LDL integrated size (nm)	25.627 ± .040	25.615 ± .020	−0.05 ± 0.11	.678
$LDL\%_{>26\ nm}$ (%)	20.93 ± 2.34	20.07 ± 1.37	−4.12 ± 6.20	.574
$LDL\%_{26\text{-}25.5\ nm}$ (%)	36.96 ± 1.47	37.95 ± 1.34	2.68 ± 4.07	.317
$LDL\%_{<25.5\ nm}$ (%)	42.26 ± 2.27	42.10 ± 1.81	−0.38 ± 5.36	.909
$LDL\text{-}C_{>26\ nm}$ (mmol/L)	0.80 ± 0.10	0.68 ± 0.05	−15.35 ± 7.96	.043
$LDL\text{-}C_{25.5\text{-}26\ nm}$ (mmol/L)	1.39 ± 0.07	1.31 ± 0.06	−5.87 ± 3.79	.048
$LDL\text{-}C_{<25.5\ nm}$ (mmol/L)	1.58 ± 0.09	1.46 ± .09	−7.82 ± 5.37	.100

[a] Mean of day 13, 14, and 15 values.
[b] Mean of day 167, 168, and 169 values.
[c] Percent change comparing the mean of day 13, 14, and 15 values to the mean of day 167, 168, and 169 values.
[d] P value within group: Student paired t test comparing the mean of day 13, 14, and 15 values to the mean of day 167, 168, and 169 values.

with exercise, decreases the estimated cholesterol concentrations of large and medium LDL particles. The relative proportion of small, medium, and large particles, however, were not significantly altered by this decrease in body weight.

Results of several recent clinical trials show that losing a considerable amount of body weight has favorable effects on certain indicators of cardiovascular disease risk [17-19]. Therefore, it would be expected that in the present intervention trial, which produced a substantial amount of weight loss, certain cardiovascular risk factors such as LDL particle size would be ameliorated. Although such favorable alterations have been previously reported [6-9], the results of the present study demonstrate that this magnitude of weight loss has a minimal effect on LDL particle distribution. More specifically, this study reports a decrease in cholesterol concentrations in both large and medium LDL particles, as a result of a 15% diet/exercise-induced weight reduction. No increase in the cholesterol content of small LDL particles, however, was noted in response to these cholesterol shifts between subfractions. There are several possible explanations that may account for these unexpected findings. First and foremost, these results could partially be explained by the low-fat diet regimen. Recent findings suggest that LDL particles shift from larger, less atherogenic, to smaller, more atherogenic, particles in direct proportion to the degree to which dietary fat is replaced by carbohydrate [20]. Thus, because the subjects were required to lower their fat intake to less than 30% of total energy, and hence, increase their carbohydrate intake to approximately 60% of total energy, it can be assumed that this shift in macronutrient distribution may be responsible for the present findings. Secondly, it is possible that the decrease in cholesterol within large and medium LDL subfractions may be attributed to the physical activity component of the study. More specifically, in a recent study by Varady et al [21], it was demonstrated that participation in a moderate-intensity exercise program decreased the peak particle size of previously sedentary, hypercholesterolemic adults. However, contradictory to these findings [21], other studies that have examined this relationship have reported either an increase in LDL peak particle size as a result of exercise [22-25] or no effect [26]. For instance, in 2 trials [22,23] that examined the effect of endurance training on LDL particle size in obese men, the number of small, dense LDL particles was shown to decrease as a result of training. In line with these findings, Kang et al [24] demonstrated that physical activity had a beneficial effect on LDL particle diameter in obese adolescents. In contrast, Elosua et al [26] demonstrated that after a 16-week training period, no changes were observed with regard to LDL particle diameter in previously sedentary men and women. In view of these findings, because most of the studies in the area suggest a beneficial effect of exercise on LDL electrophoretic characteristics, it is unlikely that the exercise intervention produced these slightly deleterious changes in LDL particle size. Thus, before it can be concluded that exercise is responsible for these unfavorable effects, it is essential that the results of the Varady et al [21] trial be supported by other independent studies testing similar objectives.

Interestingly, correlational analysis revealed a weak but significant association between greater weight loss and an increase peak LDL particle size ($r = -0.14$, $P = .03$).

These associative findings are, evidently, contrary to the causal findings reported in the present article. The reason for these conflicting results is not clear. However, one possible explanation for these contradictory findings may again be the change in diet composition. Because replacing fat with carbohydrate leads to a decrease in particle size, it can be hypothesized that, if this change in diet was not implemented, LDL size may have increased in response to weight loss. Then again, because these findings are merely associative, and also quite weak, these correlational results should not put into question the results of the causative findings. Nevertheless, these contradictory results may suggest that, when attempting to assess the effect of weight loss on vascular disease risk, LDL particle size analysis should be performed in conjunction with other more well-established indicators [27].

In summary, results of the present study demonstrate that a substantial amount of weight loss resulting from a low-fat diet and exercise regimen has no effect on the distribution of small, medium, and large LDL particles. In addition, these data suggest that this weight loss regimen decreases the estimated cholesterol concentrations of large- and medium-sized LDL particles. However, no increase in cholesterol within small LDL particles was observed in response to these cholesterol shifts between subfractions. These findings suggest that low-fat diet/exercise-induced weight loss only minimally affects LDL particle size and distribution. Therefore, when viewed in terms of modulating LDL electrophoretic characteristics, the effect of low-fat diet/exercise-induced weight loss on cardiovascular risk reduction cannot be inferred from the present data.

