HIGHER PROTEIN AND DAIRY DIETS FOR HIGH QUALITY WEIGHT LOSS

BODY COMPOSITION AND BONE HEALTH DURING HYPOENERGETIC DIET- AND EXERCISE-INDUCED WEIGHT LOSS ARE ENHANCED BY DIETS HIGHER IN DAIRY FOODS AND DIETARY PROTEIN

BY

ANDREA R. JOSSE, B.Kin., M.Sc.

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AUTHOR: Andrea R. Josse, B.Kin. (McMaster University), M.Sc. (University of Toronto)

SUPERVISOR: Dr. Stuart M. Phillips

SUPERVISORY COMMITTEE: Dr. Stephanie A. Atkinson

Dr. Mark A. Tarnopolsky

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ABSTRACT

Obesity is a major health concern. Strategies to reduce obesity including weight loss by energy restriction have disease risk reduction benefits, however, energy restriction alone often leads to the loss of muscle mass. Muscle is a very important tissues in the body particularly from a metabolic standpoint, thus, efforts to maintain it by promoting weight loss with the greatest ratio of fat:lean mass loss should be implemented. Also, bone health may be negatively affected by weight loss if hypoenergetic diets are suboptimal in calcium. Hence, the objective of this thesis was to determine how hypoenergetic diets varying in protein (amount and type) with exercise impacted the composition of weight lost and bone health in premenopausal, overweight and obese women. Ninety women were randomized to three groups (n=30/group): HiDairyPro, DairyPro and Control, differing in the quantity of total protein consumed (30%, 15% or 15% of energy, respectively) and the amount from dairy foods (high, moderate or low, respectively). Body composition was measured by DXA and fourier-transform near infrared spectroscopy (FT-NIR) at 0, 8 and 16 weeks, and visceral adipose tissue by MRI (*n*=39) at 0 and 16 weeks. Blood and urine samples were taken at 0 and 16 weeks. All groups lost similar body weight, but HiDairyPro lost significantly more total and visceral fat, and gained significantly more lean mass than Control (Chapter 2). HiDairyPro significantly improved bone health and vitamin D status compared to Control (Chapter 3). DXA and FT-NIR measured fat mass correlated and agreed well with each other (Chapter 4). Therefore, diet- and exercise-induced weight loss with higher protein and dairy promoted more favourable body composition changes and

improved bone health versus diets with lower protein and no dairy. These data have strong implications for the design of weight loss programs to combat obesity.

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For Jonathan. My love and my inspiration.

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FORMAT AND ORGANIZATION OF THESIS

This thesis was prepared in the "sandwich format" as outlined by the School of Graduate Studies in the 'Guide for the Preparation of Doctoral Theses'. This thesis is comprised of 3 original research papers (Chapters 2-4), preceded by a general introduction and followed by a general discussion/conclusion. Two papers are published and one is under review. All papers have the candidate as first author.

CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORSHIP

Chapter 2

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Contribution

AR Josse and SM Phillips conceived the design of the experiment. The principal investigator for this study was SM Phillips, co-investigator was AR Josse. Assistance in study planning was provided by SA Atkinson and MA Tarnopolsky. AR Josse was the main clinical trial study coordinator. Data collection, analytical analyses and statistical analyses were carried out by AR Josse and SM Phillips. AR Josse, SM Phillips, SA Atkinson and MA Tarnopolsky helped interpret the data and write the final manuscript, and all authors read and approved the final version of the paper.

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Contribution

AR Josse, SM Phillips, H Azizian and JKG Kramer conceived the design of the experiment. AR Josse and SB French coordinated the body fat measurements. H. Azizian acquired the FT-NIR measurements and AR Josse performed the DXA scans. Data was organized by AR Josse and SB French, analyzed by AR Josse, H Azizian, JKG Kramer and SM Phillips, and statistical analyses were carried out by AR Josse, SM Phillips and H Azizian. AR Josse, SM Phillips, SB French, H Azizian and JKG Kramer helped interpret the data and write the final manuscript, and all authors read and approved the final version of the paper.

CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

The prevalence of obesity is increasing in Canada and worldwide, which poses an important public health concern (1, 2). According to the Canadian Health Measures Survey from 2007 to 2009, 23% of Canadian women between the ages of 18-39 were overweight and 20% were obese as measured by BMI (3). Importantly, rates of abdominal obesity in women of the same age, measured by waist circumference, indicated that 31% had measures that put them at increased risk for metabolic disease (≥88 cm) (3, 4). Accompanying the growing problem of obesity is the burgeoning increase in the prevalence of associated co-morbidities such as coronary heart disease (CHD), type 2 diabetes, hypertension and certain cancers (5, 6). Some obesity treatment strategies include pharmacotherapy and different surgical options (7-9), however these treatments are not suitable for everyone nor practical, from a financial standpoint, for widespread use. Thus, to achieve weight loss, lifestyle-oriented strategies focusing on reducing energy intake and increasing physical activity should be implemented (10) since they may represent, from a global health perspective, a more viable solution.

Weight loss through diet and exercise in overweight and obese persons can confer a host of benefits including decreased diabetes and heart disease risk, increased vitality,

increased self confidence and efficacy with particular tasks, and improved quality of life (11, 12). However, calorie restriction alone, while it leads to weight loss, may be of concern since the composition of tissue mass that is lost includes both fat and lean (muscle) tissue (13), and possibly bone, particularly if calcium intakes are suboptimal during the period of energy restriction (14). Muscle is a metabolically active tissue, and its loss can have both short and long term health consequences. By virtue of its mass, skeletal muscle is a large contributor to resting metabolic rate (RMR) (15), and the largest site in the body for postprandial glucose uptake and storage, as well as lipid oxidation. Hence, the loss of muscle mass and subsequent decline in RMR, during diet-only weight loss may be responsible for the 'weight loss plateau' commonly seen during a conventional program or the tendency for weight regain following its completion (16, 17). In terms of metabolic health, the loss of skeletal muscle can affect the capacity for glucose uptake, and lipid storage and oxidation (13, 18), which may oppose the benefits of losing body weight. The importance of maintaining muscle mass during weight loss highlights that weight loss strategies should focus more on the tissue composition of what is lost rather than just body weight on a scale. Thus, weight loss programs should be tailored towards promoting fat mass loss, especially visceral fat and the sparing of muscle and bone.

The major aim of the study outlined in this thesis was to examine how best to achieve weight loss of the highest 'quality'. High 'quality' weight loss can be defined as the loss of body weight with the greatest ratio of fat to lean mass loss. This pattern, as outlined above, is very important not only for short-term weight loss efficacy but also for long term metabolic health and disease risk reduction. Thus, we designed and undertook a prospective

randomized controlled parallel clinical trial of differing diets to induce weight loss. Our approach was multifaceted and incorporated several aspects that have proven to be important in helping to preserve muscle and bone mass and preferentially promote the loss of fat mass. These included: dietary energy restriction, daily exercise (with both aerobic and resistance components), higher dietary protein (and lower carbohydrate) and higher dairy (with a concomitant increase in dietary calcium and vitamin D). In addition, since dairy, calcium and protein intakes were graded between groups, we assessed bone health outcomes in premenopausal women. As an extension of the primary endpoint, we also assessed several different ways to measure body composition using new and well established techniques.

1.2. THE ROLE OF DAIRY IN BODY COMPOSITION AND WEIGHT LOSS

Dairy foods contain components that, for the most part, either alone or in combination, can help to promote weight and fat loss. Epidemiological and clinical evidence, and several well-studied mechanisms exist in support of a dairy 'advantage' in weight loss which are detailed below in the following sections.

1.2.1. Dairy, body composition and weight loss: epidemiological evidence

In 1984, after examining the first National Health and Nutrition Examination Survey (NHANES), McCarron et al. demonstrated that higher calcium intakes were negatively associated with BMI and that those in the highest quartile of dietary calcium intake had a significantly reduced odds ratio (OR) of 0.16 of also being in the highest quartile for body

fat (19). Subsequently, Zemel et al. examined the NHANES III database and demonstrated the same association for both calcium intakes and dairy product consumption (20). Since then, many other cross-sectional, observational, retrospective and prospective studies have examined the association between dairy consumption and body weight, body composition or central adiposity (21-28). Most of the studies discussed here are those that examined associations with dairy or dairy-derived calcium intakes primarily in relatively healthy younger women or in women with a wider age range encompassing the adult premenopausal time period.

Dairy product and or milk consumption correlated inversely with BMI/obesity in several independent cohorts of younger women including those from Iran (21), Brazil (22), Israel (23), and the USA (24). However, this association was not observed in a cohort of lean (mean BMI of 20.8 kg/m^2), young women from Japan with habitually low dairy consumption (25), possibly suggesting a minimum threshold level for either dairy consumption or body weight. Two other studies examined the relationship between dairy consumption and central obesity and found dairy to be inversely associated with larger waist circumferences (26, 27). Interestingly, in a study by Beydoun et al., the type of dairy seemed to affect the associational relationship between dairy intake and obesity such that low fat dairy (milk and yogurt) were negatively correlated whereas cheese was positively correlated (28).

In addition to data from cross-sectional studies, retrospective analyses of four clinical trials also demonstrate a positive effect of dairy consumption on body weight in young to middle-aged women (29-32). Based on data from a two-year prospective

exercise intervention, Lin et al. predicted that young normal-weight women consuming 1900 kcal per day with a low calcium intake (600 mg per day) would only lose 0.26 kg of body fat over two years versus a 2.8 kg fat loss if they had a calcium intake of 1000 mg per day (and a vitamin A intake in both cases of 4000 IU per day) (29). In another two-year trial in women and men with either obesity, diabetes, or CHD assigned to various weight loss diets, both higher dairy-derived calcium intakes and increased serum vitamin D levels were independently associated with greater weight loss (30). Davies et al. showed similar results with respect to body weight, and also reported that in young women, a 100 mg increase in dietary calcium (mostly from dairy sources) may result in ~0.80 kg weight loss per year (31). Lastly, in the eighteen months following a twenty two week weight loss regimen, and after controlling for changes in energy intake, greater calcium intakes from dairy were inversely associated with weight regain in obese premenopausal women (32). Moreover, the authors calculated that, at a constant energy intake, a 100 mg per day increase in dietary calcium was associated with 1.6 kg less weight regain over the eighteen months.

Two important prospective studies in young adults with follow-up periods of at least six years were carried out to assess the effect of dairy intake on obesity; the Coronary Artery Risk Development in Young Adults (CARDIA) study (33), and the Quebec Family Study (QFS) (34, 35). After ten years of follow-up, the CARDIA study revealed a 20% lower incidence of obesity between the lowest and highest quintiles (0-1 servings per day versus ≥ 5 servings per day) of dairy intake in young adults who were overweight at baseline (33). In the QFS, BMI, body weight, fat mass, percent body fat,

waist circumference, and abdominal adipose tissue (assessed by CT) were all significantly greater in women reporting low calcium intakes (< 600 mg per day) over six years versus women with higher calcium intakes (> 600 mg per day) (34). According to food frequency questionnaires and food records, ~62% of the daily calcium intake in women was from dairy foods. Moreover, when eating patterns were assessed in the whole study, only the consumption of whole fruit, skim milk and partly skim milk were negatively associated with gains in body-weight, percent body fat and waist circumference (35).

Epidemiological evidence for the link between higher dairy and obesity may not be as strong in men and possibly also in postmenopausal women (not discussed here). However, results from the aforementioned studies do show an effect in younger women, most of whom were overweight, with a relatively consistent positive association between higher intakes of dairy or dairy-derived calcium and several indices of obesity. Of note, a prospective study with twenty three years of follow up carried out in a young Dutch population observed no associations between dairy and body composition (36), however, habitual calcium intakes (~1100 mg per day; most from dairy) were found to be at least 200 mg per day higher than those seen in Western populations. Thus, it is likely, given data from the QFS and the Dutch study that a threshold level for calcium of ~800 mg per day may exist, above which little additional benefit with respect to weight loss or body fat loss is observed.

1.2.2. Dairy, body composition and weight loss: evidence from clinical trials

Only a handful of clinical trials have been undertaken examining the effect of increasing dairy product consumption on body weight loss or body composition change in the context of energy restriction in young women. Other well-designed clinical trials with dairy consumption have been conducted during weight maintenance (recently reviewed in (37, 38)), and with calcium supplementation alone (not dairy) during weight loss (39, 40), but these will not be discussed here.

The relationship between dairy and weight loss, and dairy and body composition change has been explored in five clinical trials in overweight or obese young women (41-45). In the first study by Zemel et al., twenty nine obese women were randomized to energy restricted diets (-500 kcal per day) with low (0-1 serving per day) or high dairy (3 servings per day) for twenty four weeks. Participants consuming 3 servings of dairy per day versus only 1 serving per day lost significantly more body weight (-11 kg vs. -9 kg, respectively), body fat (-9 kg vs. -4 kg, respectively) and trunk fat (-4.2 kg vs. 0.8 kg, respectively). As well, lean body mass was preserved to a greater extent in the group consuming dairy (-0.15 kg vs. -2.0 kg) (45). Similar results were observed in a second study in young obese adults where subjects were given yogurt or placebo for twelve weeks revealing an 81% greater reduction in trunk fat in the yogurt group (-3.2 kg vs. 1.7 kg) (44). Despite these positive results, three other clinical trials failed to demonstrate an effect of increasing dairy intake on body weight or body fat loss with energy restriction. In these studies there are various reasons cited as to why this occurred (41-43). In the first study, Bowen et al. determined that in the context of higher protein intakes (34% protein),

the type of protein (dairy or mixed) did not disparately affect weight loss (41). In the second, authors noted two possible reasons for why they did not see an effect; baseline calcium intakes were closer to 700 mg per day not ~500 mg per day, and they may have been under powered to detect smaller differences in body weight or fat (42). In the third, when comparing subjects consuming 2 servings of dairy (~800 mg calcium per day) with those consuming 4 servings of dairy, (~1400 mg per day) there was no enhanced weight loss, possibly because the 'lower dairy' group was above the proposed calcium-benefit threshold (43).

Four other clinical trials assessed the effects of diets high in dairy-derived calcium versus supplemental calcium on weight loss during energy restriction (46-49). Following twenty four weeks of energy restriction with dietary calcium and supplemented calcium at comparable dosages (1200 mg per day), those consuming dairy had significantly greater weight and fat loss than those who took a supplement, and both calcium groups showed significantly greater losses compared to the low calcium group (~500 mg per day) (48). These data were subsequently supported by a recent multicentre study of ninety three subjects (47). In this trial, the high dairy group (1400 mg per day calcium) showed significantly greater total and trunk fat loss compared to a high calcium (1400 mg per day) and low calcium (<600 mg per day) group; however no differences in weight loss between groups were observed. A third clinical trial of eight weeks duration put low fat milk (3 servings per day), calcium-fortified soy beverage (3 servings per day) and calcium carbonate (800 mg per day) head to head, with all subjects consuming ~1300 mg per day calcium in different ways, and demonstrated that milk had the greatest positive

effect on body weight, BMI and waist circumference versus the other treatments (49). On the other hand, Wagner et al. observed no differences between groups for fat or weight loss; in fact, the milk group lost the least amount of body fat compared to the other groups (46). In this study, baseline calcium levels for all groups were ~800 mg per day, therefore, all groups except the placebo consumed ~1500 mg per day of calcium during the intervention. It is possible that no additional benefit was seen by those supplemented with calcium/dairy because the control group had higher baseline intakes. Moreover, in this study, no additional information was provided regarding actual macronutrient intakes and daily calories consumed, and this may be able to explain, at least in part, the lack of fat mass loss in the milk group.

A recent review by Van Loan put forth four important elements apparent in the trials with energy restriction mentioned above that have demonstrated a positive effect of dairy on weight loss and/or body composition (37); these elements are: 1) all individuals were overweight or obese at baseline; 2) calcium intakes were habitually inadequate (<600 mg per day); 3) an appropriate control group that maintained the status quo (low) calcium intake was used; and 4) a moderate caloric deficit was used and maintained to promote weight loss (~500 kcal per day). Clinical trials that did not necessarily follow this framework (some of which are detailed above) have shown mixed results (40-43, 46, 50). It is also important to note that only two clinical trials (43, 46) assessed dairy and weight loss with some form of exercise, and both showed no added benefit with increased dairy consumption possibly owing to the lack of a properly structured exercise regimen and, as mentioned above, less of a difference in dairy/calcium intakes between the groups.

1.2.3. Dairy, body composition and weight loss: mechanisms of action

Mechanistic evidence exists illustrating a plausible link between dairy, and/or its associated components, in promoting high quality weight loss during an energy deficit. The proposed mechanisms involve one or more of the following: a hormonally mediated calcium and vitamin D interaction that affects adipocyte lipogenesis and lipolysis as well as fat oxidation; a calcium-mediated reduction in intestinal fat absorption and increase in fecal fat excretion; and a calcium-mediated regulation of appetite and food intake. Dairy foods also contain high quality protein (branched chain amino acids particularly in the whey fraction), and other bioactive peptides which, through independent mechanisms from those outlined above, may further promote fat mass loss and/or muscle mass retention. This discussion will be restricted to the three main mechanisms listed above as well as a discussion on the effect of dairy protein on body composition and appetite. It is noted that other potential anti-obesity mechanisms do exist, including the effects of dairy carbohydrates (lactose) and dairy fats (conjugated linoleic acid and other medium chain fatty acids) on body composition (38), but these will not be discussed here.

1.2.3.1. Hormonally mediated calcium and vitamin D interaction that affects adipocyte metabolism (lipogenesis and lipolysis) and fat oxidation

Dietary calcium (from dairy foods) may regulate energy metabolism and adiposity through its direct relation to the calcitropic hormones, parathyroid hormone (PTH) and

calcitriol (1,25-dihydroxyvitamin D [1,25[OH]2D]) (51, 52). Reduced serum calcium, in response to a low dietary calcium intake, stimulates the release of PTH from the parathyroid gland. PTH activates renal 1α-hydroxylase which converts 25-hydroxyvitamin D (25[OH]D) to its active metabolite, 1,25[OH]2D (53). These hormones collectively stimulate the intestine, kidneys and bone to respectively absorb, reabsorb and release calcium thereby restoring serum calcium levels (53). In the context of low calcium intakes, 1,25[OH]2D directly affects the adipocyte by allowing calcium channels to open thus increasing intracellular calcium levels (54). The increase in cytoplasmic calcium in the adipocyte stimulates the expression and activity of the key enzyme responsible for intracellular lipogenesis; fatty acid synthase (FAS) (52, 55, 56). As shown in **Figure 1,** high intracellular calcium levels also inhibit lipolysis which further promotes triglyceride storage (56). Thus, decreasing the circulating levels of PTH and calcitriol, by consuming an adequate amount of dietary calcium, may act to decrease intra-adipocyte calcium resulting in less stimulation of *de novo* lipogenesis and relief of the lipolytic inhibition. This would favour fat mobilization and a subsequent decrease in body fat mass. The intra-adipocyte fat metabolism hypothesis is supported by several cell culture and animal studies (55, 57-60), but controversy exists regarding its direct effect on increasing fat oxidation in humans with some studies showing positive effects (61, 62) and others not (63, 64). Nevertheless, several clinical studies (44, 45, 47, 48), but not all (as discussed above), demonstrate that feeding higher dairy under conditions of energy restriction significantly decreases total fat and visceral fat mass. In support of a role for other

nutrients beyond calcium, studies have shown that dairy-derived calcium may be more efficacious in decreasing total fat and visceral fat mass than supplemental calcium.

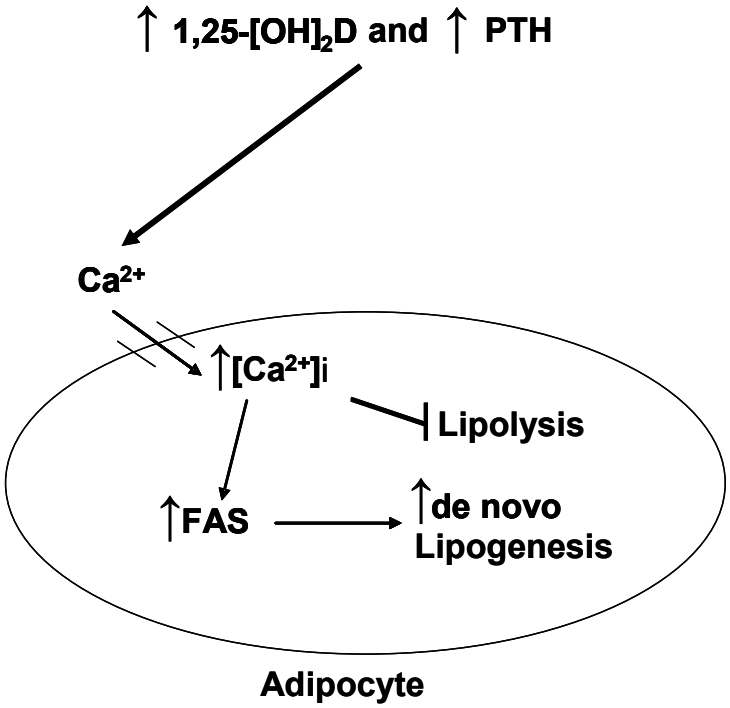

Figure 1. The effect of low dairy and dietary calcium on the modulation of adiposity. Adapted from Zemel et al. (65).

1.2.3.2. Calcium-mediated reductions in intestinal fat absorption and increases in fecal fat excretion

It has been suggested that calcium (including that from dairy sources) reduces fat absorption in the gastrointestinal tract in two ways: by producing insoluble calcium-fatty acid soaps, and by binding to bile acids which impairs the formation of micelles (66-69). The reduction in fat absorption results in increased fecal fat excretion, and a recent meta-analysis by Christensen et al. determined that an increased dairy calcium intake to at least

1241 mg per day resulted in an increased fecal fat excretion of ~5.2 g per day compared to low calcium intakes (~700 mg per day) (70). A daily excretion of this amount of fat would translate to approximately 1.9 kg of body fat loss (or not gained) per year. In a short-term human intervention study, Jacobsen et al. demonstrated that increasing calcium intakes from low fat dairy sources to ~1800 mg per day increased fecal fat excretion by 8.2 g per day versus diets with lower calcium intakes (500 mg per day) (71). Similar results with dairy-calcium were seen by Bendsen et al. (72). Other studies using calcium supplements have shown similar effects (67, 73, 74). Examining the effect of fat absorption and also using calcium from dairy sources, Lorenzen et al. demonstrated that higher meal intakes (~800 mg/meal vs. 70 mg/meal) significantly decreased lipemia measured as the postprandial (area under the curve) plasma concentrations of chylomicrons (66). Although these results support the aforementioned mechanism, the effect on total fat loss, while significant, was minimal. Unlike the pancreatic lipase inhibitor, Orlistat, which can decrease fat absorption and increase fecal fat excretion by ~30% (75), it is promising yet unknown whether increasing calcium intakes in the longer term would also produce clinically meaningful results with respect to weight and fat loss by this mechanism.

1.2.3.3. Calcium-mediated appetite and food intake regulation

The notion of a ‘calcium-specific appetite’ was first put forth by Tordoff (76) and can be defined as the motivation or desire to seek out calcium-containing foods when intakes or stores are deficient. Paradis et al. (77), and others (78), demonstrated this phenomenon in

rats where those fed diets low in calcium for six weeks subsequently preferred a drinking solution with the highest concentration of calcium carbonate over three others with lower calcium concentrations. While this effect has not been directly tested in humans, other effects have, including the notion that deficiencies in calcium may disrupt appetite regulation by affecting spontaneous energy/nutrient intake (79), and that consuming dairy products may attenuate the orexigenic effect induced during energy restriction (80). In the first situation, Major et al. demonstrated that when overweight, initially low calcium consumers were given a calcium and vitamin D supplement during fifteen weeks of energy restriction, their *ad libitum* fat intake (g and % kcal) at a buffet meal at the end of the study was significantly lower than those given placebo supplements. The behaviour observed at the buffet meal was consistent with the supplemented group achieving greater fat loss over the fifteen weeks versus the placebo group. Thus, adequate intakes of calcium and vitamin D during weight loss may attenuate food intake over time resulting in greater weight and fat loss (79). With respect to appetite sensations measured by visual analogue scales (VAS), during a six month weight loss study, those consuming milk (1000 mg per day of calcium) showed a smaller increase in the desire to eat and were less hungry compared to the placebo group (80). Dairy and calcium's effect on appetite control certainly requires further investigation, and it has been suggested that the effect on satiety may also be mediated by dairy protein, although, this does not explain the results seen by Major et al. where calcium supplements were given. Several other studies have assessed the effects of dairy food and dairy protein consumption on appetite control, food intake regulation and satiety under acute or one-week timeframes and in non-weight loss

conditions. These have been summarized by Dougkas et al. (38) and most (but not all) studies showed that consumption is associated with increased plasma concentrations of gut hormones known to reduce gastric emptying, gut motility and appetite (81) and decreased glucose concentrations as milk is a low glycemic index food (82); however, these studies are not discussed in further detail here.

1.2.3.4. The effect of dairy protein on body composition and appetite

Dairy protein, another important functional component in dairy products, may act independently or synergistically with calcium to modulate body adiposity (83), and to increase or maintain skeletal muscle anabolism and mass (84-86) during periods of energy restriction (44, 45). A large portion of the dairy derived bioactivity has been suggested to be due to the whey protein fraction which is rich in branched chain amino acids (BCAA) (87). In fact, BCAAs make up about 25% of total dairy proteins (88). Whey protein peptides have been shown to exert inhibitory effects on angiotensin converting enzyme (ACE), and this may affect adiposity since angiotensin II had been shown to upregulate FAS (65, 83). Isolated whey protein was also shown in a recent acute study to have the greatest postprandial thermogenic effect versus either soy or casein (89). As well, several studies have demonstrated that a whey protein beverage and a complete milk pre-load induced satiety and suppressed subsequent short-term food intake at a buffet meal to a greater extent than other protein drinks (90-92).

Leucine, a BCAA found in relative abundance in whey protein, has been shown to inhibit lipogenesis and promote lipolysis in adipose tissue cells, and to increase fat

oxidation in muscle cells (59). In addition, leucine also stimulates the translation-initiation machinery for muscle protein synthesis. This mechanism will not be discussed further here, but it is put forward as a plausible mechanism for the muscle sparing effect of dairy during weight loss; please refer to the following reviews for more information (93-96).

To assess the acute and chronic effect of dairy on protein synthesis and muscle mass accretion, three studies were undertaken in our laboratory; and these studies formed part of the basis for this current thesis because they confirmed the efficacy of milk in promoting lean mass gains and fat mass losses (particularly in women) during heavy resistance exercise training. In an acute study, we observed that when young men consumed fat-free milk after resistance exercise, they had a significantly greater rate of muscle protein synthesis versus when they consumed an isoenergetic and isonitrogenous soy beverage (84). To assess whether these acute changes would translate into longer term phenotypic gains in lean mass, two twelve-week resistance training studies were conducted, one in men (85) and the other in women (86). Results from these studies showed that consuming milk post-exercise did indeed result in greater lean mass gains but also promoted significantly greater fat mass losses. This finding was particularly evident in women, and despite a significant 2 kg lean mass gain during the program, no weight gain was apparent due to an almost identical fat mass loss **(Figure 2)**. On the other hand, the women consuming the carbohydrate drink post exercise showed no significant fat loss and thus gained weight on the program due to their lean mass gains (which were still significantly less than those consuming milk). The additional fat mass loss observed in

the milk-drinking women, although not directly tested, may be attributed to the other lipolytic mechanisms relating to calcium, vitamin D and leucine discussed above. Moreover, in the context of energy restriction, several clinical studies by Zemel's group showed that the consumption of dairy foods protect against muscle mass loss in overweight people, although these studies were carried out without exercise (44, 45).

Thus, given the multiple mechanistic effects that dairy and its components have on body adiposity, muscle mass sparing, and satiety and food intake regulation it is evident that the consumption of dairy foods can help to promote high quality weight loss in many different and possibly synergistic ways.

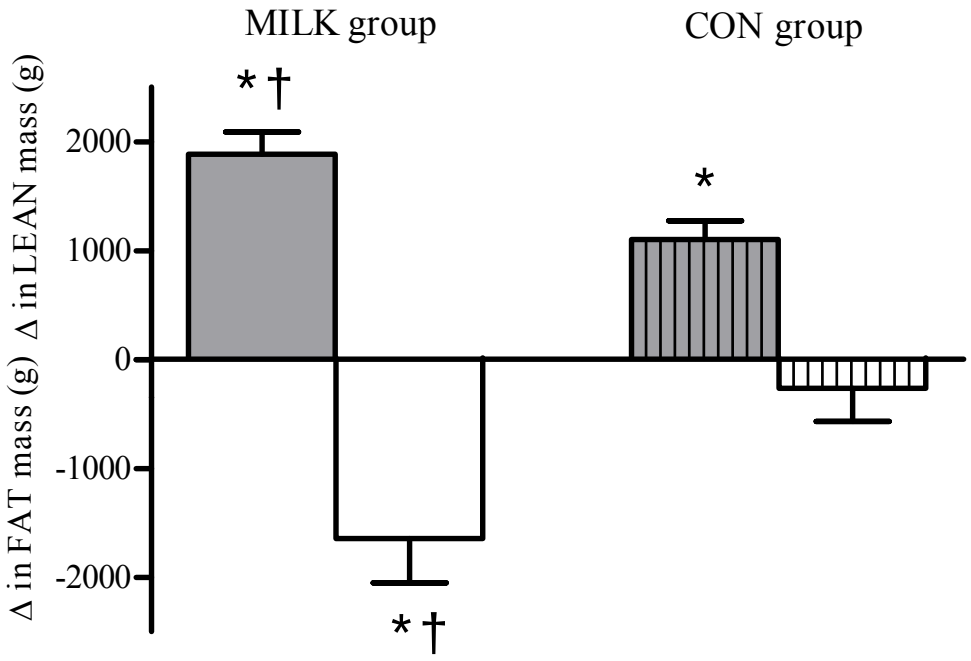

Figure 2. Change from baseline in lean mass (positive y axis of graph) and fat mass (negative y axis of graph) after young normal weight women underwent twelve weeks of resistance training with either milk or carbohydrate drink consumption post-exercise in Josse et al. (86). * Significantly different from baseline, P<0.05. †Significantly different from CON, P<0.05.

1.3. THE ROLE OF DAIRY AND ITS COMPONENTS ON BONE HEALTH

Dairy foods and their main constituents, calcium, vitamin D (fortified) and protein, positively influence bone health in many ways, and consuming dairy, by virtue of its nutrient density has been shown to increase the overall quality of young womens' diets (97, 98). In addition, it has been suggested that it would be difficult to achieve the recommended daily intakes of several key nutrients, especially calcium, if dairy was not part of a regular diet (99). Peak (or adult) bone mass is mostly accrued by the end of adolescence with final consolidation achieved between 20-30 years of age (53, 100). Because of this, young women should focus on consuming low fat dairy products to ensure adequate intakes of these and other bone supporting nutrients to help prevent disease later in life. Osteoporosis is a complex bone disease characterized by decreased bone mass and microarchitectural deterioration of bone tissue leading to, as the most deleterious outcome, an increased risk of fracture (101). Although genetics may account for as much as 80% of the variance in attaining peak bone mass, nutrition is believed to be the most modifiable factor that can aid in the protection against bone loss and maintenance of bone mass with increasing age (53). Loading-based physical activity is also a potent osteogenic stimulus, and as such, is another modifiable lifestyle factor that could enhance peak bone mass (102-104). Further discussions on the etiology and treatment of postmenopausal osteoporosis are beyond the scope of this thesis; however, it is important to understand the basis and severity of the disease in order to supply a good rationale for studying bone health in premenopausal women. Thus, as modifiable and

easily implementable lifestyle choices, nutrition and physical activity are both important ways to promote positive bone health in young women.

1.3.1. Dairy, calcium and vitamin D and their recommendations for premenopausal women

The adult human body contains ~1300 g of calcium and 99% of it is located in the bones in the form of hydroxyapatite (105). It is for this reason that calcium is the most studied nutrient in relation to bone health. It is well established that calcium is of vital importance to bone health, and several meta-analyses and systematic reviews of observational, retrospective, prospective and clinical trials carried out in all age groups have demonstrated this positive relationship (53, 106, 107). As well, optimal skeletal heath requires the intake of other key nutrients, not just calcium and vitamin D (97, 108); for more detailed overview of each of these nutrients (e.g. potassium, vitamin K, magnesium, vitamin B12), please see references (53, 105).

Current recommendations for dairy intake in Canada and the USA call for three servings of dairy products per day to help achieve adequate calcium intakes and to maintain structural integrity of the skeleton (53, 97, 99, 109). Dairy foods represent a good choice for consumption because, aside from their calcium content and fortification with vitamin D, they also contain other healthful compounds (protein, vitamins and minerals). In addition, these compounds seem to be more bioavailable from food sources (53). For example, 3 cups of skim milk contains many essential vitamins and minerals in significant quantities including approximately 900 mg calcium (90% of the dietary reference intake [DRI]), 270

international units (IU) of vitamin D (45% of the new DRI), 1500 IU of vitamin A (45% of the DRI), 3.24 μg B_{12} (135% of the DRI), 93 mg magnesium (30% of the DRI) and 27 g of protein. Calcium recommendations for women ages 19-50 years have remained the same since 1997 at 1000 mg per day (110). Yet, according to the most recent data from the Canadian Community Health Survey (CCHS), young women are only consuming an average (±SE) of 850±23 mg per day from food sources, and when supplement use is taken into account, intake increases to 965 mg per day (SE not reported) (111). Despite the latter intake being closer to the recommendation, data show that only 40% of women are actually achieving intakes greater than the DRI (111). In 2010, the adequate intake (AI) for vitamin D was increased from 200 IU per day to 600 IU per day (112). As well, serum 25[OH]D levels of 50 nM were deemed sufficient as a concentration indicating that the needs for vitamin D were being met at this level in at least 97.5% of the population (112). Fortified milk and other dairy products provide the greatest dietary sources of both calcium and vitamin D (97, 112); however, in order to reach 25[OH]D levels that meet the needs of almost all healthy young people (50 nM), greater vitamin D intakes may be necessary as this level may not be achievable through food intake alone (86, 113). In recent years, much more research has been done on the complex role of vitamin D in skeletal and especially non-skeletal diseases. Further discussions on the effects of vitamin D beyond skeletal health are outside the scope of this thesis, but for more detailed information please see reference (114).

1.3.2. The regulators of blood calcium: vitamin D, parathyroid hormone and calcitonin

There are three main hormonal regulators of blood calcium: PTH, vitamin D (and its metabolites), and calcitonin (53). When blood calcium levels are low, PTH is release from the parathyroid gland and acts on the kidneys, the intestines (indirectly *via* 1,25[OH]2D) and on the bones to absorb or release calcium into the blood. In addition, signalled by PTH, 25[OH]D is converted in the kidney by 1-α-hydroxylase to 1,25[OH]2D which stimulates calcium absorption and resorption in the intestine and kidney, respectively, to restore blood calcium concentrations (53). Vitamin D also acts directly on the bone by stimulating osteoclast formation and bone resorption, and a severe deficiency of vitamin D results in osteomalacia, a disease where bones become soft due to defective mineralization and osteoid accumulation (115). In contrast, calcitonin is released from the thyroid in response to high serum calcium levels. It opposes the action of PTH especially at the bone where it inhibits osteoclast resorption (53).

1.3.3. Protein and bone health in premenopausal women

Adequate provision of high quality dietary protein is of central importance to bone health for a few main reasons. First, protein is (apart from the bone mineral itself) the other primary structural component of bone; bone collagen. Second, protein ingestion results in a mild stimulation of hepatic insulin like growth factor 1 (IGF-1) production and release which stimulate osteoblasts and supports ‘anabolism’ in bone. Finally, protein acts to increase intestinal calcium absorption (as opposed to purportedly only increasing urinary calcium

excretion) (108, 116). Many studies have reported positive relationships between increased protein intakes and bone mineral density (BMD), bone mineral content (BMC), reduced bone resorption and reduced fracture risk in different populations (53, 116-118), and a detrimental effect of low protein intakes on calcium homeostasis (119). A recent meta-analysis supports a modest positive association between total protein intake and BMD and BMC, a result which was not evident with soy protein intake alone (120). It is important to highlight here that a negative association between protein and bone was not observed despite the notion that increased protein (particularly from meat sources) disturbs calcium balance by increasing urinary calcium excretion (116, 121). The purported negative effects (118, 119) of higher meat protein consumption on bone are incorrect. In fact, higher protein intakes actually stimulate intestinal calcium absorption, and the increased urinary calcium excretion is secondary to this effect (122). Well designed tracer studies also indicate that the calcium excreted following higher animal protein intakes was not derived from bone (122). In addition, meat protein consumption has been associated with increased serum IGF-1 levels, whereas soy protein was not (123). While it is true that animal protein, because of its higher content of sulphur-containing amino acids, can increase the metabolic acid load and slightly lower the body pH, it has been suggested that the real issue, especially in Western populations, stems from inadequate intakes of alkaline foods such as vegetables and some fruits that can act to offset these small perturbations in systemic pH (108).

It has been widely accepted that increased dietary protein results in increased urinary calcium excretion, and on average, every 50 g increase in protein is associated with an increase of 1.6 mmol/L per day in 24h urinary calcium excretion (119, 121). Because of this,

it was thought that higher protein intakes were detrimental to bone and that lower intakes should be recommended. However, a series of studies by Kerstetter et al. unequivocally describe the underappreciated negative effect that low protein diets (not high protein diets) have on calcium homeostasis (124, 125). In the first study, young women who consumed a low protein diet (0.7 g/kg/d) for only four days saw significant elevations in PTH and 1,25[OH]2D versus those consuming a higher protein diet (1.0 and 2.1 g/kg/d), even when controlling for adequate calcium intakes (124). In a second study, stable isotope tracers of calcium confirmed that lower protein intakes impaired intestinal calcium absorption by demonstrating that calcium absorption was 26% after high protein intakes (2.1 g/kg/d) and 18% after low protein intakes (0.7 g/kg/d) (125). Mechanistically, PTH directly targets bone to mobilize stored calcium and in this case, hyperparathyroidism secondary to low protein intakes probably occurred to restore serum calcium levels because intestinal absorption was low. Therefore, these data provide direct evidence that low protein diets, not high protein diets are detrimental for bone health since they independently impair calcium balance even when calcium intakes are adequate.

1.3.4. Bone turnover and biomarkers of turnover

Bone turnover or bone remodelling is characterized by the dynamic and coordinated actions of osteoclasts to degrade bone (resorption) and osteoblasts to replace it (formation). The greatest rates of bone turnover occur during growth of the skeleton but once fully grown, skeletal renewal occurs at a rate of ~5-10% per year in order to repair microdamaged bone from normal daily activity. Thus, bone remodelling is a necessary

process that, when balanced, acts to maintain the structural integrity and strength of bone (126). However, when turnover increases and resorption outweighs formation, the net result is a loss of bone mass accompanied by micro-architectural deterioration (126). If this period of time is prolonged, bones become more fragile and the risk of fracture increases.

The most common method used to assess bone health and fracture risk is dual energy x-ray absorptiometry (DXA) or bone densitometry. However, some limitations do exist in that BMD only provides a static measure of skeletal status, with no information on bone micro-architecture, and may not be useful in shorter-term clinical studies (127). On the other hand, bone turnover biomarkers (BTMs) are biochemical metabolites that provide dynamic information on skeletal status by reflecting the metabolic activity of bone during remodelling (127, 128), and can respond quicker than BMD to treatment. They are relatively easy to measure from a simple blood or urine sample, and assays for their quantification/concentration are readily available. Moreover, they have been useful in clinical settings as ways to monitor the efficacy and response to therapy particularly in postmenopausal osteoporosis (128). Further discussion on this topic in relation to postmenopausal osteoporosis is beyond the scope of the thesis, but has been reviewed elsewhere (127, 128). When measuring BTMs in clinical studies, certain sources of pre-analytical variability should be controlled for including circadian rhythm variability (higher turnover in the morning), fasted versus fed status (feeding generally decreases turnover), seasonal variations (higher turnover in the winter relating to decreased vitamin D concentrations), exercise habits (acute exercise increases turnover), and others (126-

128). As well, inherent limitations do exist when measuring BTMs. First, a small proportion of some BTMs are not derived from the skeleton, and second, there is no way to measure the activity at a specific site in the body since BTMs reflect the activity of the whole skeleton.

BTMs are reflective of either bone formation or bone resorption. Bone formation markers are chemical indicators of the activity of osteoblasts during different stages of development from the actual formation of bone (collagen and non-collagen bone matrix formation) to post-osteoid maturation and modification (128). To obtain a more detailed picture of bone formation, it is important to measure more than one formation marker (127). With respect to bone resorption, the most common markers are those of collagen degradation that are either free or bound to peptides and can be measured in the blood or urine (128). **Table 1** lists some more commonly measured BTMs (126-130).

In December 2010, the International Osteoporosis Foundation (IOF) published a position paper on the utility of BTMs. Their recommendations were to measure the amino-terminal propeptides of type 1 collagen (P1NP) and the carboxy-terminal cross-linking telopeptides (CTX) in clinical studies to get a good measure of dynamic bone activity (128). Both of these markers measure type 1 collagen turnover with P1NP measuring collagen synthesis and CTX measuring collagen breakdown. In fact, the IOF have named P1NP and CTX as the 'reference standards for bone formation and resorption', respectively (128). Reasons for this include the wide availability of easy-to-use enzyme-linked immunosorbent assays (ELISAs) and well characterized biological and analytical variability. While it is true that not all formation or degradation products

come from bone (including P1NP and CTX), it is likely that they both strongly reflect activity in bone since pharmacological therapy designed to decrease bone resorption (e.g. Denosumab) also reduces CTX to very low levels (128).

Table 1. The roles and functions of bone biomarkers.

BIOMARKER	FUNCTION
Bone Formation	
Osteocalcin (OC)	-The most abundant non-collagen protein in bone, likely involved with bone mineralization. -Most is incorporated into the bone matrix but a small amount is released by osteoblasts and can be measured as an index of formation. -In some instances, OC may reflect bone turnover; especially when bone resorption markers are also increased (131).
Bone specific alkaline phosphatase (BSAP)	-Alkaline phosphatase is present in other tissues but there is a bone isoform (BSAP). -It is involved in promoting the mineralization and calcification of the newly formed bone (post-osteoid formation modifications).
N-terminal propeptide of type 1 collagen (P1NP)	-A peptide marker of type 1 collagen synthesis originating primarily from bone (since it has a faster turnover), but it can also come from other collagenous tissues (skin, vessels, tendons, ligaments). -It is secreted from bone as procollagen and then cleaved at the amino-terminal.
Bone Resorption	
C-terminal cross-linking telopeptides (CTX)	-CTX is a C-terminal, non-helical collagen degradation fragment. -It is the peptide-bound form of pyridinoline and DPD. -Urine and serum assays correlate well with less variability in serum.
N-terminal cross-linking telopeptides (NTX)	-NTX is an N-terminal, non-helical collagen degradation fragment. - It is the peptide-bound form of pyridinoline and DPD. -More widely measured in urine and corrected for creatinine levels.
Deoxypyridinoline (DPD)	-Pyridinium cross-links formed during the breakdown of bone collagen and are released into the circulation. -DPD originates almost entirely from bone unlike pyridinoline (PYD) which can come from cartilage, vessels, and ligaments. -DPD is usually measured in urine and corrected for creatinine levels.
Other Markers	
Osteoprotegerin (OPG)	-OPG preferentially binds to RANKL on osteoblasts so that RANKL does not bind to RANK on osteoclast precursor cells. This inhibits osteoclast differentiation and activity. -higher levels of OPG and a higher ratio of OPG/RANKL are protective for bone (decreases osteoclast action and thus bone resorption). -*Denosumab* is a pharmacological agent (monoclonal antibody against RANKL) that mimics the action of OPG and is used to treat osteoporosis.
Receptor activator of nuclear factor kappa B ligand (RANKL)	-RANKL is expressed on osteoblasts and usually binds to RANK on osteoclast precursor cells. This causes a series of cellular events that stimulates osteoclast differentiation and activity. -*Denosumab* inhibits RANKL from binding to RANK and is used to prevent excessive bone resorption.

1.3.5. Receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) as markers of osteoclast activity

The OPG/RANKL system is a key mediator of osteoclastogenesis. RANKL in bone stimulates osteoclast differentiation and activity, and prevents osteoclast apoptosis, whereas OPG acts to inhibit RANKL (130). **Figure 3** summarizes the relationship between these molecules and shows how they are regulated. Briefly, when OPG is in abundance, it preferentially binds to RANKL and prevents the binding of RANKL to RANK, its primary receptor on osteoclast precursor cells. This in turn inhibits the usual cascade of cellular events that occurs to promote osteoclast formation (129). Therefore, increased OPG (and decreased RANKL) and particularly an increased OPG:RANKL ratio is protective for bone. Calcitropic hormones (PTH and 1,25[OH]2D) also affect this dynamic by increasing RANKL production (130).

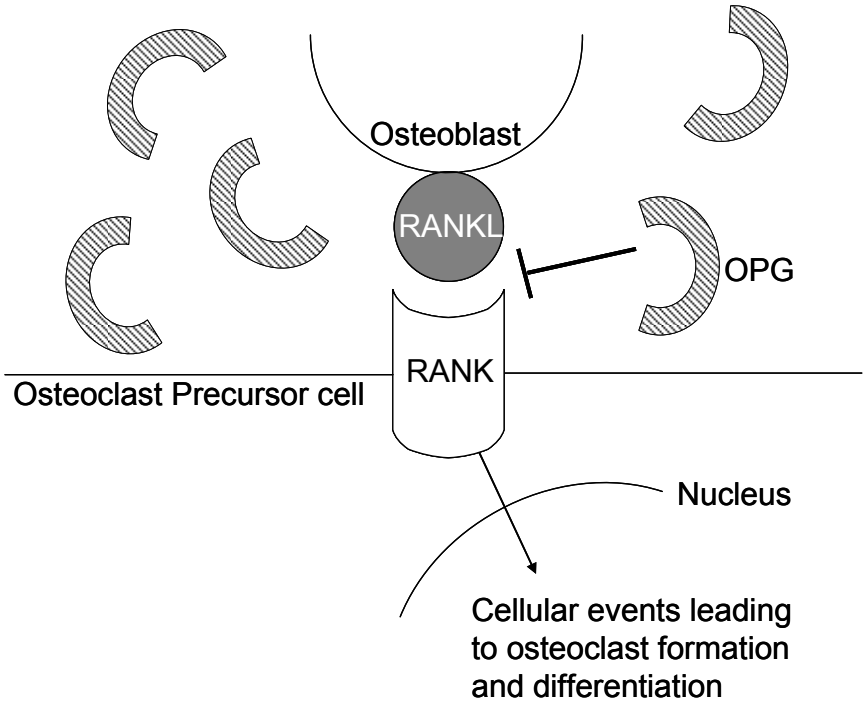

Figure 3. The interaction between OPG, RANKL and RANK. OPG blocks the binding of RANKL to RANK and thus inhibits the downstream cellular events leading to osteoclastogenesis and osteoclast differentiation. Adapted from Boyce et al. (129).

1.3.6. Clinical studies measuring bone outcomes with energy restriction in premenopausal women with and without exercise

It has been documented that weight loss can have a negative effect on bone health (14, 132, 133), but that this effect can be offset by calcium supplementation (134). The decrease in energy intake and also the decrease in the intake of critical bone-supporting nutrients are the main reasons why energy restriction affects bone loss, as well, reductions in the usual mechanical loads placed on bone are directly related to a reduced body mass (14, 135). To date, several clinical trials have examined the effect of energy restriction with and without exercise on bone health in premenopausal women, and most studies

show that bone turnover and markers of bone resorption respond differently depending on calcium and protein intakes during the intervention. For example, Shapses et al. demonstrated a significant increase in urinary deoxypyridinoline (uDPD) but no change in BMD or BMC with weight loss over six months in those receiving placebo tablets versus those taking 1000 mg of calcium per day (136), and Rector et al. showed that lower calcium intakes (range: 420 to 822 mg per day) during six weeks of weight loss increased both osteocalcin (OC) and CTX in all treatment groups regardless if they exercised or not (102). On the other hand, four separate trials have demonstrated that weight loss with at least adequate calcium intakes did not adversely affect bone mass or bone turnover in young overweight women (46, 135, 137, 138). In addition, high protein diets with an emphasis on dairy (~2400 mg per day calcium) minimized bone turnover with significantly smaller or no increases in OC and uDPD versus diets high in protein from a variety of other sources with no dairy and low calcium (~500 mg per day) over sixteen weeks (138). Similar results were seen in two other studies as higher versus lower protein intakes preserved BMC and BMD at various sites during weight loss after six months (139) and one year (140), respectively. It is worth mentioning that one study carried out in a mixed population of pre- and postmenopausal women also demonstrated no bone loss during weight loss if adequate calcium, from either dairy or supplemental sources was consumed (141). In summary, Shapses and Riedt suggest in a recent review that weight loss studies examining bone outcomes in premenopausal women show less pronounced results compared to postmenopausal women, and that the timeframe and amount of weight lost makes a big difference (14). For example, rapid and more extreme

weight loss (i.e. by diet or surgery) has the greatest negative effect on bone (and also likely on muscle mass), whereas slower weight loss, especially with adequate provision of dietary calcium (as is congruent with the aforementioned trials), has the smallest or no effect on bone.

The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) study, as well as one other study, demonstrated that the addition of aerobic exercise and/or low-impact weight bearing exercise to a weight loss program did not further affect bone beyond the effect achieved without exercise (102, 135). However, Villareal et al. demonstrated that in middle aged subjects, exercise-induced weight loss but not calorie-restricted weight loss was associated with a preservation of BMD at clinically important sites after one year despite adequate calcium and conventional protein intakes in both groups (142). Several systematic reviews carried out in both pre- and postmenopausal women on the effect of different types of exercise on bone mass with and without caloric restriction have reported positive effects (103, 143), but this is not a consistent finding (104).