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References

[1] Scaglione R, Argano C, Di Chiara T, Licata G. Obesity and cardiovascular risk: the new public health problem of worldwide proportions. Expert Rev Cardiovasc Ther 2004;2:203-12.

[2] Gerhard GT, Ahmann A, Meeuws K, McMurry MP, Duell PB, Connor WE. Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. Am J Clin Nutr 2004;80:668-73.

[3] Yancy Jr WS, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. Ann Intern Med 2004; 140:769-77.

[4] Noakes M, Foster PR, Keogh JB, Clifton PM. Meal replacements are as effective as structured weight-loss diets for treating obesity in adults with features of metabolic syndrome. J Nutr 2004;134:1894-9.

[5] Brook RD, Bard RL, Glazewski L, Kehrer C, Bodary PF, Eitzman DL, et al. Effect of short-term weight loss on the metabolic syndrome and conduit vascular endothelial function in overweight adults. Am J Cardiol 2004;93:1012-6.

[6] Archer WR, Lamarche B, St-Pierre AC, Mauger JF, Deriaz O, Landry N, et al. High carbohydrate and high monounsaturated fatty acid diets similarly affect LDL electrophoretic characteristics in men who are losing weight. J Nutr 2003;133:3124-9.

[7] Markovic TP, Campbell LV, Balasubramanian S, Jenkins AB, Fleury LA, Simons LA, et al. Beneficial effect on average lipid levels from energy restriction and fat loss in obese individuals with or without type 2 diabetes. Diabetes Care 1998;21:695-700.

[8] Katzel LI, Coon PJ, Rogus E, Krauss RM, Goldberg AP. Persistence of low HDL-C levels after weight reduction in older men with small LDL particles. Arterioscler Thromb Vasc Biol 1995;15:299-305.

[9] Purnell JQ, Kahn SE, Albers JJ, Nevin DN, Brunzell JD, Schwartz RS. Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. J Clin Endocrinol Metab 2000; 85:977-82.

[10] Wood RJ, Volek JS, Liu Y, Shachter NS, Contois JH, Fernandez ML. Carbohydrate restriction alters lipoprotein metabolism by modifying VLDL, LDL, and HDL subfraction distribution and size in overweight men. J Nutr 2006;136:384-9.

[11] Donnelly JE, Jacobsen DJ, Heelan KS, Seip R, Smith S. The effects of 18 months of intermittent vs. continuous exercise on aerobic capacity, body weight and composition, and metabolic fitness in previously sedentary, moderately obese females. Int J Obes Relat Metab Disord 2000;24:566-72.

[12] McNamara JR, Schaifer EJ. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. Clin Chim Acta 1987; 166:1-8.

[13] Wang TY, Haddad M, Wang TS. Low triglyceride levels affect calculation of low-density lipoprotein cholesterol values. Arch Pathol Lab Med 2001;125:404-5.

[14] Iannotti RN, DeAngelis K, Law T. Analytical and clinical performance of a homogeneous enzymatic LDL-cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg^{2+} method. Clin Chem 1998;44:1242-50.

[15] St-Pierre AC, Ruel IL, Cantin B, Dagenais GR, Bernard PM, Despres JP, et al. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. Circulation 2001;104:2295-9.

[16] Santosa S, Demonty I, Lichtenstein A, Cianflone K, Jones PJ. Weight loss and its effects on lipids, leptin, and adiponectin in overweight and obese, hyperlipidemic women. J Am Coll Nutr 2006:1-7.

[17] Tanaka K, Okura T, Shigematsu R, Nakata Y, Lee DJ, Wee SW, et al. Target value of intra-abdominal fat area for improving coronary heart disease risk factors. Obes Res 2004;12:695-703.

[18] Janssen I, Fortier A, Hudson R, Ross R. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. Diabetes Care 2002;25:431-8.

[19] Anderson JW, Konz EC. Obesity and disease management: effects of weight loss on comorbid conditions. Obes Res 2001;9:326-34.

[20] Desroches S, Lamarche B. Diet and low-density lipoprotein particle size. Curr Atheroscler Rep 2004;6:453-60.

[21] Varady KA, St-Pierre AC, Lamarche B, Jones PJ. Effect of plant sterols and endurance training on LDL particle size and distribution in previously sedentary hypercholesterolemic adults. Eur J Clin Nutr 2005;59:518-25.

[22] Halle M, Berg A, Garwers U, Baumstark MW, Knisel W, Grathwohl D, et al. Influence of 4 weeks' intervention by exercise and diet on low-density lipoprotein subfractions in obese men with type 2 diabetes. Metabolism 1999;48:641-4.

[23] Williams PT, Krauss RM, Vranizan KM, Wood PD. Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. Circulation 1990;81: 1293-304.

[24] Kang HS, Gutin B, Barbeau P, Owens S, Lemmon CR, Allison J, et al. Physical training improves insulin resistance syndrome markers in obese adolescents. Med Sci Sports Exerc 2002;34:1920-7.

[25] Beard CM, Barnard RJ, Robbins DC, Ordovas JM, Schaefer EJ. Effects of diet and exercise on qualitative and quantitative measures of LDL and its susceptibility to oxidation. Arterioscler Thromb Vasc Biol 1996;16:201-7.

[26] Elosua R, Molina L, Fito M, Arquer A, Sanchez-Quesada JL, Covas MI, et al. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. Atherosclerosis 2003; 167:327-34.

[27] Sacks FM, Campos H. Clinical review 163: cardiovascular endocrinology: low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab 2003;88:4525-32.