1.4. DIETARY PROTEIN AND EXERCISE IN WEIGHT LOSS: EFFECTS ON BODY COMPOSITION

Higher protein (~30% total energy intake), energy restricted (~500 kcal per day) diets for obese persons have been recommended over conventional protein (~15% total energy intake) higher carbohydrate diets (~55% total energy intake) for weight loss and other

reasons including improved glycemia and lipidemia, prevention of lean mass loss, increased dietary thermogenesis, greater satiety and increased diet satisfaction (144-148). Shorter-term, more comprehensive diet studies have shown greater weight loss and more beneficial metabolic effects in young men and women with the consumption of higher protein (149-152), and two longer term randomized controlled trials have also demonstrated this effect (153, 154).

1.4.1. Weight loss studies with higher protein

Two randomized controlled trials of one and two years duration, respectively, demonstrated that higher protein diets produced greater weight loss compared to conventional protein diets (153, 154). The first study confirmed that consuming an Atkins diet (low carbohydrate; ~10% total energy) produced significantly greater weight loss, increased high density lipoprotein cholesterol (HDL-C), decreased triglycerides and improved systolic and diastolic blood pressure to a greater extent than popular diets with less extreme macronutrient manipulations or those high in carbohydrates (153). The second study, although weight loss was not significantly different between groups, the high protein group (25% total energy) still lost more weight than the group consuming average protein (15% total energy), and those consuming higher protein also significantly improved HDL-C (154). Despite these findings (153, 154), several studies assessing the longer-term (≥ one year) effectiveness of diets varying in macronutrient composition on weight loss have reported similar weight loss between groups consuming diets with varied macronutrient contents (149, 154-156). One interpretation of these results is that

weight loss is just a function of energy restriction and adherence to a particular program regardless of the dietary macronutrient composition. While such a conclusion may be true in the context of weight loss *per se*, an underappreciated point is the tissue composition of the weight that was lost. As outlined previously, with respect to disease risk and continued weight loss success (18), minimizing the loss of skeletal muscle with weight loss is a very important outcome, and the studies most often cited in support of deemphasizing the importance of higher protein and lower carbohydrate eating patterns have not measured losses of lean mass. It is possible that if these aforementioned studies employed measures of lean mass, they may have seen preservation of this tissue mass which would have of course resulted in less weight loss. Thus, as a future consideration, to assess the important effect of protein on body composition, long-term weight loss studies with higher protein intakes should incorporate measures of lean mass.

With respect to body composition, only shorter-term studies (four weeks - six months) have assessed this with higher protein consumption during energy restriction. Results show that higher protein not only promotes greater weight loss (149-152) and fat loss (157, 158), but also helps to offset most of the obligatory loss of lean mass seen with conventional (~55%) carbohydrate-based energy restricted diets (145, 159-161). According to a meta-regression analysis by Kreiger et al. (2006), protein intakes ≥1.05 g/kg/d in studies that were more than twelve weeks in duration were associated with a 1.2 kg greater lean mass retention than studies where protein consumption was at or close to the recommended dietary allowance (RDA; 0.8 g/kg/d) (160). Therefore, the increased

consumption of protein with energy restriction in shorter term studies significantly improved the quality of weight lost by maintaining lean mass.

It is well known, primarily based on controlled isotope infusion trials, that amino acids (particularly leucine (87)) independently stimulate muscle protein synthesis, and this, along with the proven effect of exercise on muscle, may be two primary mechanisms driving the observed lean mass retention with energy restriction (161, 162). Moreover, higher protein diets increase thermogenesis (83, 89) and have been deemed more palatable and satiating (145). This may help promote greater dietary adherence and possible reductions in energy intake. Therefore, in the context of energy restriction, a comprehensive strategy including an increase in dietary protein (to ~30% of total energy) with exercise (both resistance and aerobic) would promote weight loss, greater body compositional change and better improvements in several metabolic and cardiovascular parameters than diets with conventional protein (15% of total energy) and no exercise. In addition, this pattern would produce weight loss where muscle mass is spared, and would be more palatable and satiating, which contributes to greater weight loss potential and long-term diet adherence.

1.4.2. Exercise and weight loss

In addition to macronutrient modifications, exercise has been shown to benefit weight loss efforts not necessarily though weight loss *per se*, but through changes in the tissue composition of the weight loss (i.e. increasing fat loss), increasing energy expenditure, improving insulin sensitivity and glycemic regulation and other cardiovascular

parameters (144, 158, 163). A number of studies have shown that the conventional loss of lean mass with energy restriction can be offset with exercise (both resistance and aerobic) and that the quality of weight loss can be further improved if exercise and higher protein are combined (146, 158, 164). Two recent studies have assessed this effect in overweight and obese young women and demonstrated that higher protein plus exercise produced the greatest weight loss and greatest improvements in body composition (146, 158). Both studies employed a resistive component to the exercise program.

1.5. THE UTILITY OF THE BODY MASS INDEX (BMI)

Obesity is a common problem worldwide and its prevalence continues to increase. The most widely used classification of obesity is BMI and values of $\geq$25 kg/m^2 are considered overweight and $\geq$30 kg/m^2 obese (1). BMI is a quick and easy calculation that measures a ratio of weight per unit of height squared to assess disease risk. While it can be argued that BMI is suitable for classifying morbidity and mortality risk in larger-scale epidemiological studies (1, 165, 166), a major inherent flaw exists in its application since it does not account for differences in body composition (167, 168). This often leads to the misclassification of those with greater muscle mass or more extreme body phenotypes. For example, the 'body builder' versus the 'couch potato' with the same height and weight – both would be considered obese according to BMI, but their disease risk profiles are drastically different. The main issue is that BMI is not a good indicator of body fat content, and as a result, BMI measures have been shown to correlate poorly with heart

disease risk, morbidity and mortality (169, 170). Therefore, to improve disease risk classification, it is important to develop appropriate ways to accurately measure and monitor adiposity since it is a better indicator of health and disease than just height and weight alone (167, 171).

1.5.1. Subcutaneous and visceral/intra-abdominal adipose tissue

Generally speaking, adiposity can be divided into two main compartments comprising subcutaneous (SAT) and visceral/intra-abdominal (VAT) with the former located under the skin and the latter surrounding the internal organs of the viscera (i.e. intestines, kidneys, liver, etc). Research has firmly established VAT as more metabolically adverse than SAT, and its presence is more strongly associated with diabetes, inflammation and cardiovascular disease (167, 171). Waist circumference, a surrogate anthropometric measure for abdominal/visceral obesity, has also been shown to strongly predict the risk of myocardial infarction (169) and mortality (170, 172), and it is an important risk factor for the metabolic syndrome (4). In the context of energy restriction, both exercise (163, 173) and increased dairy consumption (44) (versus energy restriction alone) have been shown to promote greater reductions in visceral adipose tissue in clinical trials.

1.6. IMAGING TECHNIQUES USED TO MEASURE ADIPOSITY

There are several different techniques now available to measure body fat with some methods being more reliable and accurate than others. This discussion will be restricted to

the modalities that were used in this thesis: DXA, which was used to measure total body fat and abdominal fat (abdominal adipose tissue; AAT); magnetic resonance imaging (MRI) to measure visceral fat (visceral adipose tissue; VAT); and Fourier-transform near infrared (FT-NIR) spectroscopy to measure total subcutaneous fat. Currently, MRI is the gold standard for body composition assessment, but it is also quite costly and inaccessible making it an unsuitable option for routine body fat determination. DXA is an accurate alternative, it is very reliable and more accessible, however its upkeep can also be costly and it uses ionizing x-rays which increase low-level radiation exposure (albeit to a very minimal extent; (165)). Using FT-NIR spectroscopy to measure body fat is a relatively new technique. FT-NIR is a rapid, low risk and portable method for body fat determination, and fat mass measured by this method has been shown to correlate well with MRI indirectly in age and sex matched individuals (174). More research is still needed to validate FT-NIR spectroscopy in other populations and in clinical settings. These three methods are discussed in more detail below.

1.6.1. Dual energy x-ray absorptiometry (DXA)

DXA has been and still is the most widely used method for measuring BMD to assess osteoporosis risk, but it is also an increasingly popular and well established technique for measuring regional and total body composition (165, 175). The physics of DXA is based on the absorption and subsequent signal detection of two opposing x-ray photons of different energy levels (165). The differential attenuation of the two x-ray beams is then used to estimate the composition of the different body tissues (i.e. bone, muscle and fat)

(176). Unlike other methods used to measure body composition such as underwater weighing, the BodPod, and bioelectric impedance analysis which only yield information on two body compartments (fat and fat free mass), DXA is a more intricate technology as it measures body composition using three compartments: fat mass, bone mass and fat and bone free (lean) mass (165, 176). DXA is quick and relatively non-invasive with minimal radiation exposure (5-7 μSv per whole body scan), it is easy to use, and can accurately measure body composition in individuals varying in age, size and health status (165, 177).

While DXA provides accurate and reliable measures of body fat mass, it cannot differentiate between the subcutaneous and intra-abdominal fat depots. On the other hand, VAT can be directly assessed with MRI and computed tomography (CT) (175, 178), but these two methods are impractical for widespread use for a variety of reasons. As a result, several studies have used the surrogate measure of AAT with DXA by manually isolating a specific anatomical region of interest between the first and fourth lumbar vertebrae on a whole-body scan (175, 178, 179). In three recent studies, DXA-derived AAT was shown to correlate significantly with MRI-derived VAT in healthy men (179) and CT-derived VAT in both normal and overweight subjects of different ages (175, 178). Glickman et al. (2004) demonstrated that DXA is sensitive enough to detect small changes in AAT, and also demonstrated that measuring AAT with DXA is easy and precise since different operators were able to reproduce the same region of interest on the same scans (175). Collectively, these studies show that DXA provides an accurate, albeit indirect, measure of VAT in different populations compared to gold standard methodology (MRI and CT),

and thus clearly highlights the utility and usefulness of DXA in classifying those at greater risk for disease based on central adiposity.

1.6.2. Magnetic resonance imaging (MRI)

MRI is a medical imaging technique that uses a powerful magnetic field to align the magnetization of atoms in the body, and radio frequency fields to systematically alter the alignment of this magnetization. This causes the nuclei to produce a rotating magnetic field detectable by the scanner, and this information is used to construct an image of the scanned area of the body (176). Detailed three-dimensional images can be obtained with MRI based on how these electromagnetic fields function in different planes (X, Y and Z) and how they affect the protons within the cells in the area of interest (176). MRI is an excellent modality for quantifying body fat since it provides good contrast between soft tissues (180) (**Figure 4**). To detect fat tissue on an MRI scan, the use of a particular imaging sequence called 'IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation)', is recommended as it is specifically designed to characterize and separate water and fat-filled compartments based on their different resonant frequencies and chemical signals (181). The MR images obtained using the IDEAL sequence are maximized to detect fat and suppress water (and hence also tissues that are made up of water like muscle, organs, etc.), which is why in **Figure 4**, fat is easily detected in bright white or light grey and the other tissues as black or darker grey.

Unlike DXA, MRI provides three-dimensional images that allow for the quantification of adipose tissue in different compartments (e.g. visceral vs. subcutaneous).

In this thesis, we took scans of the area of interest (from the top of the liver to the iliac crest; **Figure 5**) in three contiguous blocks consisting of 11 axial scans, each of 8 mm thick. To quantify visceral adipose tissue from a scan, whiter intra-abdominal/visceral fat can be traced (excluding the subcutaneous band) using specialized software (SanteSoft Medical Imaging DICOM Editor; **Figure 6)**. The software quantifies the voxel areas (cm^2) and subsequently calculates the volume (cm^3) of fat by multiplying the area by each slice thickness.

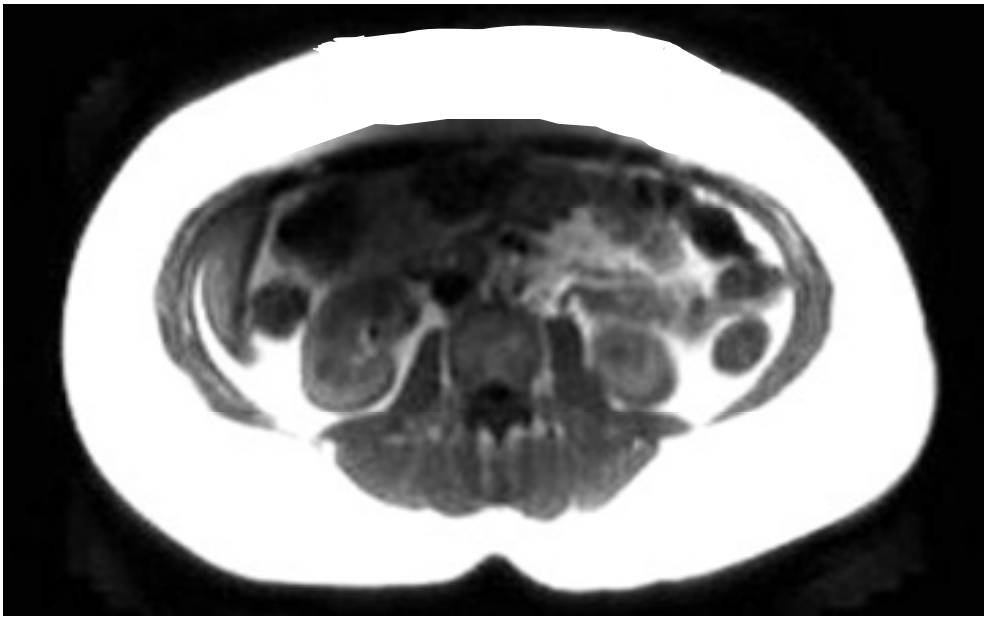

Figure 4. Axial slice of the abdomen showing the contrast between the white fat and darker muscle and organ tissue.

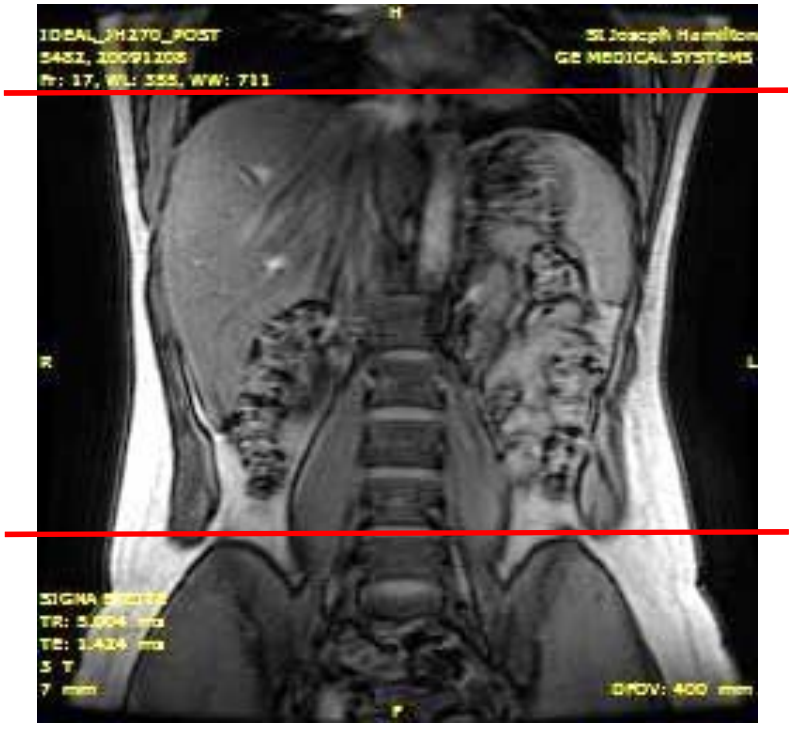

Figure 5. A coronal MRI section. Between the lines is the visceral region of interest; from the top of the liver to the top of the iliac crest.

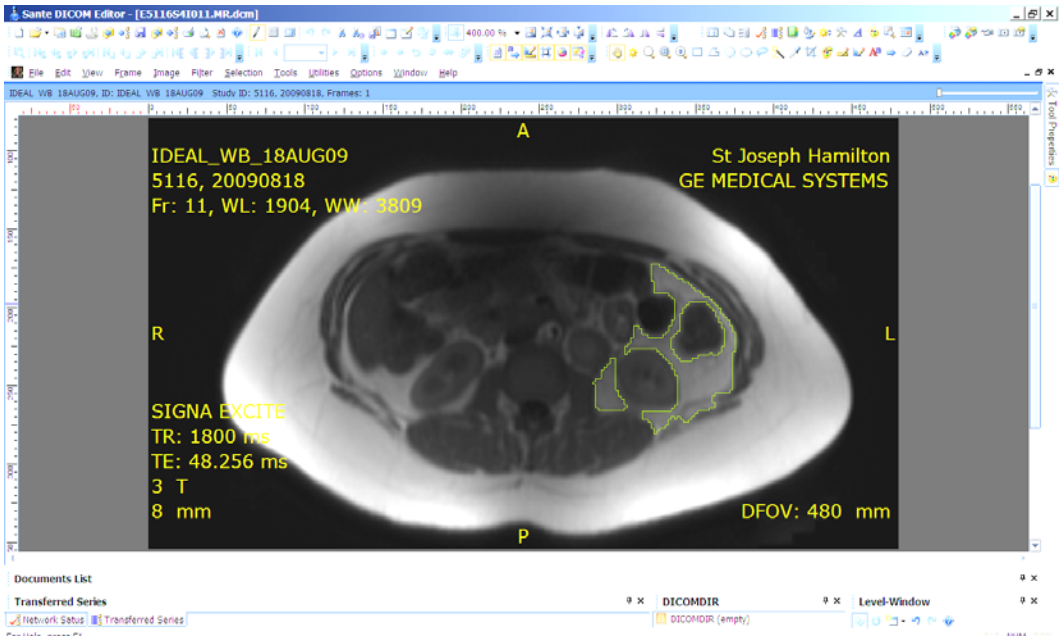

Figure 6. Screen-capture of adipose tissue tracing on an axial slice using Santesoft Medical Imaging Software (DICOM editor).

1.6.3. Fourier-transform near infrared spectroscopy (FT-NIR)

FT-NIR spectroscopy is a relatively new method for measuring subcutaneous body fat. It is quick, easy, portable and non-invasive, and measurements of body fat by this method have been shown to correlate well with whole-body MRI in different age- and sex-matched individuals (174). FT-NIR spectroscopy for the quantification of fat is based on detecting and analyzing the reflected signal in the near infrared region of the electromagnetic spectrum from a substance containing fat (182, 183). For each measurement, a standard fibre-optic probe carrying the near-infrared light (similar to a laser pointer) is shone from the back through the upper ear (cartilaginous area), and the resulting wavelengths and intensities in the reflected rays are measured (184). Like other analytical spectroscopic techniques, due to the unique chemical properties of fatty acids (i.e. organic functional groups), fat has a distinct reflective fingerprint on the spectrum which differentiates its signal from those of other substances found in the body (i.e. protein, bone, water). The reflected ray from the subject is Fourier-transformed and compared to signals from appropriate reference materials to determine the amount of fat or % of fat in a person (174). **Figure 7** shows several calibration spectra from substances with known fat contents; the deeper the trough between the 5750 and 5800 wavenumber, the greater % fat. **Figure 8** shows two transformed spectra from volunteers with different % body fat. To ascertain the amount of fat, the signals in **Figure 8** are compared to those in **Figure 7**.

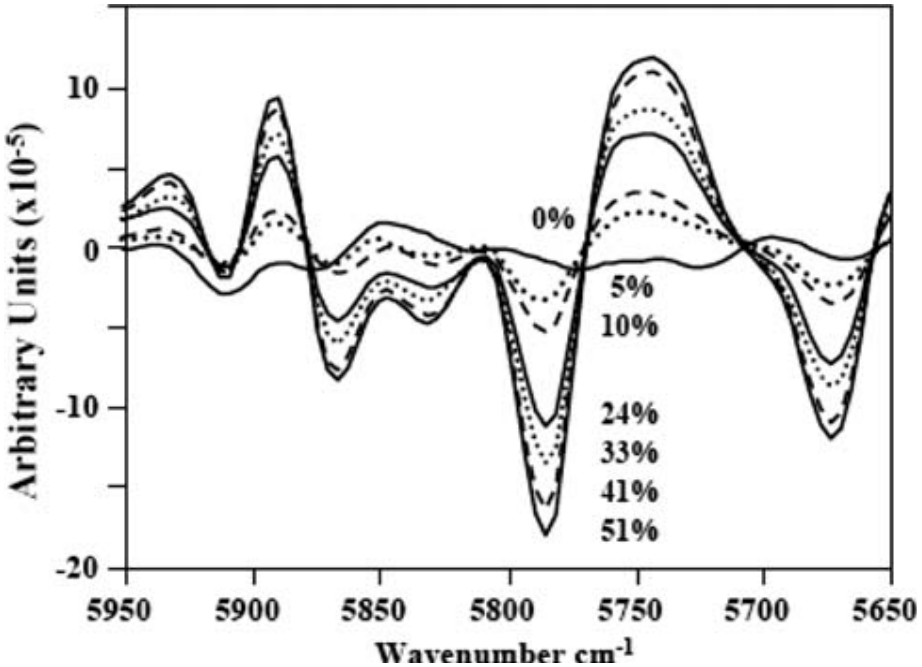

Figure 7. Several calibration FT-NIR spectra from different substances with known fat contents. The deeper the trough between 5750 and 5800 wavenumber, the greater % fat.

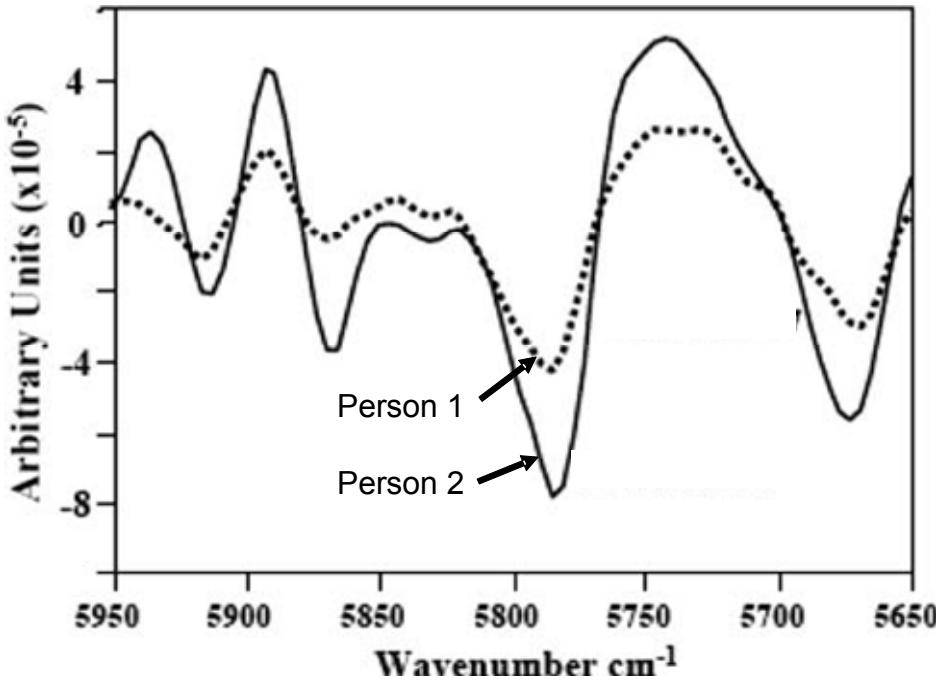

Figure 8. FT-NIR Spectra output from two people with different fat contents.

The human upper ear was chosen as the appropriate site of measure compared to other sites on the body by trial and error. This is believed to be the case for two reasons. First, the human upper ear is a thin, relatively stiff, hairless area of the body that contains a uniform distribution of two major tissues layers – fat and protein (cartilage) surrounded by skin; second, it was shown to yield the strongest and cleanest reflectance signal (because of its depth) with the least amount of light dispersion and background interference (174, 184). A usable reflectance spectrum cannot be obtained from thicker body parts such as the arm or abdomen because there is too much dispersion and tissue absorption of the infrared light (174).

1.7. STUDY OBJECTIVES AND HYPOTHESES TESTED

The primary objective of the clinical trial outlined in this thesis was to characterize the optimal condition for achieving weight loss with the most favourable change in body composition from a metabolic health standpoint. To achieve this goal, our study participants underwent a diet- and exercise-induced hypocaloric program for sixteen weeks; all of our subjects were overweight or obese, but otherwise healthy, premenopausal women. The Chapters that follow describe the effect of our unique weight loss program: the 'I.D.E.A.L. (Improving Diet, Exercise, and Lifestyle) for Women' study, which combined energy restriction, daily exercise (aerobic and resistance), higher protein and dairy food consumption, and assessed its effect on fat and lean mass (Chapter 2) and bone health (Chapter 3). In addition, Chapter 4 outlines a direct comparison (in the

same subjects) between body fat measured by DXA versus the new FT-NIR spectroscopy method.

1.7.1. Specific hypothesis and objectives - Chapter 2

This Chapter highlights the fat mass and lean mass changes (the primary endpoints) in the sixteen week I.D.E.A.L. for Women study after the consumption of hypoenergetic diets that varied in the amount (30% or 15% of total energy) and type (dairy or non-dairy) of protein consumed. We hypothesized that the most favourable condition for the highest ratio of fat to lean mass loss would be seen in those consuming higher protein and higher dairy along with daily exercise (HiDairyPro treatment group) versus those consuming adequate protein and medium dairy (DairyPro treatment group) or adequate protein and low dairy (Control treatment group). Although other studies have demonstrated the utility of higher protein (160) or higher dairy (44, 45, 49) or exercise (146, 158) in weight loss, fat loss and/or lean mass maintenance during energy restriction, no study has combined higher protein, higher dairy and exercise into one regimen. Furthermore, previous mechanistic and animal studies (55, 57-60), and clinical findings (44, 45, 47) demonstrated that dairy, potentially through its constituents such as protein (leucine), calcium and vitamin D, decreased *de novo* lipogenesis (55, 57-60) and visceral adipose tissue (44, 45, 47), respectively. Therefore, we also sought to ascertain the effect of our intervention on MRI-derived visceral adipose tissue volume and compare this to DXA-determined trunk fat mass in the same individuals. This has never been done before in overweight and obese young women.

1.7.2. Specific hypothesis and objectives - Chapter 3

This Chapter highlights the effect of the I.D.E.A.L. for Women study on outcomes of bone health and calcium metabolism. Given that dairy foods are excellent sources of the three most important nutrients for bone health (protein, calcium and vitamin D) (53, 97, 108), and that our study design featured three treatment groups that consumed varying levels of these nutrients, we decided to investigate bone health as an important outcome. We hypothesized that daily exercise along with higher dietary protein and higher daily intakes of dairy from low fat milk, fat free yogurt and regular fat cheese, will provide enough bone supporting nutrients to positively affect bone health (bone turnover and OPG/RANKL ratio) and calcium metabolism (serum 25[OH]D and PTH) in overweight and obese young women. Previous work from our laboratory demonstrated that the consumption of one supplemental litre of milk per day over twelve weeks improved serum 25[OH]D concentrations and reduced PTH levels in women of similar age undergoing a rigorous resistance training program (86). Importantly, clinical trials of overweight premenopausal women have shown that bone health can be maintained during weight loss so long as calcium intakes are sufficient (134); however, no study has combined weight loss and exercise with varying levels of dairy and particularly not with higher protein intakes to assess bone health outcomes. Because of our study design, we were also able to assess whether bone health status can improve with even greater intakes of dairy and calcium (with daily exercise) which had not been investigated before. In addition, ours is the first study to assess the responses of the OPG/RANKL signalling

system, which directly affects osteoclastogenesis and bone resorption (129), in young women after this type of lifestyle modification.

1.7.3. Specific hypothesis and objectives - Chapter 4

In this Chapter we investigated how well a newly developed non-invasive method for measuring body fat – FT-NIR – compared to the commonly used and reliable DXA method for measuring body fat. Although fat mass measures from FT-NIR spectroscopy have been compared to those from MRI indirectly with age and sex matched individuals (174), FT-NIR spectroscopy had never before been validated against a gold standard modality of fat mass determination in the same subjects; hence, this was the objective of our investigation. We hypothesized that body fat measured by FT-NIR spectroscopy and DXA taken at the same time in the same overweight and obese young women would yield similar results, and that these measures would agree with and correlate well with each other.

Another important reason for conducting this study was to assess the degree of overlap in body fat, or lack thereof, in women within the same BMI category of overweight or obesity (25-29 kg/m^2 or 30-39 kg/m^2, respectively) in our study. Various inherent issues with using BMI to determine disease risk are well documented (167-170), and pertain to the fact that BMI is not a measure of fat mass. Studies show that fat mass is more strongly associated with disease risk (167, 169, 171), thus we aimed to corroborate this notion by simply demonstrating the varying degrees of fat mass in our subjects with similar BMIs. We hope this finding will add to the body of evidence advocating for

routine body composition measures versus a reliance on BMI for disease risk assessment and reduction in clinics and in research.

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CHAPTER 2

TITLE: Increased consumption of dairy foods and protein during diet- and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. J Nutr. 2011 Jul 20. [Epub ahead of print]. PMID: 21775530

AUTHORS: Andrea R. Josse, Stephanie A. Atkinson, Mark A. Tarnopolsky and Stuart M. Phillips

N.B. In this publication, the HiDairyPro group is referred to as High-Protein-High-Dairy (HPHD); the DairyPro group is referred to as Adequate-Protein-Medium-Dairy (APMD); and the Control group is referred to as Adequate-Protein-Low-Dairy (APLD).

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Increased Consumption of Dairy Foods and Protein during Diet- and Exercise-Induced Weight Loss Promotes Fat Mass Loss and Lean Mass Gain in Overweight and Obese Premenopausal Women[1–4]

Andrea R. Josse,[5] Stephanie A. Atkinson,[6] Mark A. Tarnopolsky,[7] and Stuart M. Phillips[5]*

[5]Exercise Metabolism Research Group, Department of Kinesiology, [6]Department of Pediatrics, and [7]Department of Pediatrics and Medicine, McMaster University, Hamilton, ON, Canada

Abstract

Weight loss can have substantial health benefits for overweight or obese persons; however, the ratio of fat:lean tissue loss may be more important. We aimed to determine how daily exercise (resistance and/or aerobic) and a hypoenergetic diet varying in protein and calcium content from dairy foods would affect the composition of weight lost in otherwise healthy, premenopausal, overweight, and obese women. Ninety participants were randomized to 3 groups (n = 30/group): high protein, high dairy (HPHD), adequate protein, medium dairy (APMD), and adequate protein, low dairy (APLD) differing in the quantity of total dietary protein and dairy food-source protein consumed: 30 and 15%, 15 and 7.5%, or 15 and <2% of energy, respectively. Body composition was measured by DXA at 0, 8, and 16 wk and MRI (n = 39) to assess visceral adipose tissue (VAT) volume at 0 and 16 wk. All groups lost body weight ($P < 0.05$) and fat ($P < 0.01$); however, fat loss during wk 8–16 was greater in the HPHD group than in the APMD and APLD groups ($P < 0.05$). The HPHD group gained lean tissue with a greater increase during 8–16 wk than the APMD group, which maintained lean mass and the APLD group, which lost lean mass ($P < 0.05$). The HPHD group also lost more VAT as assessed by MRI ($P < 0.05$) and trunk fat as assessed by DXA ($P < 0.005$) than the APLD group. The reduction in VAT in all groups was correlated with intakes of calcium (r = 0.40; $P < 0.05$) and protein (r = 0.32; $P < 0.05$). Therefore, diet- and exercise-induced weight loss with higher protein and increased dairy product intakes promotes more favorable body composition changes in women characterized by greater total and visceral fat loss and lean mass gain. J. Nutr. doi: 10.3945/jn.111.141028.

Introduction

Weight loss in overweight and obese persons through diet and/or exercise can confer a host of metabolic benefits (1,2). Although energy restriction alone often leads to weight loss, the tissue composition of the loss includes lean tissue (3–5), which could have deleterious metabolic consequences. The loss of lean tissue with weight loss may be responsible for the tendency toward a plateau in weight loss during, or weight regain following, a weight loss program (6,7). Moreover, skeletal muscle plays a number of important roles in the regulation of glycemia (8) and lipidemia (9), suggesting that the loss of this tissue may have adverse effects on long-term metabolic health (10). We propose that weight loss strategies should be focused on the tissue composition of the weight lost and not based merely on scale weight; instead, weight loss interventions should be aimed toward loss of fat, especially visceral fat, and the preservation of muscle mass.

The optimal dietary macronutrient composition to achieve weight loss remains controversial. Several large clinical trials have shown that adherence to an energy-restricted diet, not the macronutrient composition per se, seems to be the primary factor driving weight loss (11–13). However, none of these studies addressed the tissue composition of the participants' weight loss, especially lean mass loss, the sparing of which would actually reduce weight lost; thus, in these trials, the investigators potentially missed important effects of the different diets beyond weight change (11–13). For example, higher protein, lower carbohydrate, energy-restricted diets have been shown to help offset the lean mass loss observed with conventional (~55% of energy intake) carbohydrate diets (14–16), and pairing higher protein intakes with exercise, especially resistance

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[3] This trial was registered at clinicaltrials.gov as NCT00710398.
[4] Supplemental Figure 1 and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: phillis@mcmaster.ca.

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exercise, worked synergistically to further maintain lean mass during weight loss (17–19). Insofar as weight maintenance is concerned, individuals who consumed higher protein (and reduced the glycemic index of their dietary carbohydrates) following 8 wk of weight loss were better able to maintain their initial loss for an additional 26 wk compared to those who consumed maintenance diets lower in protein (20).

Trials in humans have linked the consumption of dairy foods and dairy-derived calcium to greater weight loss and fat mass loss (21–25), a mechanism that is supported by in vitro experimental studies for the antiadipogenic effects of dairy foods (26–28). In young, recreationally active men and women, we observed an advantage of consuming low-fat milk with resistance exercise in promoting lean mass gains both acutely (29) and over time in conjunction with fat mass loss, despite participants being in positive energy balance (30,31). Thus, as a strategy for weight loss and lean mass retention, dairy foods would appear advantageous.

In this study, we aimed to achieve weight loss with a high ratio of fat:lean mass loss. This pattern is important not only for short-term efficacy but also for long-term metabolic health and body weight maintenance (6,7). Our approach was multifaceted and incorporated aspects shown in previous programs to help preserve or increase muscle mass and yet still promote fat mass loss (30–32). We combined energy restriction, higher dietary protein (lower carbohydrate), increased intakes of dairy foods, and exercise (aerobic and resistance) into one program. Our specific objectives were to test: the effect of consuming high-dairy protein and calcium compared to a typical dietary pattern, the effect of doubling the consumption of dietary (and dairy) protein as a percent of total energy intake, and the effect of protein source (dairy vs. nondairy) in diets that contain moderate amounts of protein. We hypothesized that the optimal condition for a high ratio of fat:lean mass loss would occur in the individuals who consumed higher protein and a greater amount of dairy foods.

Methods

Participants. Our study, the Improving Diet, Exercise and Lifestyle (I.D.E.A.L.) for Women Study, was approved by the Research Ethics Board of the Hamilton Health Sciences and conformed to the most recent Canadian government tri-council funding policy statement on the use of human subjects in research (33). The study was conducted from June 2008 to May 2010. A total of 246 women were recruited from McMaster University and the surrounding Hamilton area through posters and local newspaper advertisements. Of those, 95 participants gave their written, informed consent to participate. Prior to beginning the trial, 5 women declined participation and 90 were randomized into 3 groups. Information regarding recruitment and participant flow is in **Supplemental Figure 1**.

Participants were all premenopausal and overweight or obese women (BMI between 27 and 40 kg/m^2) between the ages of 19 and 45 y. Other general inclusion criteria were: low dairy product consumption, sedentary lifestyle, regular menstrual cycle, no vitamin or mineral supplementation, and otherwise healthy. Participants were deemed healthy and eligible to participate based on their responses to a short medical screening questionnaire that inquired about metabolic risk factors (cholesterol, insulin, and glucose concentrations and blood pressure), heart and other organ disease, orthopedic injury that would interfere with exercise, gastrointestinal disease, clinically diagnosed dairy protein allergy, clinically diagnosed lactose intolerance, and prescription medication use. Participants were excluded if they had any of the aforementioned conditions.

Prior to the study commencing, participants' height and weight were measured (Healthweigh 140–10 Eye Level Digital Physician scale). They then completed a FFQ validated for calcium and dairy foods to verify their low consumption at baseline (as per the study inclusion criteria). Participants were also instructed on how to accurately complete a 7-d food record and were given their own set of measuring cups and spoons to use throughout the study.

Study protocol. Participants were randomly assigned to 1 of 3 groups: APLD[8], APMD, or HPHD. The randomization was stratified by BMI (27–29, 30–34, and 35–40 kg/m^2), the most commonly used criterion for classification of overweight/obesity, to ensure that an equal number of participants from each BMI category were in each treatment group. Our stratification scheme was adequate, because there were no between-group differences in any baseline variable. The 3 groups differed in the amount and type of protein consumed and their dietary macronutrient distribution. The APLD group maintained their stable baseline dairy food consumption at <2% energy/d from dairy protein corresponding to 0–1 serving/d [1 dairy serving represents 250 mL of milk, 50 g cheese, or 175 mL of yogurt according to Canada's Food Guide (34), and 250 mL of milk or yogurt and 45 g of cheese according to the USDA Food Pyramid (35)]. The APMD group was instructed to consume 7.5% energy/d from dairy protein at 3–4 servings/d of dairy products and the HPHD group to consume 15% of energy/d from dairy protein at 6–7 servings/d of dairy products. All dairy products needed to control dairy and calcium intakes were provided to participants in the APMD and HPHD groups and were generously donated by Agropur Dairy Cooperative (**Table 1**). The APLD and APMD groups consumed a diet consisting of 55:30:15 (percent energy from carbohydrate, fat, and protein) and the HPHD group consumed a diet consisting of 40:30:30. Thus, the study was designed such that the HPHD group consumed twice the amount of protein (and lower carbohydrate) than the other 2 groups and twice the amount of dairy foods as the APMD group. The APMD group consumed the same amount of protein as the APLD group (15%), with one-half of that protein from dairy sources and the other one-half from high-quality nondairy sources such as lean red meat, eggs, fish, chicken, pork, legumes, soy, and wheat. The APLD group was counseled to consume all protein from the same nondairy sources mentioned above. All participants were prescribed the same exercise regimen and underwent individual biweekly dietary counseling during the 16 wk.

Maintenance energy requirements were calculated per participant using the Mifflin St Jeor equation (36) with a sedentary activity factor. Once this energy level was determined, it was reduced by 500 kcal/d and used as the participant's targeted total energy intake throughout the study; thus, all women consumed a hypoenergetic diet.

All groups consumed 2 study drinks/d; one immediately postexercise and another drink at least 5 h before or after exercise. Each drink was 375 mL and was prepared daily in opaque, reusable, plastic bottles. Bottles were prepared and labeled for each participant by study staff in our metabolic kitchen. All drinks provided similar energy and were chocolate flavored so that they looked, smelled, and tasted similar to keep the participants unaware of their contents. Known to study staff only, the drinks were either 1% chocolate milk (APMD); Splenda-sweetened 1% chocolate milk (HPHD) (Table 1); or a custom-blended, nondairy, vitamin D- and calcium-free, carbohydrate-based, chocolate-flavored beverage (APLD). Splenda-sweetened chocolate milk was provided to the HPHD group to keep their carbohydrate intake from study foods low. This enabled them to consume their restricted level of dietary carbohydrate (40%) from other food sources (e.g. fruits, vegetables, and whole grains).

All participants received individualized diet counseling by study dieticians and research nutritionists on a biweekly basis. The initial 7-d food record was analyzed using ESHA (Food Processor SQL, ESHA Research) (37) and it served as the starting point for which the diet counseling was based. Each participant was provided with an individualized plan outlining their required macronutrient intakes in grams corresponding to their new daily energy requirements and the respective macronutrient distribution of the group to which they were randomized.

[8] Abbreviations used: AMDR, acceptable macronutrient distribution range; APLD, adequate protein, low dairy; APMD, adequate protein, medium dairy; CRP, C-reactive protein; HPHD, high protein, high dairy; HR, heart rate; VAT, visceral adipose tissue.

TABLE 1 Nutritional information of the dairy foods provided to the participants in the adequate protein, medium dairy (APMD) and high protein, high dairy (HPHD) groups in the Improving Diet, Exercise and Lifestyle for Women Study, including the products used, the quantities of dairy foods consumed, and their nutritional contents

	Quantity	Energy[1]	Calcium	Vitamin D	Protein
		Kcal	*mg*	*μg*	*g*
APMD					
1% chocolate milk (Sealtest)[2]	3 x 250 mL	450	750	6.8	21
Source yogurt (Yoplait)	2 x 100 g	70	200	1.5	8
Total amount		520	950	8.3	29
HPHD					
1% Splenda-sweetened chocolate milk (Sealtest)[2]	3 x 250 mL	300	750	6.8	21
1% white milk (Sealtest)	1 x 250 mL	100	300	2.3	9
Source yogurt (Yoplait)	4 x 100 g	140	400	3.0	16
Cheddar cheese (Bright Bites)	2 x 21 g	180	200	0.0	10
Total amount		720	1650	12.1	56

[1] 1 kcal = 4.18 kJ.
[2] Chocolate milk used as the study drinks for the APMD and HPHD groups.

Every 2 wk thereafter, participants provided a 3-d food record to track compliance with the nutrition protocol. All 3-d food records were analyzed with ESHA and participants were provided with feedback in their next biweekly private counseling session. To ensure that the results were due as much as possible to the differing dietary components, participants were instructed not to take any vitamin or mineral supplements during the study.

Exercise training. Participants exercised in our own exercise and testing center and at the main fitness center at McMaster University. They engaged in various modes of aerobic exercise every day, 5 d/wk with us and 2 d on their own on the weekend. At each workout participants were to expend 250 kcal. During the week (Monday–Friday), they reported to our study office daily and were often given a SenseWear Pro energy expenditure device (BodyMedia) to wear, programmed for them, so they could track their energy expenditure during their workout. Compliance with weekend workouts was assessed by having women take home and wear the SenseWear Pro device on random occasions. In addition to aerobic exercise, participants engaged in a progressive resistance training regimen 2 d/wk (upper body, lower body split) with trained study personnel (personal trainer or kinesiologist). Weight lifted by each participant was recorded every session and increased once they could complete 3 sets of 10 repetitions or more at any given weight. The majority (i.e. ~70%) of the aerobic exercise sessions were individually or small-group (i.e. 1 trainer to 2–3 participants) supervised and all of the resistance exercise sessions were individually supervised. Resistance exercise logs were completed by the trainer and aerobic exercise logs were completed by the participant and checked frequently by study personnel to ensure compliance.

Body composition. Whole-body DXA scans (QDR-4500A; Hologic, software version 12.31) were carried out at wk 0, 8, and 16 to determine total body weight, fat mass, and (fat and bone free) lean mass. Women were scanned wearing light clothing at the same time of day and were instructed to follow the same prescan diet and exercise instructions before each scan. After study completion, pre- and poststudy DXA scans were further analyzed for the determination of trunk fat. The abdominal region of interest on all scans was isolated between the lumbar vertebrae L1–L4 by the same trained individual and fat mass was recorded. Investigators were unaware of the participants' group assignment during analysis.

A subset of women (n = 39) underwent MR imaging (3T Signa Scanner, GE Healthcare) at wk 0 and 16 to directly ascertain the change in VAT volume during the intervention. Of these 39 women, 8/12 in the APLD group, 8/13 in the APMD group, and 9/14 in the HPHD group were Caucasian. MRI scans were performed on the participants' torso region from the top of their liver to the iliac crest (determined by an axial scan), corresponding to 3 sectional scans of 10–12 slices each. Participants were instructed to hold their breath after exhalation for the duration of the sectional scan (~20 s each) to ensure that the movement of breathing did not adversely affect the quality of the image. The MR images were analyzed with Santesoft Medical Imaging software (Sante DICOM Editor) using a standardized tracing protocol. To reduce variability, all scans were analyzed by the same trained and blinded technician who, on random scans, showed an inter-scan variability of $<5\%$.

Strength and aerobic fitness testing. Strength was tested by determining each participant's single lift voluntary maximal strength or 1 repetition maximum on the leg extension, hamstring curl, seated row, and chest press pre- and postintervention. All testing was done by trained study personnel using the same standardized protocol described elsewhere (31).

Aerobic fitness was measured using a modified Astrand submaximal fitness test protocol (38). Participants were asked to cycle on a stationary bicycle (Monark Ergomedic 828E, HealthCare International) at 2 consecutive workloads for 6 min each and HR was recorded every minute.

Anthropometry. Waist and hip circumference was measured 3 times throughout the study: at wk 0, 8, and 16. We used the circumference of the waist at the participant's umbilicus as the site of measure. Two separate research assistants took measurements independently with the same tape measure (Gulick II Tape Measure, FitnessMart) and the 2 measurements were averaged. The average difference between the 2 measures was always <1.5 cm.

Blood samples and laboratory analyses. Fasting blood samples were obtained on 2 occasions (pre- and postintervention) between 0630 and 1000 h after an overnight fast of 10–12 h in tubes containing either sodium heparin or no additives to obtain plasma and serum, respectively. Serum insulin (chemiluminescent microparticle immunoassay; Architect System, Abbott Laboratories) and lipids and plasma glucose and calcium (colorimetric assay; Roche COBAS MIRA Clinical Analyzer, Roche Diagnostics) were analyzed at the Core Laboratory at the McMaster University Medical Centre. The remaining samples were stored at −20°C for later analysis. At study completion, serum samples were batch-analyzed for IL-6 (high sensitivity ELISA; R&D Systems), and CRP (ELISA; ALPCO Immunoassays). All investigators and laboratory technicians were unaware of the participants' group assignment during analysis.

Statistics. Statistical analyses were performed using SPSS (version 18.0). Prior to hypothesis testing, primary endpoint data were examined for normality. Non-normally distributed variables were log-transformed before analysis. Analysis was conducted on all participants who completed the study as well as on those who did not complete (all of

whom dropped out after 8 wk). In accordance with recently suggested practices for randomized weight loss trials (39), we conducted an intent-to-treat analysis where missing data values were imputed using model-based multiple imputation. Differences between groups in all baseline variables were compared by univariate ANOVA. Significance was set at $P < 0.05$. Data in the text are means ± SE.

Initial analysis was performed using a 2-way, repeated-measures ANCOVA with baseline bodyweight as the covariate for the primary outcome variables. Two-way, repeated-measures ANOVA was also performed on the change values (wk 0–8 and 8–16) for the primary outcome variables and waist circumference. Significant F ratios were further analyzed using planned contrast ANOVA (repeated measures or univariate) to determine differences both within and between groups, respectively. Significant differences were isolated with Tukey's post hoc test with a Bonferroni correction for multiple comparisons. Pearson correlation coefficients were also calculated for several measures. The MRI portion of the study was carried out on a smaller cohort of women (n = 39; 12 in APLD, 13 in APMD, and 14 in HPHD); therefore, only these women were included in the MRI statistical analysis.

Results

Participants. Ninety women participated in this study. Nine participants (6 in the APLD group and 3 in the HPHD group) dropped out after wk 8 (half-way) for reasons unrelated to the study (Supplemental Fig. 1). The participants represented a multiracial/multiethnic population with n = 15 (APLD), n = 13 (APMD), and n = 18 (HPHD) Caucasian women in each group. The other women were East Indian (n = 15), Asian (n = 9), African, African American, Native Canadian, Portuguese, European, or Hispanic and were equally distributed throughout the groups. None of the baseline values for any variables studied significantly differed between the groups (**Table 2**).

Body composition (DXA). Total body mass decreased by similar amounts across all groups over time with no differences between groups (pooled mean change over time: −4.3 ± 0.7 kg; $P < 0.05$). The loss of body fat in all groups was significant when expressed in kilograms and percent of initial weight ($P < 0.01$), but the fat mass and percent fat reductions in the latter half of the study (wk 8–16) in the HPHD group were significantly greater than in the other 2 groups (**Fig. 1**). The loss of percent fat expressed as the change from baseline in the HPHD group was similar in the first and second halves of the study. Lean mass increased over the 16 wk in the HPHD group (0.7 ± 0.3 kg; $P < 0.05$), remained unchanged in the APMD group (−0.2 ± 0.2 kg), and decreased in the APLD group (−0.7 ± 0.3 kg; $P < 0.05$). During the latter half of the study, the accretion of lean mass in the HPHD group was greater than in the APLD ($P < 0.01$) and APMD groups ($P < 0.05$). DXA-determined trunk fat mass [regional analysis of L1–L4; a surrogate measure of VAT (40)] decreased in all groups ($P < 0.05$), with the HPHD group losing more trunk fat than the APLD group ($P < 0.005$; **Fig. 2***A*).

VAT volume (MRI). All groups had a reduction in VAT volume over time ($P < 0.05$); however, the HPHD group lost more VAT than the APLD group ($P < 0.05$; Fig. 2*B*). The change in trunk fat mass, obtained by DXA, was correlated with the change in MRI VAT measured in the same women (Fig. 2*C*). This indicates that determination of changes in trunk fat mass by DXA in this population provides a reasonable proxy measure for changes in VAT.

Strength and fitness. All groups improved their strength for all exercises over time (**Table 3**). The HPHD group had greater strength at wk 16 than the APLD group in the seated row and the hamstring curl ($P < 0.05$) and a tendency for greater strength in the chest press (P = 0.059). Cardiovascular fitness improved similarly in all groups with a mean HR reduction pre- to postintervention of ~15 bpm ($P < 0.05$).

Anthropometry. BMI decreased across all groups over time with no differences between groups (pooled mean change over time: −1.8 ± 0.3 kg/m^2; $P < 0.05$). Waist circumference also decreased in all groups throughout the study (pooled mean change over time: −4.9 ± 0.9 cm; $P < 0.05$). When expressed as change from baseline, that in the APLD group during the latter half of the study (−0.6 ± 0.8 cm; wk 8–16) was less than that during the first half (−4.2 ± 1.0 cm; wk 0–8; $P < 0.05$). On the other hand, both the APMD (−3.0 ± 0.7 and −1.8 ± 0.7 cm) and HPHD (−3.2 ± 0.8 and −1.9 ± 0.7 cm) groups showed similar significant reductions in waist circumference in the earlier and later halves of the study.

Blood results. IL-6 decreased over time in the APMD group (−0.39 ± 0.27 ng/L; $P < 0.05$) and tended to decline in the HPHD group (−0.37 ± 0.12 ng/L; P = 0.071). There was no change in the APLD group (−0.10 ± 0.14 ng/L). CRP decreased in all groups over time with no differences between groups (pooled mean change over time: −2.32 ± 0.66 mg/L; $P < 0.05$). Baseline levels in all groups for glucose, insulin, cholesterol (total, LDL, and HDL), and TG were within normal ranges (41,42) and did not significantly differ from one another (Table 2). During the study, total cholesterol, LDL cholesterol, and TG decreased in the HPHD and APLD groups ($P < 0.05$). Insulin levels decreased in the APMD group ($P < 0.05$). Plasma calcium remained unchanged during the study in all groups (**Supplemental Table 1**).

Diet. Baseline diets were analyzed from the prestudy 7-d food records. Calcium intake at the start of the study was approximately one-half of the recommended intake (43) (Table 2) and did not differ between groups. At study entrance, participants were consuming close to the RDA for protein (44) (Table 2), which corresponded to a mean intake of 15% of daily energy from protein across all groups. Carbohydrate consumption in all groups was ~52% and fat was ~31% (Table 2).

Calcium intake at wk 16 (which reflected dietary intakes throughout the study; **Table 4**) correlated with changes in percent body fat (r = 0.21; $P < 0.05$), trunk fat mass (r = 0.30; $P < 0.01$), and VAT volume (r = 0.40; $P < 0.01$). Protein consumption at wk 16 (expressed as a percentage of daily energy intake; Table 4) was also correlated with changes in body fat mass (r = 0.22; $P < 0.05$), percent body fat (r = 0.25; $P < 0.05$), trunk fat mass (r = 0.33; $P < 0.005$), and VAT volume (r = 0.32; $P < 0.05$).

Discussion

In overweight and obese premenopausal women, significantly greater fat loss accompanied by a gain in lean (muscle) mass was achieved during a 16-wk hypoenergetic period, mediated by diet (−500 kcal/d) and exercise (−250 kcal/d), that provided higher dietary protein (30% of total energy intake) within the AMDR (44), with one-half of the total dietary protein intake from dairy foods. Although all treatment groups lost a significant amount of trunk fat, higher dairy food and dietary protein consumption were associated with significantly greater reductions in trunk fat mass and VAT volume than those consuming no dairy foods and

TABLE 2 Baseline characteristics of participants in the Improving Diet, Exercise and Lifestyle for Women Study[1]

Variable	APLD	APMD	HPHD
Body composition			
BMI, *kg/m²*	31.5 ± 0.6	31.8 ± 0.6	31.4 ± 0.6
Height, *cm*	163 ± 1	164 ± 1	166 ± 1
Age, *y*	28 ± 1	26 ± 1	30 ± 1
Body weight, *kg*			
DXA	84.0 ± 2.1	85.3 ± 2.1	87.1 ± 2.1
Scale	83.8 ± 2.1	85.2 ± 2.0	86.7 ± 2.2
Fat mass, *kg*	33.1 ± 1.4	34.8 ± 1.2	35.5 ± 1.3
Body fat, *%*	39.1 ± 0.9	40.6 ± 0.7	40.5 ± 0.6
Lean mass, *kg*	48.5 ± 1.1	48.2 ± 0.9	49.2 ± 0.9
Waist circumference, *cm*	99 ± 2.2	102 ± 1.80	102 ± 2.01
Waist:hip ratio	0.86 ± 0.01	0.88 ± 0.01	0.87 ± 0.01
Trunk fat, *DXA, kg*	3.3 ± 0.2	3.8 ± 0.2	4.0 ± 0.3
Visceral fat,[2] *MRI, cm³*	655 ± 80.0	796 ± 77.2	666 ± 43.6
Heart rate (HR) in fitness test			
1st workload, HR, *bpm*	kp[3] = 1.2; 138 ± 3	kp = 1.2; 141 ± 3	kp = 1.3; 139 ± 3
2nd workload, HR, *bpm*	kp = 1.9; 166 ± 3	kp = 1.8; 163 ± 3	kp = 2.1; 167 ± 3
Daily dietary intakes[4]			
Carbohydrate			
g	234 ± 9	243 ± 8	250 ± 9
% of total energy	51 ± 1	53 ± 1	53 ± 1
Protein			
g	69 ± 3	66 ± 3	69 ± 4
g/kg body weight	0.81 ± 0.05	0.78 ± 0.03	0.80 ± 0.05
% of total energy	16 ± 1	14 ± 1	15 ± 1
Fat			
g	68 ± 4	65 ± 3	63 ± 4
% total energy	33 ± 1	31 ± 1	30 ± 1
Energy			
MJ (Mcal)	7.66 ± 0.31 (1.83 ± 0.07)	7.63 ± 0.22 (1.82 ± 0.05)	7.69 ± 0.33 (1.84 ± 0.08)
kJ/kg body weight	92 ± 4.1	90 ± 3.1	90 ± 4.4
Calcium, *mg*	555 ± 39	480 ± 26	520 ± 28
Vitamin D, *μg*	1.4 ± 0.2	1.7 ± 0.3	1.7 ± 0.3
Blood analytes			
Glucose, *mmol/L*	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Insulin, *pmol/L*	57 ± 7	69 ± 8	51 ± 6
Hemoglobin A1c, *%*	5.6 ± 0.2	5.4 ± 0.1	5.4 ± 0.1
Cholesterol, *mmol/L*			
Total	4.72 ± 0.15	4.48 ± 0.11	4.67 ± 0.15
LDL	2.75 ± 0.13	2.58 ± 0.09	2.77 ± 0.13
HDL	1.40 ± 0.05	1.39 ± 0.05	1.43 ± 0.05
TG, *mmol/L*	1.10 ± 0.07	1.11 ± 0.07	1.00 ± 0.07

[1] Values are means ± SE, n = 90 (30/group) unless otherwise noted.
[2] n = 39: 12 in APLD, 13 in APMD, 14 in HPHD.
[3] kp = Kilopond; 1 kp at 50 rpm = 50 watts; 1.5 kp = 75 watts; 2 kp = 100 watts.
[4] From baseline 7-d food records.

lower protein despite undergoing the identical exercise regimen. Reductions in trunk fat mass were reflected in reduced waist circumferences, which were also greater in the APMD and HPHD groups compared to the APLD group. The observed greater fat loss and the increase in lean mass in the HPHD group, in the face of similar weight loss across all groups, highlights the beneficial body composition changes in the HPHD diet intervention in support of our original hypothesis. Because all groups performed resistance exercise (2 d of 7 d/wk), which is a potent stimulus for skeletal muscle anabolism (32,45,46), our results likely represent a loss of skeletal muscle (lean) tissue that was minimized in the APLD and APMD groups (3,32) but that translated into a gain, with a measurably greater strength gain, in the HPHD group.

Favorable associations between dairy food consumption and adiposity, weight loss, body compositional change, prevention of weight gain, and a decreased incidence of insulin resistance and other metabolic syndrome risk factors have been reported in observational studies and population surveys (47–49). Data from a recent meta-analysis also emphasizes the benefit of calcium on body weight in premenopausal women (25), and clinical trials highlight a possible threshold effect for calcium intake such that those with habitual calcium intake levels > ~600–700 mg/d do not seem to derive the same body compo-

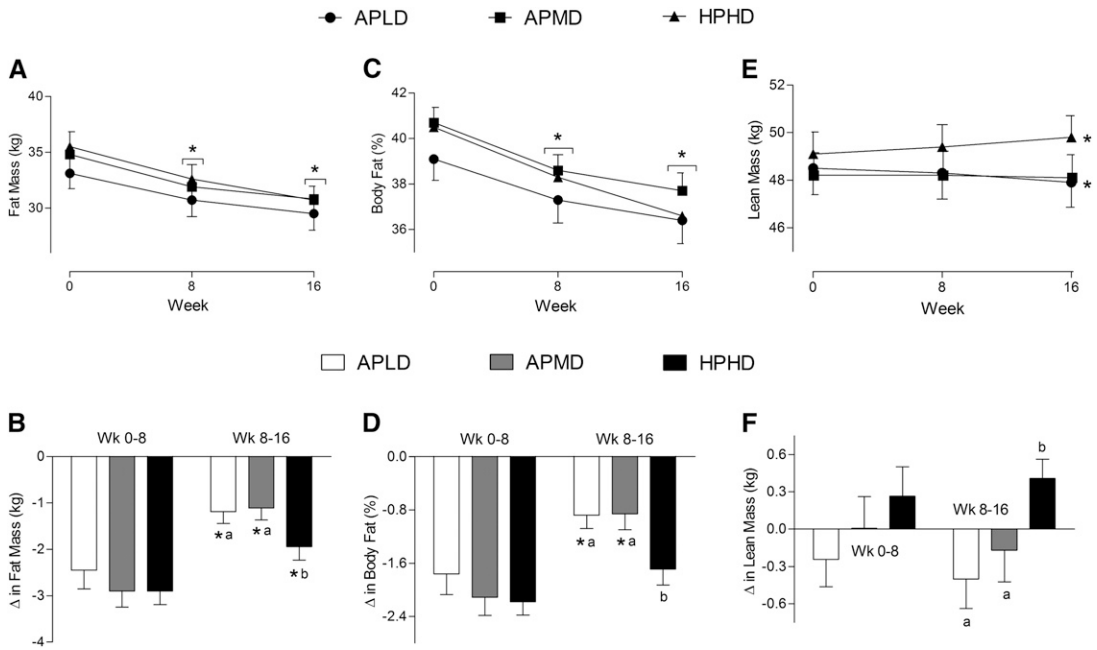

FIGURE 1 Absolute (*A,C,E*) and relative-to-baseline (*B,D,F*) changes in body fat mass (*A,B*), percent body fat (*C,D*), and lean mass (*E,F*) in overweight and obese premenopausal women who underwent a 16-wk intervention of diet- and exercise-induced weight loss. Values are means ± SE, $n = 90$ (30/group). Means at a time without a common letter differ, $P < 0.05$. *Different from the preceding time point, $P < 0.05$.

sition effect from calcium consumption as those with lower baseline intakes (50,51). Thus, as an entrance criterion, using a FFQ and baseline food records, we measured and verified that our participants were consuming <555 mg/d calcium at study entry. As such, our data can still be generalized to North American women, of whom a large percentage, according to the NHANES (52,53) and the Canadian Community Health Survey (54), do not consume adequate calcium and are overweight or obese (55).

The decision to use dairy foods in this trial was premised on evidence demonstrating that consumption of calcium from dairy foods as opposed to elemental calcium, with energy restriction, has a greater effect on weight loss and body composition in humans (56,57). The greater fat loss observed with dairy foods compared to calcium supplementation may relate to the fact that dairy foods contain other bioactive components acting independently or synergistically with calcium and vitamin D to enhance fat loss. In premenopausal obese women consuming low-fat milk (providing 1200 mg/d calcium) on an 8-wk energy-restricted diet, greater reductions were observed in weight, BMI, and waist:hip ratio than in groups who consumed calcium carbonate or a soy beverage with equal amounts of calcium (56). The authors concluded that the additional effect observed with milk consumption likely related to other bioactive components such as the protein fraction rich in branched-chain amino acids (leucine, isoleucine, and valine) (58). Other effects of calcium and dairy foods that could potentially be contributing to a greater fat loss include an increased fecal fat excretion (59), a decrease in fat absorption (60), increase in fat oxidation (61), and an increased thermic effect of food (62).

In vitro mechanisms have been shown to exist in adipocytes that support an antiadipogenic and prolipolytic effect of dairy components, including leucine and calcium (26,63,64). Similar effects have been demonstrated in rodents (65,66). These mechanisms may help to explain the greater total and visceral fat mass loss experienced by those women consuming the most dairy foods and dairy-derived calcium (the HPHD group). Interestingly, it may not require an energy deficit to see such effects. We have reported similar effects under anabolic conditions (i.e. energy surplus with intense resistance training) in young women consuming an additional 1 L/d of low-fat milk (31). Our trial design featured changes in consumption of whole foods with varying contents of macronutrients to affect fat loss as opposed to isolated individual nutrients that comprise dairy; thus, the significance of calcium per se as a causative factor in fat loss is hard to determine. However, we also observed that dietary calcium intakes during the study were significantly correlated with changes in percent body fat and visceral fat assessed by both DXA and MRI, which is similar to the relationships between calcium intake and fat loss during energy restriction reported by others (26,63,64). Reductions in inflammatory biomarkers (CRP and IL-6), which also may relate to visceral fat loss, were seen in all groups, with the HPHD and APMD groups having greater reductions in IL-6 than the APLD group.

The consumption of higher protein (lower carbohydrate) compared to conventional protein (i.e. 15% of energy) with energy restriction has yielded mixed results with respect to weight loss and several studies have shown that the macronutrient composition of a weight loss diet does not seem to matter as much as compliance to the regimen itself (11–13). However, when assessing the effect of weight loss diets with different macronutrient ratios on hypoenergetic-induced changes in body composition, higher protein diets appear to be advantageous. In a meta-analysis (14), energy restriction (without exercise) with higher protein intakes [125% of the protein RDA (44)] were associated with a greater lean mass retention than studies where protein consumption was at the RDA (44). In our study, the HPHD group consumed ~1.3 g/(kg·d) protein for 16 wk (Table 4), which could have contributed to the lean mass gain in this group. In addition, the protein and carbohydrate intakes of the HPHD group (~30 and 40% daily energy intake, respectively) were not as excessive as those employed in more extreme protocols (11,12) and they were close to intakes recommended in the AMDR (44). Of note (and not by design), during the study the APLD group was consuming protein at a level slightly below

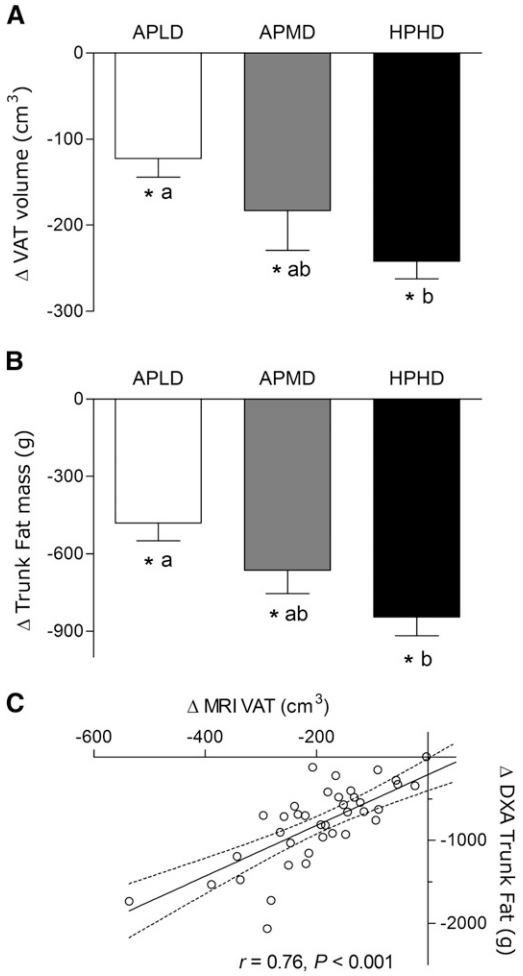

FIGURE 2 Relative to baseline changes in trunk fat mass measured by DXA (*A*) and visceral fat volume measured by MRI (*B*) in overweight and obese premenopausal women who underwent a 16-wk intervention of diet- and exercise-induced weight loss, and the correlation between the 2 measures (*C*). Values are means ± SE, $n = 39$. Means without a common letter differ, $P < 0.05$. *Different from wk 0, $P < 0.05$.

the RDA (44) (Table 4) and an additional 0.08 g/(kg·d) corresponding to ~8 g/d of protein would have rectified this. However, it has been suggested that even protein intakes around the RDA may be insufficient during weight loss to spare muscle mass (16). Nevertheless, protein intake in the APLD group did correspond to 16% of their daily energy intake, which is within the AMDR (10–35%) (44) and at the appropriate a priori level per the study protocol. Other studies have assessed the combined effect of high protein and physical activity during a period of hypoenergetic weight loss (17,19) and reported that exercise does not actually affect total weight loss; however, exercise increases fat mass loss and also promotes lean mass retention greater than that seen with the intake of higher protein alone (17), which parallels our observations (17,19).

With 16 wk of training composed of both aerobic and resistance exercise, strength and fitness increased substantially in all groups. HR reductions at the same absolute workload averaged 15 bpm, which is indicative of a clinically meaningful increase in aerobic fitness. Improvements in cardiovascular fitness show a strong negative association with all-cause mortality in women (67); therefore, the importance of the increase in fitness shown here should not be overlooked. Insofar as muscular strength is concerned, although gains were evident in all groups for all exercises performed, the HPHD group had greater increases in strength in various exercises (seated row, hamstring curl) compared to the APLD group (Table 3). It is entirely possible that these strength gains relate to the body composition changes in that the group that lost muscle mass (the APLD group, albeit minimal) had the most modest improvements in strength, whereas those that lost no muscle (the APMD group) or even gained muscle (the HPHD group) showed greater improvements. The improvement or even preservation of muscle function with weight loss could have important implications in populations such as the elderly, in whom muscle mass preservation is critical. Similar results for strength gains were demonstrated in a previous study performed in our laboratory in women consuming milk compared to an isoenergetic carbohydrate-containing drink post resistance exercise (31).

In summary, higher intakes of dietary protein and dairy foods, during dietary energy restriction combined with an exercise intervention, improved the composition of weight lost compared to those who consumed diets lower in protein and lower or devoid of dairy foods. We observed what we view as a highly beneficial profile of weight loss in the HPHD group: greater total fat and visceral fat losses, greater lean mass gains, and increases in strength despite identical body weight loss. Correlations between self-reported dietary calcium and protein intakes with total fat, and DXA- and MRI-derived trunk/visceral fat losses indicate potential mechanisms of action for these nutrients in fat loss. Our data suggest that higher protein intakes with an emphasis on increased intakes from dairy foods, modest energy restriction, and combined aerobic and resistance training result in favorable body composition, strength, and fitness changes that are not captured by simple measures of body weight or BMI. These data provide evidence for the promotion of low-fat dairy food consumption not only to ensure adequate calcium intakes in populations previously deficient (52–54), but to promote the provision of high-quality protein that can aid in fat loss and lean mass retention during energy restriction.

TABLE 3 Single repetition voluntary strength test results from overweight and obese premenopausal women before and after a 16-wk intervention of diet- and exercise-induced weight loss[1]

	APLD		APMD		HPHD	
Variable	wk 0	wk 16	wk 0	wk 16	wk 0	wk 16
			kg			
Seated row	34 ± 1	36 ± 1[a*]	35 ± 1	40 ± 1[b*]	35 ± 1	41 ± 1[b*]
Chest press	28 ± 2	30 ± 1*	28 ± 2	33 ± 1*	28 ± 1	34 ± 1*
Leg extension	77 ± 3	89 ± 3*	79 ± 3	89 ± 3*	80 ± 3	95 ± 3*
Hamstring curl	46 ± 2	52 ± 2[a*]	49 ± 2	55 ± 2[a,b*]	49 ± 2	58 ± 2[b*]

[1] Values are means ± SE, $n = 90$ (30/group). Means at a time with superscripts without a common letter differ, $P < 0.05$. *Different from wk 0, $P < 0.01$.

TABLE 4 Dietary intakes from overweight and obese premenopausal women consuming hypoenergetic diets during a 16-wk intervention of diet- and exercise-induced weight loss[1]

Dietary variable	APLD	APMD	HPHD
Energy intake,[2] *kcal/d*	1320 ± 40[a]	1430 ± 42[a,b]	1500 ± 36[b]
Energy restriction achieved,[2] *kcal /d*	−498 ± 41	−477 ± 42	−435 ± 37
Protein			
%/d	16 ± 1[a]	18 ± 1[b]	28 ± 1[c]
g/d	55 ± 1[a]	66 ± 2[b]	108 ± 3[c]
g/(kg·d)	0.72 ± 0.02[a]	0.84 ± 0.02[b]	1.33 ± 0.04[c]
Fat, *%/d*	28 ± 1[a]	24 ± 1[b]	31 ± 1[c]
Carbohydrate, *%/d*	56 ± 1[a]	58 ± 1[a]	41 ± 1[b]
Dietary fiber, *g/d*	21 ± 1	18 ± 1	16 ± 1
Calcium, *mg/d*	299 ± 22[a]	1200 ± 19[b]	1840 ± 13[c]
Vitamin D, *μg/d*	0.7 ± 0.1[a]	9.8 ± 0.3[b]	13.2 ± 0.2[c]
Vitamin C, m*g/d*	119 ± 19[a]	79 ± 6[a,b]	76 ± 6[b]
Vitamin A, *mg of RAE* [3] */d*	1.71 ± 0.22[a]	1.85 ± 0.16[a]	2.55 ± 0.18[b]
Iron, *mg/d*	10 ± 0.6[a]	15 ± 0.5[b]	11 ± 0.6[a]

[1] Values are means ± SE, $n = 90$ (30/group). Data were taken from post study 7-d food records. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] 1 kcal = 4.18 kJ.
[3] RAE, retinol activity equivalent.

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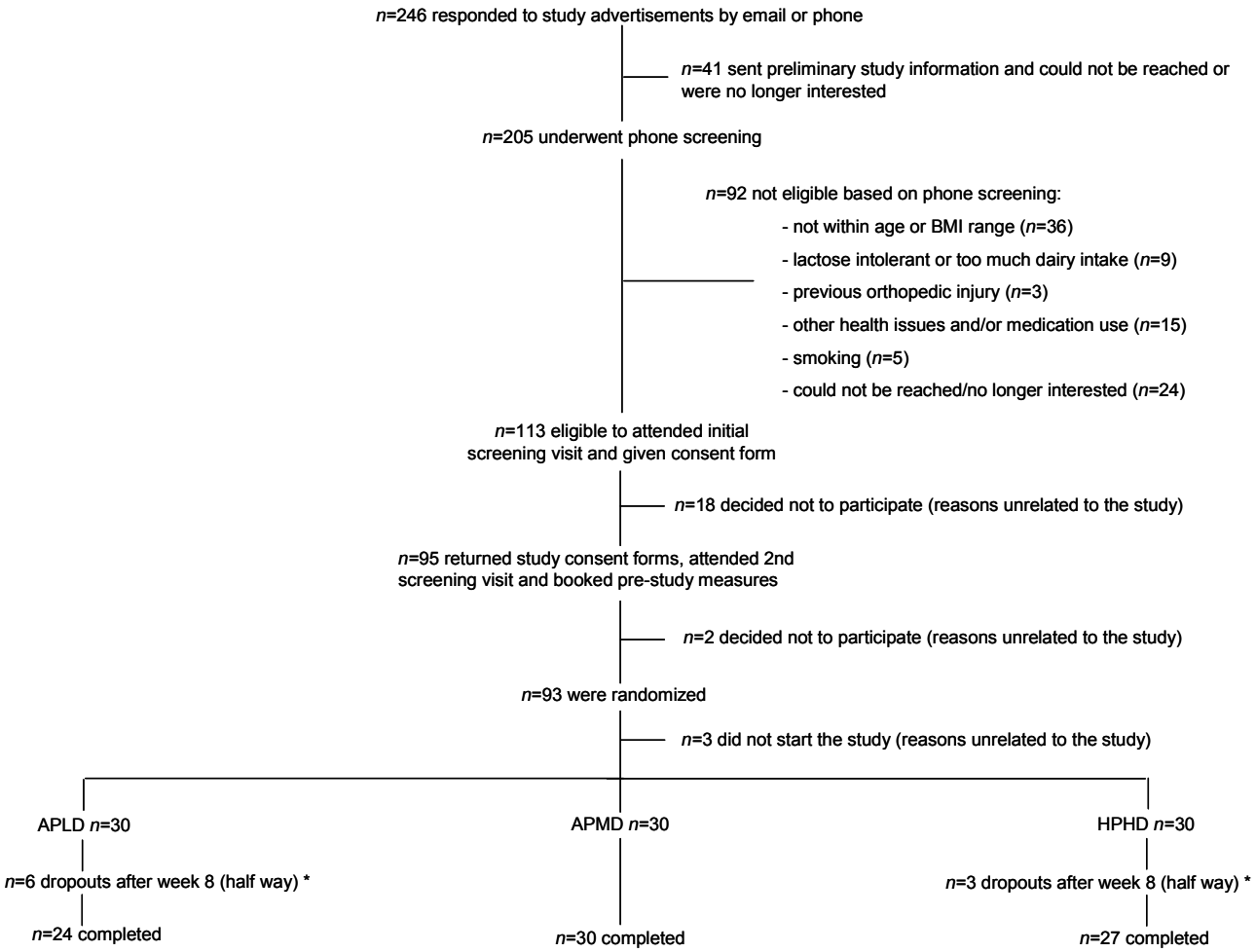

Supplemental Figure 1. Participant flow through the I.D.E.A.L. for Women study. * Six participants in APLD and three participants in HPHD dropped out for reasons unrelated to the study including: moving houses, working extra shifts, sickness of a relative, and exam time at school.

Supplemental Table 1: Circulating lipids, glucose, insulin, inflammatory markers and calcium in overweight and obese premenopausal women before and after a sixteen week intervention of diet- and exercise-induced weight loss. [1]

	APLD		APMD		HPHD	
Variable	**wk 0**	**wk 16**	**wk 0**	**wk 16**	**wk 0**	**wk 16**
Serum Cholesterol, *mmol/L*						
Total	4.72±0.15	4.39±0.16*	4.48±0.11	4.39±0.11	4.67±0.15	4.39±0.13*
LDL	2.75±0.13	2.54±0.12*	2.58±0.09	2.53±0.09	2.77±0.13	2.51±0.12*
HDL	1.40±0.05	1.42±0.06	1.39±0.05	1.35±0.04	1.43±0.05	1.47±0.06
Serum triglycerides, *mmol/L*	1.10±0.07	0.93±0.08*ab	1.11±0.07	1.09±0.11a	1.00±0.07	0.81±0.06*b
Plasma glucose, *mmol/L*	4.9±0.1	4.7±0.1	4.9±0.1	4.9±0.1	4.9±0.1	4.9±0.1
Serum insulin, *pmol/L*	57±7	50±5	69±8	59±7*	51±6	44±6
Plasma calcium, *mmol/L*	2.41±0.02	2.39±0.02	2.38±0.02	2.40±0.02	2.50±0.02	2.51±0.02
Serum IL-6, *ng/L*	1.95±0.13	1.86±0.20	2.08±0.31	1.69±0.15*	1.80±0.16	1.43±0.13
Serum C-reactive protein, *mg/L*	9.66±2.25	7.19±2.25*	6.40±1.37	4.25±0.75*	7.21±1.83	4.88±1.27*

[1] Data are presented as mean ± SE; n=90 (30 per group). Means at a time with superscripts without a common letter differ, $P<0.05$. *Different from wk 0, $P<0.05$.

CHAPTER 3

TITLE: Diets higher in dairy and dietary protein support bone health status during diet- and exercise-induced weight loss in overweight and obese premenopausal women

AUTHORS: Andrea R. Josse, Stephanie A. Atkinson, Mark A. Tarnopolsky and Stuart M. Phillips

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3.1. ABSTRACT

Consolidation and maintenance of peak bone mass in young adulthood may be compromised by inactivity, low dietary calcium, and restrictive dietary practices for weight loss. We aimed to determine how higher intakes of dairy and dietary protein during diet- and exercise-induced weight loss for sixteen weeks affected bone health in premenopausal, overweight and obese women. Ninety participants were randomized to three groups (n=30/group): HiDairyPro, DairyPro and Control, differing in the quantity of total protein consumed (30%, 15% or 15% of energy, respectively) and the amount from dairy foods (high, moderate or low, respectively). Bone mineral density and content, serum and urine bone turnover biomarkers, serum osteoprotegerin, receptor activator of nuclear factor kappa-B ligand (RANKL), parathyroid hormone and 25-hydroxyvitamin-D were measured at baseline and sixteen weeks. All treatment groups lost body weight and fat ($P<0.05$). N-Telopeptide (NTX), C-telopeptide (CTX), urinary deoxypyridinoline and osteocalcin increased in Control ($P<0.01$), whereas in HiDairyPro, only osteocalcin and procollagen 1 amino-terminal propeptide (P1NP) increased ($P<0.05$), and all resorption markers remained unchanged. NTX was slightly elevated in DP ($P<0.05$). P1NP:CTX ratio increased in HiDairyPro and DairyPro ($P<0.005$), and decreased in Control ($P<0.05$). Osteoprotegerin increased in HiDairyPro and DairyPro ($P<0.05$) and RANKL decreased in HiDairyPro only ($P<0.05$). Parathyroid hormone decreased in HiDairyPro and DairyPro versus Control ($P<0.005$), and 25-hydroxyvitamin-D increased in HiDairyPro ($P<0.05$), remained unchanged in DairyPro and decreased in Control ($P<0.05$). In conclusion, hypoenergetic

diets higher in dairy and dietary protein with daily exercise favourably affected several important markers of bone health versus diets without these bone-supporting nutrients.

3.2. INTRODUCTION

Peak (or adult) bone mass is mostly accrued by the end of adolescence with final consolidation achieved between the second and third decade of life (1, 2). Efforts to maximize the accretion of bone by early adulthood are crucial since a higher peak bone mass may help to delay the declines in bone health status with age and lessen the burden of diseases like osteoporosis later in life (3, 4). While genetics play a large role in determining an individual's peak bone mass, lifestyle factors such as nutrition and exercise can also make a considerable contribution to the enhancement and maintenance of bone mass during this time (2, 5).

Milk and other dairy products provide about 70% of the dietary calcium in our food supply (4), and it has been suggested that without the consumption of dairy foods, it would be difficult to meet the current dietary recommendations for several essential nutrients including calcium, potassium, magnesium, vitamin D (if fortified) and certain B vitamins (6, 7). Dairy foods are also good sources of high quality protein (8). With respect to bone health, all of the aforementioned nutrients contribute to the structural integrity and strength of bone either by influencing bone mineralization (*via* formation of hydroxyapatite crystals) or collagen formation (2, 7). Intakes of dietary calcium and vitamin D further promote bone health by decreasing circulating parathyroid hormone (PTH) concentrations which positively affects bone mineralization and reduces rates of bone turnover (2, 9).

While a higher body weight is associated with greater bone mass (10), weight loss by energy restriction can adversely affect bone health (11, 12). This relationship between body mass and bone likely reflects the established positive effect that weight bearing and mechanical loading have on bone (11, 13, 14), and conversely, may explain the negative effect that prolonged inactivity and unloading have on bone mass (15). Nonetheless, recent clinical trials have demonstrated that the reductions in bone mass sometimes observed with energy restriction can be offset with increased consumption of dietary protein (emphasizing dairy), increased dietary calcium intakes and/or exercise (10, 11, 16, 17).

Strategies to maintain or improve bone health during weight loss should include increased intakes of bone-supporting nutrients such as protein, calcium and vitamin D, as well as weight bearing exercise to stimulate bone. While the individual effects of dairy, calcium and protein nutrition (16-19) or exercise (11, 14) on bone during weight loss have been studied in premenopausal women, only one study investigated the effects of almost all these variables in one trial but not together in one treatment (20). With this in mind, we carried out a randomized controlled weight loss intervention trial (Chapter 2) which was primarily designed to achieve weight loss with a high ratio of fat to lean (muscle) mass loss. Our trial emphasized healthy lifestyle modification characterized by modest dietary energy restriction (-500 kcal per day) and daily exercise (aerobic and resistance) with varied intakes of protein and dairy products (and thus also calcium and vitamin D). Given the emphasis on differential dairy intakes in our weight loss study along with daily exercise, we explore here the effects of our intervention on bone health status. We hypothesized that during energy restriction with daily exercise, higher protein and recommended (or greater) daily intakes of

calcium from dairy would provide adequate levels of bone-supporting nutrients to positively affect bone health (i.e. markers of bone turnover, PTH levels, vitamin D status and other proxy markers of osteoclast activity) in overweight or obese young women participating in a sixteen week, hypoenergetic diet- and exercise-induced weight loss program.

3.3. METHODS

3.3.1. Participants, recruitment and informed consent

Participants were recruited from McMaster University and the surrounding Hamilton area. All participants were premenopausal, overweight or obese women (body mass index [BMI] between 27 and 40 kg/m^2) between the ages of 19 and 45 years. Other general inclusion criteria included participants who were otherwise healthy (as assessed through a standard medical screening questionnaire), habitually low dairy consumers (≤1 serving per day or <600 mg calcium per day), generally sedentary, regularly menstruating, not pregnant or nursing, and not taking any vitamin or mineral supplements. Before study commencement, participants filled out a food frequency questionnaire validated for calcium and dairy foods and filled out a 7-day food record to verify their low dairy/calcium intake. This trial was registered at clincaltrials.gov as NCT00710398. Further details outlining the participant recruitment procedures are written elsewhere (Chapter 2).

3.3.2. Study protocol

The 'I.D.E.A.L. (Improving Diet Exercise And Lifestyle) for Women' study was a randomized, controlled, parallel intervention study. Participants were randomly assigned to one of three groups: Control (APLD), DairyPro (APMD), and HiDairyPro (HPHD). The groups differed in the amount and type of protein consumed as detailed below. This paper will focus on the bone-specific outcomes assessed in the I.D.E.A.L. for Women study including dietary calcium, protein and vitamin D; serum 25-hydroxyvitamin D (25[OH]D), serum PTH; serum osteoprotegerin (OPG) and serum receptor activator of nuclear kappa-B ligand (RANKL); bone mineral density (BMD) and bone mineral content (BMC) obtained by dual energy x-ray absorptiometry (DXA); and serum and urinary biomarkers of bone turnover. Results for weight loss, body composition (DXA and MRI), anthropometry, strength, fitness, blood lipids, glucose, insulin and inflammatory markers have been reported elsewhere (Chapter 2).

3.3.3. Diets and Energy Restriction

Maintenance energy requirements were calculated per participant using the Mifflin St Jeor equation (21) with a sedentary activity factor. Once this energy level was determined, it was reduced by 500 kcal and used as the new maximum energy intake level per participant throughout the study. The three study groups differed in their dietary macronutrient distributions and dairy food consumption. The Control group maintained their stable baseline dairy intake by keeping it at 0-1 serving per day and consumed 15% of their daily calories from non-dairy sources of protein. The DairyPro group was instructed to consume

3-4 servings of dairy per day and 15% of their daily calories from protein (including dairy protein). The HiDairyPro group was instructed to consume 6-7 servings of dairy per day and 30% of their daily calories from protein (including dairy protein). Further details relating to the diets have been reported elsewhere (Chapter 2).

3.3.4. Study foods and daily study drinks

Two groups (DairyPro and HiDairyPro) received all dairy products needed to control their dairy and calcium intakes. All dairy foods were generously donated by Agropur Dairy Cooperative (Longueuil, QC, Canada). Please refer to **Table 1** in Chapter 2 for further details on the nutritional breakdown of the study foods. All groups consumed two 'study drinks' per day; one immediately post-exercise and another drink at least five hours before or after exercise. Each study drink was 375 mL and was prepared daily in opaque plastic bottles. Bottles were prepared and labeled for each participant by study staff in our metabolic kitchen. All drinks provided similar energy and were chocolate flavoured so that they looked, smelled, and tasted equivalent to keep the participants blinded to their contents. Known to study staff only, the drinks were either 1% chocolate milk (DairyPro), Splenda-sweetened 1% chocolate milk (HiDairyPro), or a custom-blended, non-dairy, vitamin D and calcium free carbohydrate-based chocolate flavoured beverage (Control). Splenda-sweetened chocolate milk was provided to the HiDairyPro group in order to keep their carbohydrate intake from study foods low. This enabled them to consume their restricted level of dietary carbohydrate (40%) from other food sources (e.g. fruits, vegetables and whole grains).

3.3.5. Dietary counselling

All participants received individualized diet counselling by study dietitians and research nutritionists on a biweekly basis as detailed in Chapter 2.

3.3.6. Exercise training regimen

Participants all underwent the same exercise protocol. They exercised in our own exercise and testing centre and at the main fitness centre at McMaster University. They engaged in various modes of aerobic exercise every day; five days per week with us and two days on their own on the weekend. In addition to aerobic exercise, participants engaged in a progressive resistance training regimen two days per week (upper body, lower body split) with trained study personnel (personal trainer or kinesiologist). The majority of the aerobic exercise sessions were individually or small group (i.e., one trainer to two or three participants) supervised, and all resistance exercise sessions were individually supervised to ensure safety. Resistance exercise logs (Appendix 2) were filled out by the trainer and aerobic exercise logs (Appendix 2) were filled out by the participant and checked frequently by study personnel to ensure compliance. Further details regarding the exercise program have been reported elsewhere (Chapter 2).

3.3.7. DXA

Dual-energy x-ray absorptiometry scans (DXA; QDR-4500A; Hologic Inc., Waltham, MA., software version 12.31) were carried out at the McMaster University Medical Centre, Hamilton, Ontario. Participants underwent DXA scans at baseline and weeks

eight and sixteen to determine whole-body BMD and BMC. For each scan, participants wore a standard hospital gown or the same loose clothing of their own and were scanned at the same time of day. They were also asked to follow a similar daily routine on the three scanning-days (i.e., controlling their exercise and meal times for the day).

3.3.8. Blood and urine samples

Blood samples were obtained on two occasions (pre and post intervention) between 0630h and 1000h after an overnight fast of 10-12h into tubes containing no additives (serum). They were then processed (centrifuged and aliquoted) in our laboratory within 1h of collection and stored in -20°C freezers for later analysis. Once the study was completed, frozen serum samples were taken to the Core Laboratory at the McMaster University Medical Centre (Hamilton, ON) for analysis of 25[OH]D (RIA - DiaSorin Canada Inc., Mississauga, ON; coefficient of variation [CV] was 14%), and intact PTH (Immulite 2500 autoanalyzer, Siemens Healthcare Diagnostics, Deerfield, IL; CV was 4%). Osteocalcin (OC; RIA - Nichols Institute Diagnostics, San Juan Capistrano, CA; CV was 6%), bone-specific alkaline phosphatase (BSAP; RIA - Quidel, San Diego, CA; CV was 4%), procollagen type 1 N-terminal propeptide (P1NP; ELISA - antibodies-online Inc. Atlanta, GA; CV was 4%), C-telopeptide (CTX; ELISA - Nordic Bioscience, Herlev, Denmark; CV was 1%), N-telopeptide (NTX; ELISA - Ostex International, Inc., Seattle, WA; CV was 4%), OPG (ELISA - Biomedica-Gruppe, Wien, Austria; CV was 1%) and RANKL (ELISA - Biomedica-Gruppe, Wien, Austria; CV was 6%) were batch analyzed upon study completion. Fasting spot-urine samples were obtained at the same time as the blood samples,

and aliquots were stored at -20°C for later analysis. Upon study completion, frozen urine samples were analysed for deoxypyridinoline (uDPD; ELISA - Pyrilinks-D, Metra Biosystems, Mountain View, CA; CV was 3%) and corrected for urinary creatinine (Cayman Chemical Co., Ann Arbor, MI). All investigators and laboratory technicians were unaware of the participants' group assignment during analysis.

3.3.9. Statistics

Statistical analyses were performed using SPSS (version 18.0; SPSS®, Chicago, IL). Statistical significance was set at $P < 0.05$. Data in the Text, Tables and Figures are means±SE. Differences between groups in all baseline variables were compared by univariate analysis of variance (ANOVA). Analyses of BMD, BMC, 25[OH]D, PTH, bone turnover biomarkers (OC, BSAP, P1NP, CTX, NTX, uDPD), additional markers of bone resorption (OPG and RANKL) and dietary intake data were performed using a two-way, repeated measures ANOVA with time (pre and post) as the within subject factor and group (Control, DairyPro, HiDairyPro) as the between subject factor. Significant F ratios were further analyzed and differences were isolated with a Tukey's post-hoc test. To compare the change from baseline (post-pre) between groups, univariate ANOVA with Tukey's post-hoc test were carried out.

3.4. RESULTS

The I.D.E.A.L. for Women study was designed primarily to assess the effect of diets varying in the amount (15% or 30% of daily energy) and type (dairy or non-dairy) of protein on body

composition during energy restriction. Ninety women participated in this study and were randomly assigned to one of three groups (n=30 each): Control, DairyPro and HiDairyPro. Nine participants dropped out after week eight (half-way) for reasons unrelated to the study; six in the Control group and three in the HiDairyPro group. Primary endpoint results (Chapter 2) demonstrated that, despite similar weight loss across all groups, the HiDairyPro group achieved a favourable body composition change characterized by greater total fat loss, visceral fat loss and muscle mass gain. **Table 1** shows the baseline values for age, body weight, BMI, BMD, BMC, 25[OH]D, PTH, bone biomarkers (OC, BSAP, P1NP, CTX, NTX, uDPD), OPG and RANKL. For baseline dietary intake data, please refer to **Table 2** in Chapter 2. None of the baseline values were statistically different between any of the groups. For dietary intake data during the study, please refer to **Table 4** in Chapter 2.

Table 1: Baseline characteristics of participants in the I.D.E.A.L. for Women study (mean±SE).

Variable	Control (n=30)	DairyPro (n=30)	HiDairyPro (n=30)	P-value (1-way ANOVA)
BMI (kg/m^2)	31.5±0.6	31.8±0.6	31.4±0.6	0.88
Height (cm)	163±1	164±1	166±1	0.12
Age (yrs)	28±1	26±1	30±1	0.38
Body weight (DXA; kg)	84.0±2.1	85.3±2.1	87.1±2.1	0.55
BMD (g/cm^2)	1.17±0.02	1.13±0.02	1.17±0.02	0.17
BMC (g)	2333±54	2259±47	2427±57	0.084
PTH (pmol/L)	5.0±0.3	4.7±0.2	5.4±0.3	0.22
25[OH]D (nmol/L)	48.4±3.2	49.3±3.1	51.0±4.0	0.88
OC (ng/mL)	7.8±0.4	7.7±0.4	7.5±0.5	0.90
BSAP (ng/mL)	16.3±0.2	14.5±0.1	15.7±0.2	0.36
P1NP (µg/L)	55.2±1.8	52.9±1.7	49.2±2.0	0.11
NTX (nmol/L BCE)	11.5±0.3	10.5±0.3	10.8±0.3	0.087
CTX (nmol/L BCE)	0.74±0.01	0.75±0.01	0.77±0.01	0.26
uDPD (nM/mM Cr)	5.3±0.1	5.3±0.1	5.4±0.1	0.70
OPG (pg/mL)	94.7±1.6	94.1±1.5	97.5±1.5	0.27
RANKL (pg/mL)	5.9±0.3	6.1±0.3	6.0±0.3	0.90

3.4.1. DXA

We observed no significant changes in any groups over sixteen weeks in BMD (g/cm^2: Control -0.02±0.02; DairyPro 0.01±0.004; HiDairyPro 0.001±0.004) or BMC (g: Control -8.2±7.4; DairyPro 9.9±7.0; HiDairyPro -1.9±7.3).

3.4.2. Dietary Calcium and Vitamin D

During the study, dietary calcium intakes increased in the HiDairyPro (520±28 mg to 1840±13 mg) and DairyPro (480±26 mg to 1200±19 mg; $P<0.01$ for both) groups and decreased in the Control group (555±39 mg to 299±22 mg; $P<0.05$) over sixteen weeks. The dairy products supplied to the DairyPro and HiDairyPro groups provided 950 mg per day and 1650 mg per day of dietary calcium, respectively. Dietary vitamin D followed the same pattern with the HiDairyPro group achieving higher intake levels (69±11 IU to 529±7 IU), the DairyPro group with intermediate levels (68±12 IU to 391±10 IU) and the Control group showing a reduction (57±9 IU to 26±4 IU; $P<0.05$ for all). The dairy products supplied to the DairyPro and HiDairyPro groups provided 330 IU and 480 IU of dietary vitamin D, respectively.

3.4.3. Serum 25[OH]D (Figure 1a, b)

Serum 25[OH]D was similar between treatment groups at baseline with a mean of about 50 nmol/L. Serum 25[OH]D increased significantly in the HiDairyPro group, whereas the DairyPro group remained unchanged and the Control group showed a significant reduction over sixteen weeks (% change from baseline: HiDairyPro 20.1%, DairyPro -4.6%, Control -13.2%). For both the DairyPro and Control groups, serum 25[OH]D at week sixteen was significantly lower than the HiDairyPro group ($P<0.05$) (**Figure 1a**).

3.4.4. Serum intact PTH (Figure 1c, d)

The patterns of response for PTH were in line with the observed changes in dietary intakes of calcium and vitamin D throughout the study. Both the DairyPro and HiDairyPro groups showed significant reductions in PTH over time with a greater reduction in the HiDairyPro group, whereas the Control group showed a significant rise (% change from baseline: HiDairyPro -24.1%, DairyPro -14.8%, Control 14.5%). Serum PTH at week sixteen in the Control group was significantly greater than the other two groups (P<0.001; **Figure 1c**).

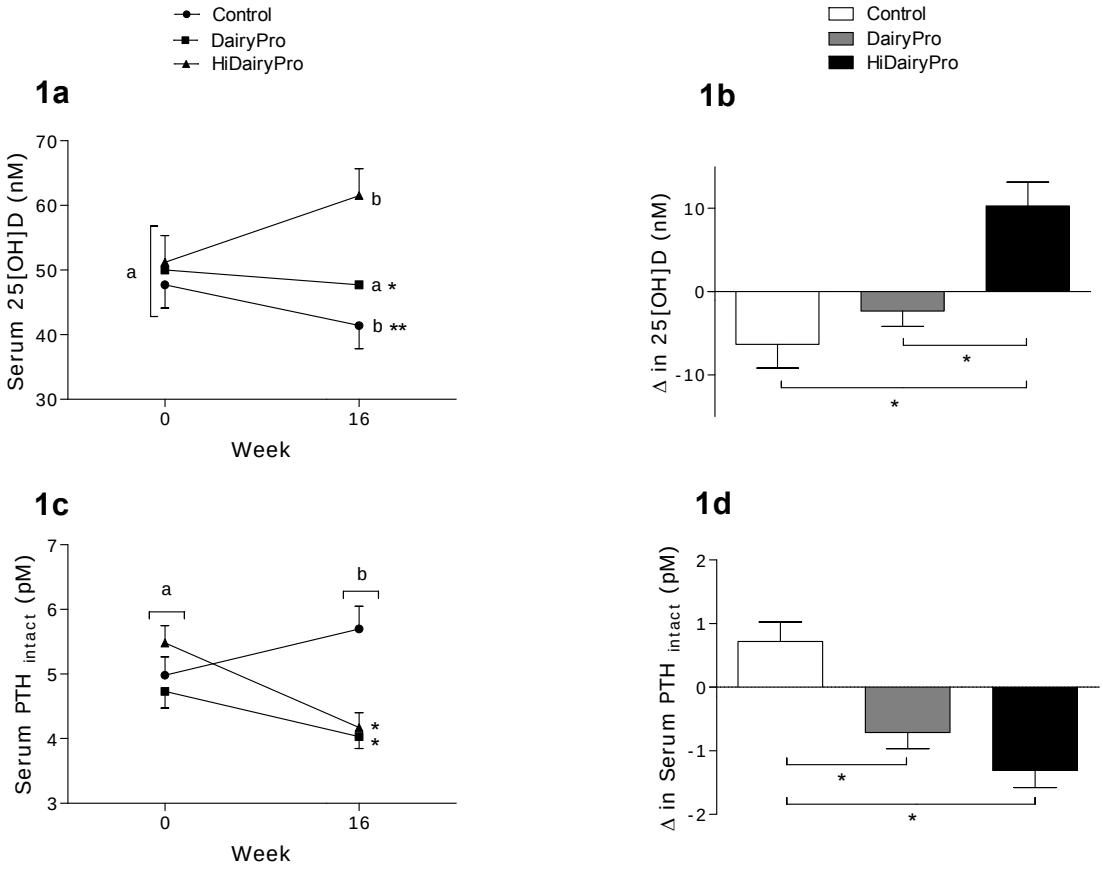

Figure 1. Serum 25[OH]D and PTH levels pre- and post-intervention. a) *P<0.05 vs. HiDairyPro at same timepoint; **P<0.005 vs. HiDairyPro at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.05. b) *P≤0.001 vs. HiDairyPro. c) *P<0.001 vs. Control at same timepoint;

different means not bearing the same letter within a group are significantly different from each other, $P<0.05$. d) *$P<0.005$ vs. Control.

3.4.5. Markers of bone formation (OC, BSAP and P1NP; Figure 2)

No formation marker was significantly different between groups at baseline (**Table 1**). OC increased significantly over time in the HiDairyPro and Control groups, and remained unchanged in the DairyPro group (% change from baseline in OC: HiDairyPro 19.5%, DairyPro 11.3%, Control 44.6%). BSAP showed no change over time within a group or between groups. P1NP increased significantly in both the HiDairyPro and DairyPro groups with no change in the Control group (% change from baseline in P1NP: HiDairyPro 33.9%, DairyPro 20.0%, Control 2.9%).

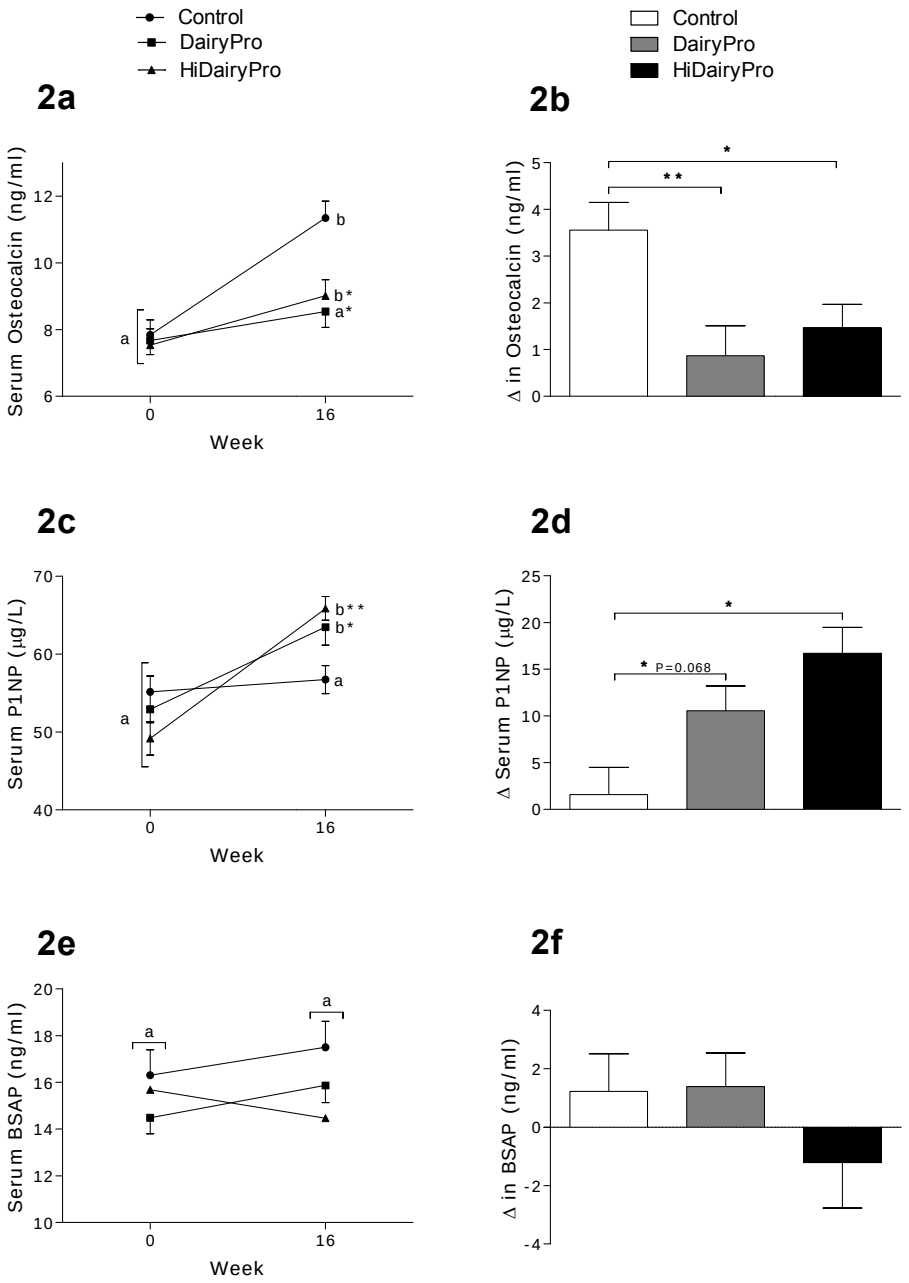

Figure 2. Serum markers of bone formation pre- and post-intervention. a) *P≤0.001 vs. Control at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.05. b) **P<0.005 vs. Control; *P<0.05 vs. Control. c) *P<0.05 vs. Control at same timepoint; **P<0.005 vs. Control at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.001. d) *P≤0.001 vs. Control. e) No statistical differences. f) No statistical differences.

3.4.6. Markers of bone resorption (NTX, CTX, uDPD, Figure 3)

No resorption marker was significantly different between groups at baseline (**Table 1**). NTX and CTX increased significantly in the Control group, and these increases were significantly greater than those observed in both the DairyPro and HiDairyPro groups. The HiDairyPro group showed no significant change in either serum resorption marker, and NTX was significantly increased in the DairyPro group (% change from baseline in NTX: HiDairyPro 8.5%, DairyPro 39.0%, Control 62.6%; % change from baseline in CTX: HiDairyPro 0.0%, DairyPro 4.0%, Control 16.2%). Urinary DPD did not change over time in the HiDairyPro group or the DairyPro group, but increased significantly in the Control group (% change from baseline in uDPD: HiDairyPro -0.2%, DairyPro 2.1%, Control 6.5%).

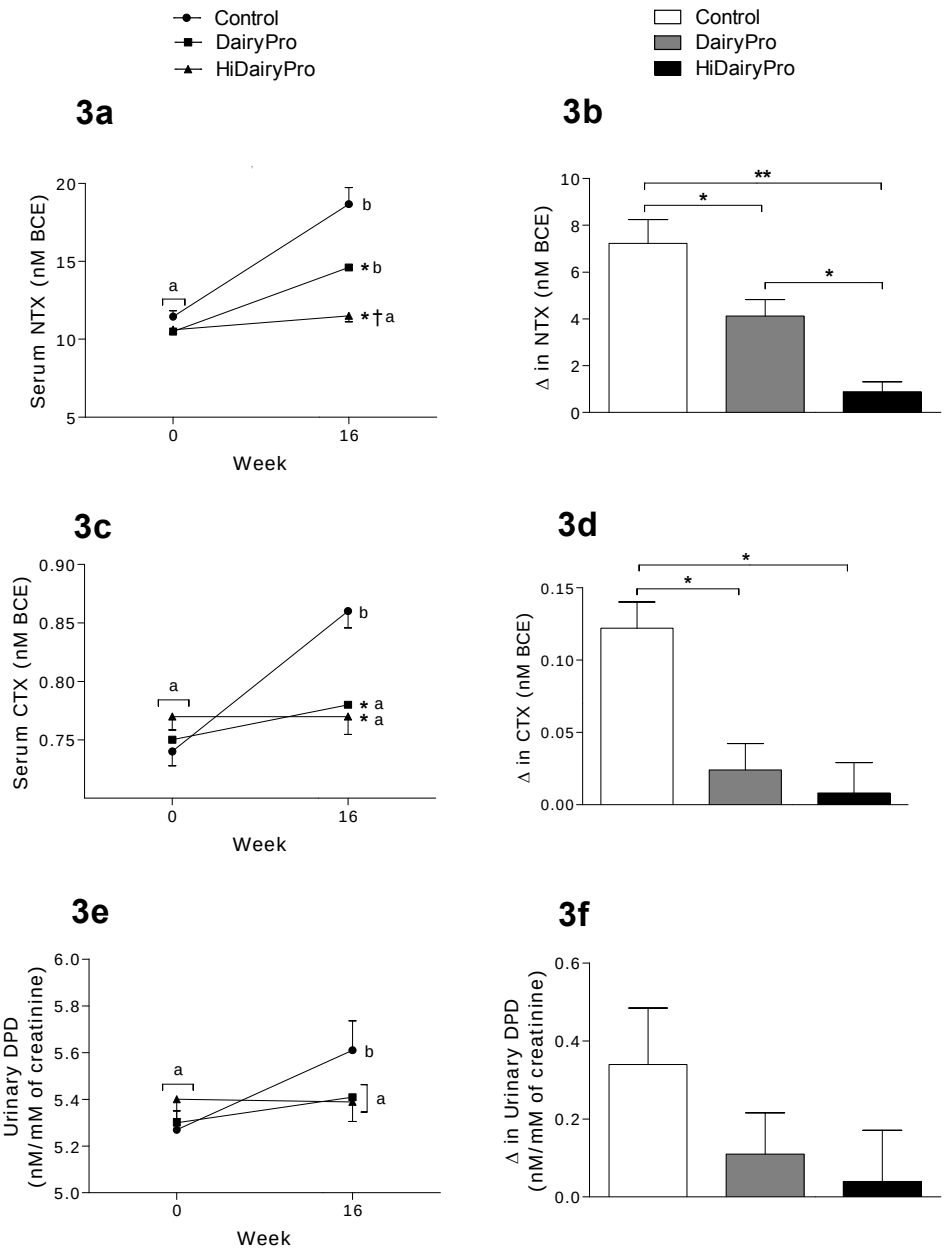

Figure 3. Serum markers of bone resorption pre- and post-intervention. a) *P<0.001 vs. Control at same timepoint; †P<0.01 vs. DairyPro at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.01. b) *P<0.05 Control vs. DairyPro and DairyPro vs. HiDairyPro; **P<0.001 Control vs. HiDairyPro. c) *P<0.005 vs. Control at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.001. d) *P<0.005 vs. Control. e) Different means not bearing the same letter within a group are significantly different from each other, P<0.05. f) No statistical differences.

3.4.7. P1NP to CTX ratio

The ratios pre- to post-intervention were (μg/nmol): HiDairyPro 64.6±2.9 to 86.2±2.9 ($P<0.001$); DairyPro 70.7±2.5 to 82.5±3.2 ($P<0.005$); Control 75.3±3.3 to 66.3±2.4 ($P<0.05$). At week sixteen, the ratios for the HiDairyPro and DairyPro groups were significantly greater than the Control group ($P<0.001$). Expressed as the net change (post-pre), the Control group (-9.0±4.1) was significantly lower than both the HiDairyPro (21.6±4.1, $P<0.001$) and DairyPro (11.8±3.8, $P<0.001$) groups. There was no significant difference between the HiDairyPro and DairyPro groups.

3.4.8. OPG and RANKL (Figure 4)

No significant differences were seen at baseline in either marker **(Table 1**). OPG increased significantly in both the DairyPro and HiDairyPro groups with no change in the Control group pre- to post-intervention (% change from baseline in OPG: HiDairyPro 12.5%, DairyPro 6.7%, Control -0.7%). The change in OPG in the HiDairyPro group was significantly greater than the Control group ($P<0.005$; **Figure 4b**). RANKL significantly decreased in the HiDairyPro group, and this decrease was significantly greater than the slight increase observed in the control group (% change from baseline in RANKL: HiDairyPro -16.0%, DairyPro -5.4%, Control 8.4%). The OPG:RANKL ratio increased significantly pre- to post-intervention only in the HiDairyPro group, and at week sixteen, the ratio in the HiDairyPro group was significantly greater than both the DairyPro and Control groups (**Figure 4e**).

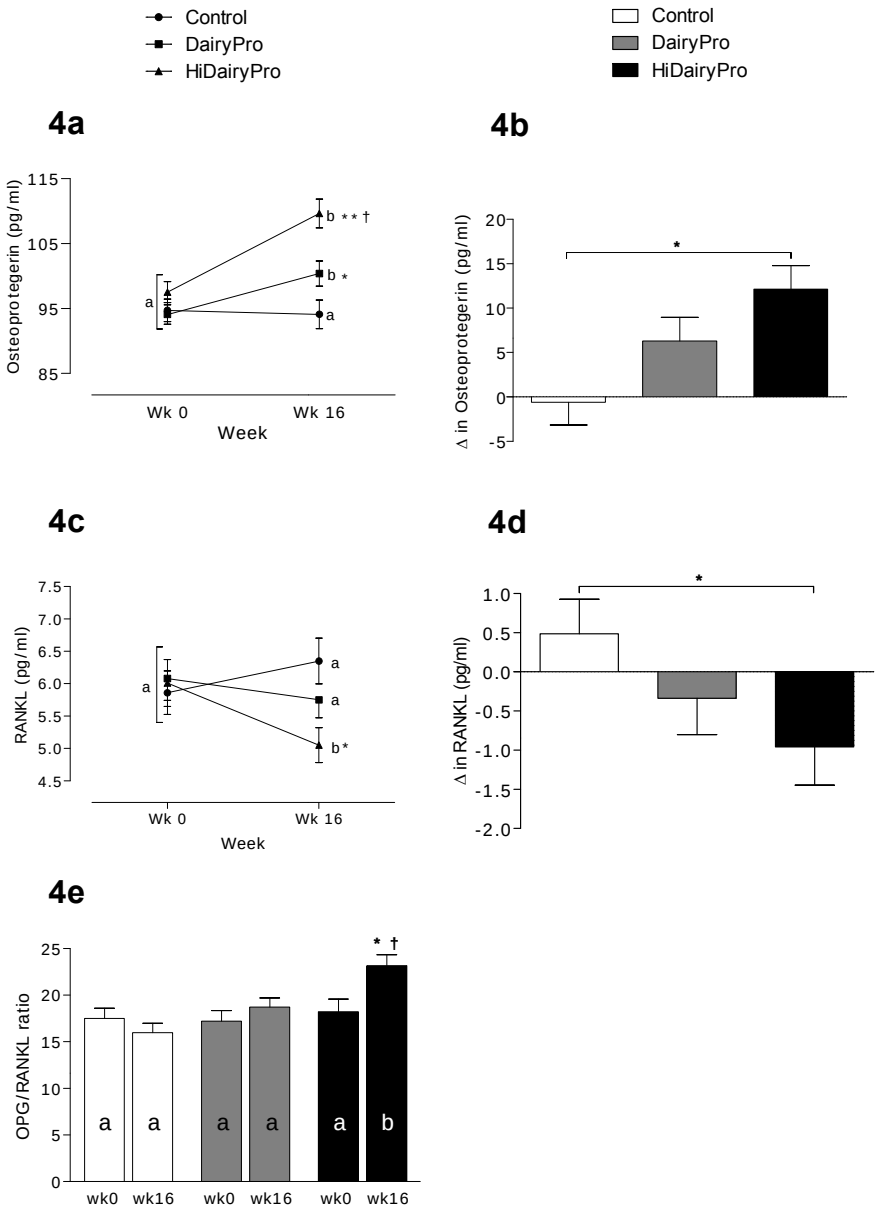

Figure 4. OPG, RANKL and OPG/RANKL ratio pre- and post-intervention. a) *P<0.05 vs. Control at same timepoint; **P<0.001 vs. Control at same timepoint; †P<0.01 vs. DairyPro at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.05. b) *P<0.005 HiDairyPro vs. Control. c) *P<0.005 vs. Control at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.05. d) *P<0.05 HiDairyPro vs. Control. e) *P<0.001 vs. Control at same timepoint; †P<0.01 vs. DairyPro at same timepoint; different means not bearing the same letter within a group are significantly different from each other, P<0.005.

3.5. DISCUSSION

This study demonstrates the importance of diet composition to the maintenance of bone health status during diet- and exercise-induced weight loss. The consumption of higher dairy and dietary protein resulted in improved markers of bone health and calcium metabolism in overweight and obese young women over sixteen weeks. Both the DairyPro and HiDairyPro groups achieved dietary calcium and vitamin D intakes that were above the 2008 dietary reference intakes (DRI; which the study was modeled on) of 1000 mg per day of calcium and 200 IU per day of vitamin D (22); however, no group achieved a dietary intake of vitamin D at the new recommended intake level of 600 IU per day (23). Despite this, serum 25[OH]D rose significantly by week sixteen in the HiDairyPro group, with a mean value in excess of the 50 nmol/L value suggested for optimal bone health in the recent DRI report (23). In contrast, the Control group experienced a small but significant decline in serum 25[OH]D over the sixteen weeks. Evidence of a benefit to bone health status is also provided by the significant reductions in serum PTH, and stability in markers of bone turnover in both dairy-consuming groups compared to the Control group. The HiDairyPro group showed further bone health benefit in that formation markers (OC and P1NP) increased significantly (**Figure 2**) with no change in any resorption marker indicating slower and more stable turnover with a modest shift towards bone formation. In addition, net bone collagen synthesis was reflected as a significant increase in the ratio of P1NP to CTX (biomarkers of type-1 collagen synthesis and degradation, respectively) for both the DairyPro and HiDairyPro groups, whereas the Control group showed a significant reduction in this ratio favouring bone resorption. OPG also increased and RANKL

decreased in the HiDairyPro group (**Figure 4**) which, through a different mechanism, may have reduced osteoclast differentiation and action, and thus bone resorption (24). In contrast, the Control group showed a significant rise in circulating PTH, and a marked increase in bone turnover as indicated by significant increases in both markers of bone formation and resorption with the latter being of greater magnitude than the former. Despite the positive changes in bone biomarkers observed in the dairy-based diet groups, we did not see significant changes in total body BMD or BMC in any group; this is likely due to the shorter duration of our study (3, 14). Thus, in accordance with our initial hypothesis, we demonstrated that diets adequate or higher in bone supporting nutrients primarily from dairy foods positively affected markers of bone turnover favouring formation, decreased circulating PTH, increased 25[OH]D, and potentially reduced osteoclast formation and function in young women who lost weight through a hypoenergetic diet- and exercise-induced weight loss program.

Energy restriction and weight loss appear to stimulate bone resorption (10, 25). However, in most cases (10, 16, 18, 20), but not all (17, 19), maintaining adequate calcium intakes and/or consuming a diet higher in dairy-source protein during weight loss minimizes bone turnover in overweight women. In our study, resistance exercise may have acted to offset the negative effect of energy restriction on bone by offering an osteogenic stimulus (11). However, since all women participated in the same exercise program and different rates of bone resorption were still evident, we surmise that even with daily exercise, the intake of higher dairy, calcium and vitamin D in the HiDairyPro group and the DairyPro

group offered greater protection against bone resorption and potential bone loss usually observed with weight loss.

Consumption of adequate dietary calcium in both the DairyPro and HiDairyPro groups may have contributed to the significant reductions observed in PTH over sixteen weeks (**Figure 1**). We have shown this previously in young women who underwent resistance training while consuming and additional 1 L of milk per day for twelve weeks (26). With respect to bone health, a higher intake of dietary calcium and vitamin D helps to maintain serum calcium, and in turn, this suppresses the secretion of PTH *via* negative feedback decreasing its resorptive effect on bone (2, 27). Furthermore, in the HiDairyPro group, higher intakes of calcium and vitamin D and lower circulating PTH may have also helped maintain stable levels of bone remodelling. In contrast, in the Control group, the low dietary calcium and vitamin D intake and greater increase in PTH, likely contributed to elevations in markers of remodelling that favoured resorption. The apparent enhanced bone remodelling, particularly in those with long-term suboptimal intakes of dietary calcium and vitamin D, could adversely affect the density and strength of bone over time and ultimately increase the risk for osteoporosis later in life (3).

Adequate provision of high quality dietary protein is of central importance to bone strength and health given its role as the primary structural component of bone (28). Positive relationships between higher protein intakes and BMD, BMC, reduced bone resorption and reduced fracture risk have been observed in different populations (2, 28-30), and a recent meta-analysis supports a modest positive association between dairy-based protein intake and bone health (31). As well, lower protein consumption has been shown to influence PTH

regulation by causing secondary hyperparathyroidism (32). Kerstetter et al. demonstrated that despite the maintenance of adequate dietary calcium and vitamin D intakes, after just four days on a low protein diet (<0.7 g/kg/d), serum PTH concentrations increased, whereas those consuming protein at ~1.0 g/kg/d, PTH did not change (32, 33). The mechanism responsible for this effect was related to impaired intestinal calcium absorption leading to secondary hyperparathyroidism (32, 33). Thus, with respect to our study, the Control group consumed protein at a lower level (0.72 g/kg/d, 55±7 g per day although still at 16% of daily energy intake) along with inadequate intakes of calcium and vitamin D, and both of these factors may provide further explanation for the significant increase in PTH demonstrated by this group. On the other hand, the HiDairyPro group showed the largest decrease in serum PTH possibly due to their greater consumption of protein averaging 1.33 g/kg/d (108±18 g per day; 28% of daily energy), as well as greater intakes of dietary calcium and vitamin D.

Bone remodelling refers to the coupled process of bone resorption and formation which generally occurs in response to changes in mechanical stress or load. A healthy, relatively low and balanced level of remodelling should not affect the overall mass of bone, and is advantageous as it helps to remove and replace the older potentially microdamaged bone with new bone while maintaining its structural integrity and strength (2, 34). However, higher rates of bone remodelling favouring resorption have been associated with more severe forms of osteoporosis and greater fracture risk in older persons (35). Bone remodelling can be assessed by measuring markers of bone turnover which reflect the metabolic activity of bone, but generally have no function in controlling skeletal metabolism (36, 37). In the Control group, the rise in bone turnover markers would favour resorption as

indicated by significant increases in NTX, CTX and uDPD (**Figure 3**), and a significant decrease in the ratio of P1NP:CTX over sixteen weeks. Of note, OC also rose significantly, but this could be for many reasons. Although OC is generally categorized as a marker of formation and correlates well with other formation markers in healthy individuals, increases in OC generally reflect greater rates of turnover or remodelling as opposed to greater rates of mineralization, especially when resorption markers are also increased (38). Several mechanisms may explain the increase in bone turnover favouring resorption seen in the Control group with the greatest effect possibly being attributed to low dietary calcium and vitamin D intakes and subsequently higher PTH levels. We recognize that a large inherent biological variability exists in the measurement of bone turnover biomarkers (34), and due to the duration of our intervention our biomarker results did not translate into DXA-measured bone mass changes. Nevertheless, we did measure multiple biomarkers, and our results do demonstrate the positive effect that consuming dairy food has on bone remodelling and bone health in young women.

To our knowledge, this is the first study to assess the effect of energy restriction and exercise in premenopausal women on the OPG-RANKL signalling system with and without the provision of dairy. Briefly, when OPG is in abundance, it preferentially binds to RANKL which prevents the binding of RANKL to RANK (its primary receptor on osteoclast precursor cells) and thus inhibits osteoclast formation (24). So, increased OPG (and decreased RANKL) and particularly an increased OPG:RANKL ratio would be protective for bone, and this was exactly what we observed in the HiDairyPro group (**Figure 4**).

In summary, we have proven our initial hypothesis by demonstrating that the consumption of diets higher in protein with an emphasis on dairy during a diet and exercise-induced energy deficit positively affected bone turnover as well as every marker of calcium and bone metabolism measured here in overweight and obese premenopausal women. Moreover, diets with protein intakes around the current RDA (i.e. DairyPro; 0.84 g/kg/d) with at least 1000 mg per day of calcium still offered a favourable benefit compared to diets with no dairy and low calcium. Our data provide good reason to recommend consumption of dairy foods to aid in high-quality weight loss (Chapter 2) and the promotion of bone health, particularly in young women who are at the age when peak bone mass is achieved (2), but also in whom adequate dairy consumption would offset relatively prevalent suboptimal nutrient intakes (39-41). Thus, in the context of weight loss, our data confirm that lifestyle programs aimed to promote fat loss, maintain or increase lean mass and substantially protect bone in young women should emphasize higher protein, particularly from dairy sources that provide adequate levels of calcium and vitamin D.

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Authors' contributions to manuscript: ARJ, SAA, MAT and SMP designed the research project. ARJ and SMP conducted the research. ARJ and SMP conducted the statistical analysis. ARJ, SAA, MAT, and SMP helped write the final manuscript and all approved the final content.

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CHAPTER 4

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AUTHORS: Andrea R. Josse, Hormoz Azizian, Shannon B. French, John K.G. Kramer and Stuart M. Phillips.

Body Fat Content Determination in Premenopausal, Overweight, and Obese Young Women Using DXA and FT-NIR

Andrea R. Josse[1], Hormoz Azizian[2], Shannon B. French[1], John K.G. Kramer[3] and Stuart M. Phillips[1]

Even though BMI is the most commonly used method for assessing and monitoring obesity, it does not take into account the individual's body fat content assuming instead that body mass is closely associated with body fat, which is a tenuous assumption. The aim of this study was to make a direct comparison between measurements of body fat content using a convenient and rapid Fourier transform near-infrared (FT-NIR) spectroscopy and dual-energy X-ray absorptiometry (DXA). We recruited 52, premenopausal women (age range 19–45), all of whom had a BMI that classified them as either overweight or obese (range: 27–40 kg/m^2, mean: 31.1 ± 3.7 kg/m^2) and indicated a statistically significant linear relationship between the fat content in kilograms measured by FT-NIR and DXA (r = 0.95, P < 0.001). Bland–Altman analysis showed that almost all the differences between two measurements fell within 2 s.d. We report here that the FT-NIR method provided comparable measurements of subcutaneous body fat content similar to those of total fat obtained using DXA. The FT-NIR method is a lower cost, easy to use and transport, and, based on comparison with DXA, an accurate method to measure body fat content. We propose that FT-NIR is an ideal method for safe repeat measurements in large trials or in screening and monitoring individuals during interventions in which changes in body fat will occur.

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INTRODUCTION

BMI is the most common variable calculated and reported as an estimate of overweight and obesity (1–4). However, it is body fat, and not body weight that is the primary physiological risk factor for morbidity and mortality (5). BMI does not take into account the individual's body composition and its guidelines assume that body mass is closely associated with body fat (6), which is not always the case. For example, individuals with a BMI of 19–24.9 kg/m^2 have been reported to have a total body fat content varying from ~23 to 42% (measured by magnetic resonance imaging (MRI)) (7). Thus, BMI is not a good indicator of fat content and there is a substantial range of body fat content in people who, according to the BMI guidelines, are considered at low risk. Interestingly, BMI is also not as strong of a predictor for cardiac mortality as either waist-to-hip ratio or waist circumference (8), both are proxy markers for visceral body fat, which is a strong predictor of disease risk. In light of this finding and the fact that body fat and not body weight is the primary risk factor for morbidity and mortality (5,8), a reliable measure of body fat is needed to rapidly assess risk.

Accurate methodologies are currently available to measure body fat content including dual-energy X-ray absorptiometry (DXA) and MRI, which show good agreement and would both correctly classify adiposity phenotype and estimate risk (9). However, neither the MRI nor DXA method would be suitable for routine fieldwork in large-scale studies where a quick and easily transportable method would be desirable. On the other hand, the bioelectrical impedance analysis method is considered to be easily transportable, although not as accurate as either MRI or DXA. Kim *et al.* (10) reported that bioelectrical impedance analysis overestimated body fat and underestimated lean body mass when compared to DXA in a 6-week herbal diet intervention program in 50 premenopausal obese (by BMI) women.

Azizian *et al.* (11,12) reported a rapid and noninvasive method to measure subcutaneous body fat using Fourier transform near-infrared (FT-NIR) spectroscopy, where the reflectance of near-infrared light from the upper part of the ear was compared to reference materials with known fat content. They also demonstrated that the results can be validated by an indirect comparison with results obtained by MRI in different individuals, or by the use of equations that incorporate body surface area, NIR response, as well as MRI data to convert subcutaneous to total body fat (11). In the present communication, we

[1]Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada; [2]NIR Technologies, Oakville, Ontario, Canada; [3]Agriculture and Agri-Food Canada, Guelph, Ontario, Canada. Correspondence: Stuart M. Phillips (phillis@mcmaster.ca) or Hormoz Azizian (hazizian@nirtechnologies.com)

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report a direct comparison between FT-NIR and DXA measurements in the same subjects; FT-NIR obtained a measure of subcutaneous body fat, which was based on the comparison to a reference material. Subjects underwent a 16-week diet- and exercise-induced weight loss program and we compared body fat content and percent body fat obtained by these two methods at various time points throughout the study.

METHODS AND PROCEDURES

Subjects

All subjects were recruited from McMaster University (Hamilton, Ontario, Canada) and the surrounding Hamilton city area through posted advertisements or word of mouth. Otherwise, healthy, premenopausal, nonpregnant women between the ages of 19–45 years, with a BMI between 27 and 40 kg/m^2 were selected. These overweight and obese women were all part of a larger clinical trial that was designed to assess the effect of a particular hypoenergetic diet- and exercise-induced weight loss program. The majority of subjects who took part in this FT-NIR vs. DXA substudy were scanned more than once and up to three times throughout their participation in the larger clinical trial. The full study protocol, which included (optional) FT-NIR scanning, was approved by the McMaster University Medical Research Ethics Board and conformed to all standards of Canada's Interagency Panel on Research Ethics for conducting human research (http://www.pre.ethics.gc.ca/english/index.cfm). All subjects gave their written consent to participate in the study having read and understood all of the risks and procedures.

DXA

Subjects underwent whole-body DXA scans (QDR-4500A; Hologic, Waltham, MA) three times throughout the study (week 0, 8, and 16) at the McMaster University Medical Centre to determine body composition with a specific focus on fat mass and %body fat. All scans were performed by the same investigator (A.R.J.). Study participants were scanned at the same time of day wearing either a standard hospital gown or light clothing (the same for each scan) and were asked to refrain from vigorous exercise that day. As per protocol, the DXA machine underwent quality control testing daily to ensure no significant deviations existed in the day-to-day variability. On some occasions, corrections had to be made to DXA measurements for subjects with higher body weights because the scanning field was slightly smaller than the subject's body. In these cases, adjustments were made whereby the side of the upper body (fingers/arm) that was in full view was used as a surrogate for the other side that was cutoff (**Figure 1**). Similar adjustments have been made previously in other studies where subjects' body dimensions exceed the length or width of the scanning bed (13).

FT-NIR methods and procedures

A matrix-F FT-NIR spectrometer equipped with a standard fiber optic probe (Bruker Optics, Milton, Ontario, Canada) in combination with signal processing software (OPUS; Bruker Optics) was used to obtain the spectra. The fiber optic probe carrying the He-Ne laser (Class IIIA, 4 mW) focused a near-infrared light that was held up to the back of the subjects' upper ear (to avoid eye contact); see Azizian and Kramer (14) for details on the technique of scanning. Several measurements per subject were taken from various parts of the upper ear (cartilage area) and sometimes from the ear lobe. The whole measure took <3 minutes. Absorption spectra were averaged and the average spectrum was used in conjunction with the calibration curve which was based on a synthetic reference material that resembled human tissue in water, fat, and protein content (**Figure 2**), to determine the subcutaneous fat content of each subject at various times in the study, generally at the beginning (0 weeks), between weeks 7 and 9, and again between weeks 15 and 16. We did not consider it necessary in this study to validate our results using the reference material with the use of the equations since we had already demonstrated the accuracy of data obtained using the reference material in a previous publication (11). In fact, we converted the subcutaneous fat content obtained using FT-NIR to total body fat using the previously determined MRI ratio of subcutaneous to total body fat (11), but the calculation only showed a small increase (data not shown), which was within the experimental error of FT-NIR and DXA. Thus, only the subcutaneous FT-NIR results were used in this study.

Some minor adjustments to this procedure were made in a few cases. The FT-NIR method is based on the reflectance of the light from the cartilage (14), therefore, in the first instance a much lower than expected reading for a subject was obtained due to some scarring on the ear cartilage from a prior ear piercing. This cartilage damage caused light scattering, therefore a new measurement was collected from a different area of the ear, avoiding the scarred part of the cartilage. In a separate instance, again due to reflectance of the light, the measurements within a person differed when the upper part of the ear was scanned closer to the outer border of

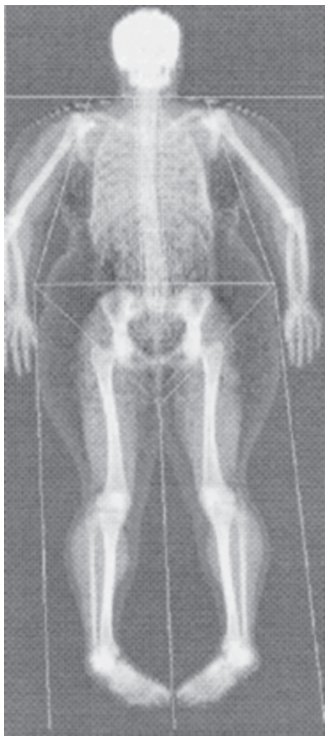

Figure 1 Dual-energy X-ray absorptiometry (DXA) scan of one subject who did not completely fit on the DXA scan bed.

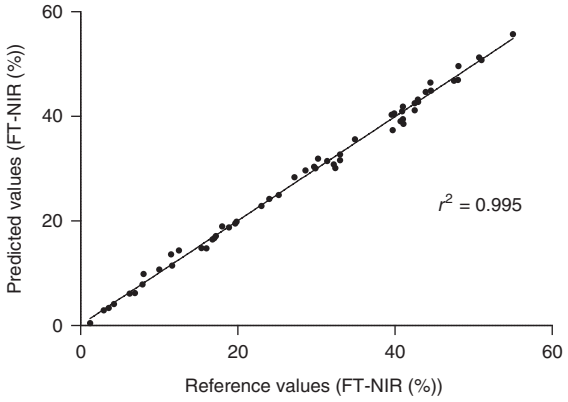

Figure 2 Fourier transform near-infrared (FT-NIR) spectroscopy calibration curve for fat content determination.

the ear vs. the middle. This was due to variations in the thickness of the skin layer near the edge of the ear. The maximum light penetration depth is about 2 mm and in this case, the edge of the ear was thicker. This would be akin to scanning without cartilage where reflectance is significantly reduced. Figure 3 shows the first-derivative spectrum of this subject's scan near the edge of the ear (solid line) and the back middle part of the ear (dotted line), which predicted a subcutaneous body fat content of 33.5 and 44.6%, respectively. These two measurements represented a substantial difference in the fat content of this subject; the comparable measurement by DXA was 43.0%. The lack of proper reflectance on the edge of the ear was confirmed by repeating the scans for several other subjects with similar results.

Statistics

Descriptive statistics of age and body composition measurements are presented as the mean ± s.d. (Table 1). We used Pearson's correlation coefficient (r) to examine the correlation between fat content (kg) determined by DXA and FT-NIR and the correlation between subjects' body weights and %body fat from both DXA and FT-NIR. We also used the Student's t-test to compare the fat content values (kg) obtained by DXA and FT-NIR. The Bland–Altman analysis (15) was used to evaluate the validity of comparing the body composition results between the two methods (FT-NIR and DXA). Data were analyzed using SIGMASTAT statistical software (version 3.10, 2004; Systat Software, San Jose, CA). P values of <0.05 were considered statistically significant.

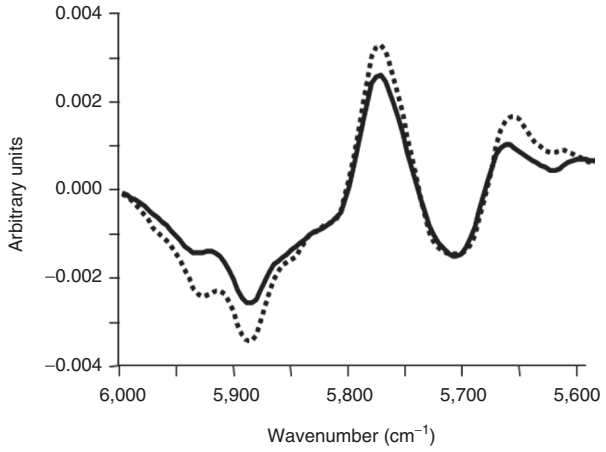

Figure 3 First-derivative spectrum of one subject measured on the back of the ear in the middle (dotted line) and closer to edge (solid line). The more accurate reading (the one that more closely matched that obtained by dual-energy X-ray absorptiometry (DXA)) was taken from the back middle part of the ear.

Table 1 Subject characteristics and DXA and FT-NIR-based fat mass variables of 91 separate measurements of individuals

	Multiple measurements per subject
Variable	**Mean ± s.d.**
Number of subjects	n = 52
Number of FT-NIR and DXA measurements	n = 91
Age (years)	30.0 ± 7
Weight (kg)	83.6 ± 14
Height (m)	1.6 ± 0.1
BMI (kg/m^2)	31.1 ± 4
Fat—DXA (%)	37.9 ± 5
Fat—DXA (kg)	32.1 ± 9
Fat—FT-NIR (%)	37.8 ± 5
Fat—FT-NIR (kg)	32.0 ± 8
Difference ((FT-NIR) – DXA) (kg)	0.13 ± 2.6
Mean ((FT-NIR) + DXA)/2 (kg)	32.6 ± 9

DXA, dual-energy X-ray absorptiometry; FT-NIR, Fourier transform near-infrared spectroscopy.

RESULTS

Fifty-two subjects were assessed in this study. Fat content was measured between one and three times in each subject throughout the study using both the FT-NIR and DXA methods. The subject characteristics are shown in Table 1. Figure 4 shows the plot of weight (kg) for each individual in relation to their fat content (%) showing both DXA (open circles) and FT-NIR (closed circles) results. The Pearson correlation coefficients obtained on comparison of the subjects' body weight (kg) with fat content (%) by both DXA and FT-NIR were 0.65 (P < 0.01) and 0.51 (P < 0.01), respectively. It is evident from this graph that individuals with the same body weight show a wide range of body fat content.

Placing the subjects into three groups (Figure 5) based on currently used BMI categories (group 1 BMI 19–24.9 kg/m^2; group 2 BMI 25–29.9 kg/m^2; group 3 BMI 30–39.9 kg/m^2) showed the extent of variation within each group. This phenomenon was observed for both DXA (open circles) and FT-NIR (closed circles). The three measurements in group 1 (BMI: 19–24.9 kg/m^2) were taken from subjects following the completion of the program. There was a wide variation in the fat content of individuals within each BMI group, with some individuals in the lower BMI range having similar body fat contents to those in higher BMI groups. This was particularly evident with the degree of overlap in body fat content in groups 2 and 3 (Figure 5).

Figure 6 shows a statistically significant relationship between DXA (kg) and FT-NIR (kg) fat content measurements for all subject scans, r = 0.95 (P < 0.001). There was no statistical dif-

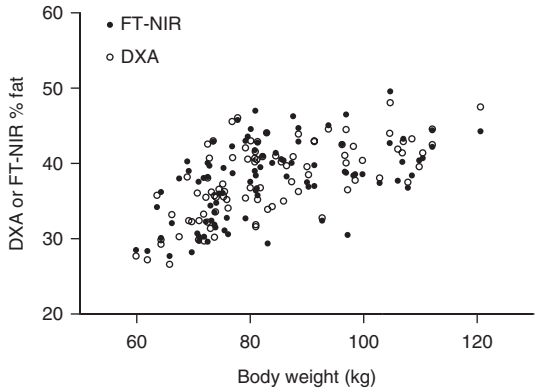

Figure 4 Relationship between body weight (kg) and fat content (%) in the same individuals determined by both Fourier transform near-infrared (FT-NIR) spectroscopy (closed circles) and dual-energy X-ray absorptiometry (DXA) (open circles). R values for body weight vs. DXA and FT-NIR were 0.65 and 0.51 (P < 0.01), respectively.

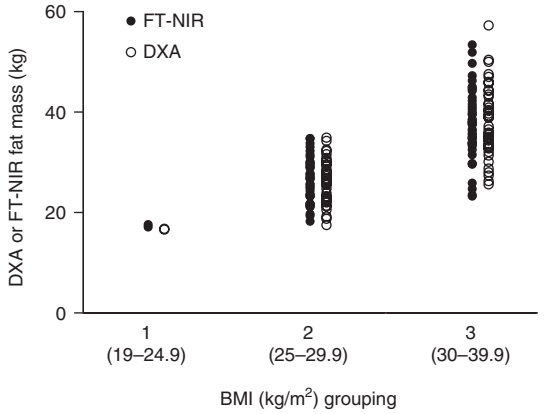

Figure 5 Relationship between weight and fat content based on BMI subgroups. Body fat (kg) determined by Fourier transform near-infrared (FT-NIR) spectroscopy (closed circles) and dual-energy X-ray absorptiometry (DXA) (open circles) for each individual grouped according to BMI: group 1, BMI 19–24.9 kg/m^2 (n = 2); group 2, BMI 25–29.9 kg/m^2 (n = 41); group 3, BMI 30–39.9 kg/m^2 (n = 48). The numbers of individuals (91) in each group are given in brackets. The FT-NIR and DXA results within each BMI group are presented on separate lines for clarity.

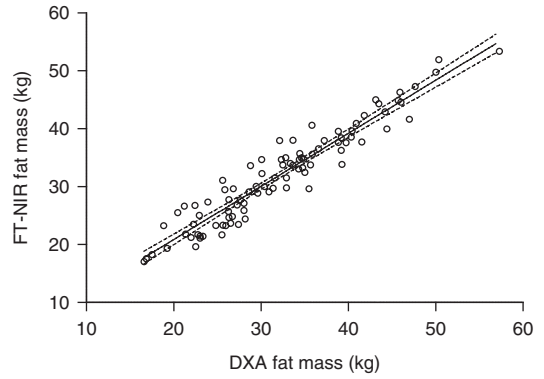

Figure 6 Correlation between individual subjects' fat content (kg) measured by dual-energy X-ray absorptiometry (DXA) and Fourier transform near-infrared (FT-NIR) spectroscopy. R value is 0.95, P < 0.001. This graph shows the line of best-fit by least squares linear regression and the 95% confidence interval.

ference between the fat content (kg) values obtained by DXA vs. FT-NIR as assessed using a paired t-test (P = 0.65).

We also performed a Bland–Altman analysis (15) of the differences between the FT-NIR and DXA (kg) results on each of the paired measurements (**Figure 7a,b**). Most of the measured differences were between ± 2 s.d. from the overall mean difference for the group (**Figure 7a**). **Figure 7b** shows the individual body fat measures from DXA and FT-NIR ± 1 s.d. The measurement bias was low (0.13, **Table 1**) indicating that the two methods produced similar results. Moreover, the plot shows no evidence of systematic bias at higher or lower body weights, which indicates no biased variability as the measured fat mass increased or decreased. The s.d. of the bias was 2.6 kg and the 95% confidence interval range was from −5.0 to 5.2 kg. This degree of variability is in line with the between-measurement technical error for % fat estimates (3–4%) by DXA reported by Boyer *et al.* (9).

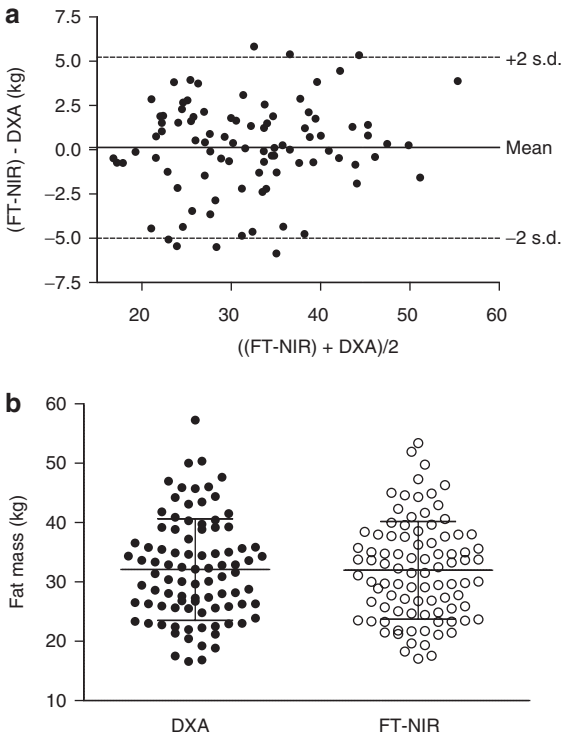

Figure 7 Agreement between the two methods for measuring fat mass. (**a**) Bland–Altman analysis of dual-energy X-ray absorptiometry (DXA) and Fourier transform near-infrared (FT-NIR) spectroscopy fat mass values (kg) showing the mean and 2 s.d. (**b**) Individual body fat content (kg) determined using FT-NIR and DXA showing the mean ± 1 s.d.

DISCUSSION

The primary purpose of the present study was to compare the FT-NIR method for body fat determination to the more established DXA method. DXA has been demonstrated as a reliable method to monitor fat mass reduction (9,16). However, DXA does have some disadvantages, which relate to a low-level radiation exposure, cost, and nonportability (13). These drawbacks make DXA somewhat impractical for large-scale trials with repeated measurements of body fat. Both DXA and FT-NIR fat content results showed a statistically significant linear relationship across a wide range of BMIs. In addition, subcutaneous and visceral fat decrease to a similar extent with diet and exercise (7). Hence, while FT-NIR only gives estimates of subcutaneous fat, its measurement alone should provide meaningful information for monitoring obesity in general and in particular during weight loss.

Even though BMI is the most commonly calculated variable in monitoring and categorizing overweight and obesity it does not take into account fat content or distribution, and clearly weight alone does not differentiate between body fat mass and lean mass. Other methods such as DXA (9,16), MRI (7,9), bioelectrical impedance analysis (10), and FT-NIR (11,12) do differentiate between fat and muscle mass and therefore should be implemented more readily in studies and clinics that assess the relationship between body composition and disease risk reduction. The wide variation in body fat content observed among the participants in this study within each of the BMI categories (**Figure 5**) is consistent with similar MRI findings

by Thomas *et al.* (7). Clearly, our data and that of others provide sufficient evidence to indicate that there is actually very little relationship between BMI and fat content making the continued use of BMI for categorizing risk as suspect.

There is substantial evidence indicating that a reasonable correlation exists between total fat, and subcutaneous and visceral fat in individuals. Thomas *et al.* (7) reported that of their total body fat, subject's subcutaneous fat content was ~85% and visceral fat content was ~15%. Azizian *et al.* (11) showed similar results based on MRI data and also found that the ratio of the two fat compartments was age-dependent with the ratio of subcutaneous to visceral fat decreasing as age increased particularly in women. In this study, we did calculate total body fat from our subcutaneous measures (data not shown) but the difference was very small so we report here our actual subcutaneous data only. We feel that this is appropriate considering the strong relationship that exists between total and subcutaneous fat contents (7).

Boyer *et al.* (9), when comparing several methods for measuring body composition, reported that the highest Pearson correlation coefficient obtained was between MRI and DXA. The variation in the differences between MRI and DXA was small and the correlation between the average and the difference was virtually zero. However, both of these methods have drawbacks that relate to nontransportability, cost, and limited accessibility. In the case of DXA, there is an additional concern of exposure to X-rays, and the physical limitations of the instrument size when conducting a whole-body scan of larger individuals (a limitation of MRI also). Although a single exposure may be around 0.3–2 μSv of radiation (17–20) (i.e., less than the amount acquired during a day from natural background radiation sources, which is ~2–8 μSv (19,20)), repeat measurements to monitor changes in body composition during weight loss may not be recommended and the use of DXA for repeat measurements in, for example, pediatric populations may also be unwarranted.

The burdgeoning prevalence of obesity and associated comorbidities such as type 2 diabeties and coronary artery disease needs to be addressed. To monitor, in large numbers, the increased prevalence and/or ability of interventions to affect change in fat mass, a rapid, safe and low cost method that accurately determines body composition is required. A common and effective recommendation for those with chronic disease is to lose body weight, but this recommendation is without recognition of the importance of lean mass. Thus, the recommendation should be to lose body fat. At this time, however, unless less expensive and more accessible technologies are available health-care practitioners and/or researchers are not routinely able to distinguish between weight loss as fat vs. weight loss as lean mass or body water. This poses a problem since disease risk reduction is strongly related to fat loss and inversely related to muscle loss, and not necessarily weight loss *per se* (21–23). In fact, the weight lost would actually be greater with greater loss of lean mass. Here, we report on a rapid, convenient, low risk methodology that uses a validated technique (11–12,14) of FT-NIR to measure body fat and we have directly compared this method with DXA methodology with good agreement. Results of the Bland–Altman analysis show very little and, importantly, no systematic bias indicating a good measurement capacity as compared to DXA. Further refinement of the FT-NIR method in this population, taking into account the newly identified measurement discrepancies outlined in this article, would likely improve the predictive capacity of this method.

We conducted our comparison in a population of overweight and obese young women who would be considered at risk for chronic disease and who also underwent a hypoenergetic diet- and exercise-induced weight loss program. FT-NIR was previously demonstrated to provide fat content measurements comparable to MRI, although these correlations were established using matched pairs of individuals (11) instead of the same subjects. It is evident from this and previous studies that the three methods, MRI, DXA, and FT-NIR, unlike BMI, provide an accurate measure of the fat content of individuals upon which meaningful health decisions can be made. The FT-NIR method is quick, easy to use, reliable, transportable and low-risk, and has been demonstrated to provide measures of fat mass that correlate strongly with those obtained using proven and reliable methods. Thus, FT-NIR technology may be able to provide the health-care community with an accurate means to repeatedly and safely monitor patients' body composition in clinics and those on weight/fat loss programs.

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DISCLOSURE

The authors declared no conflict of interest.

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CHAPTER 5

GENERAL DISCUSSION

This Chapter summarizes the key findings from the I.D.E.A.L. for Women study, highlights the implications of those findings and details additional information relating to the dietary intervention and general participant compliance. This Chapter also addresses some study limitations and missed opportunities, and suggests a future direction for this particular research.

5.1. General body composition findings in the I.D.E.A.L. for Women study

The clinical trial detailed in this thesis, was designed to answer the question of how best to achieve weight loss of the highest possible quality. We defined high quality weight loss as the loss of body weight with the highest ratio of fat to lean mass loss and asserted that this pattern of loss is important not only for short-term efficacy but also for long term metabolic health, disease risk reduction, and possibly the prevention of weight regain following a program. We took a multifaceted approach to our study design and incorporated all aspects that we thought were important in helping to promote weight and fat mass loss, preserve muscle mass, and improve bone health in young women. Hence, our weight loss regimen included daily aerobic exercise, twice weekly resistance exercise and a 500 kcal per day dietary energy restriction, but also sought additional potential benefit by manipulating the

dietary intake of some macronutrients, vitamins and minerals. Two of our groups, DairyPro and HiDairyPro, consumed 3-4 and 6-7 servings of dairy foods per day, respectively (foods included 1% white and chocolate milk, fat free yogurt and regular fat cheddar cheese). This brought their calcium intake up to and above the current adequate intake (AI) of 1000 mg per day and their vitamin D intake above 200 IU per day (1). The control group remained at their low baseline dairy intake throughout the study. In addition, the HiDairyPro group consumed 30% of their daily energy from protein whereas the other two groups (DairyPro and Control) consumed protein at 15% of daily energy. Thus, the main manipulations in the I.D.E.A.L. for Women study were exercise and nutrition; however, the between group differences were nutritional in nature with the different treatment groups consuming different amounts and types of protein and dairy products.

The rationale for our design was based on a multitude of reasons relating to the potential additive and/or synergistic benefits of high quality diet-induced weight loss and the benefits of exercise. Clinical studies have been undertaken assessing the effect of energy restriction, dairy and/or dietary calcium on weight loss and fat loss (2-14), yet only one so far (7) has combined dairy food intake with exercise during weight loss. This study showed no additional effect of dairy or calcium on body composition, but it also had a few key methodological differences that may have resulted in these researchers missing a potential positive effect of dairy (7). Exercise, both resistance and aerobic, has a host of other beneficial effects beyond aiding in weight loss that relate not only to the preservation and gain of muscle mass (15), but also to improved metabolic risk factors, vitality, mood and general quality of life (16, 17). Therefore, there are many evidence-

based reasons for employing daily exercise in our study design with the overarching goal of promoting healthy weight loss and sustainable lifestyle modification practices. Our choice to carry out this study in young, premenopausal, overweight and obese women was strategic. We thought it was important to demonstrate the patterns and habits of daily exercise and dairy consumption in a population who not only has shown relatively consistent suboptimal dairy nutrient intakes (18-20) but who generally, for various reasons, self-select to avoid both dairy (e.g. dairy foods perceived as fattening) and to some extent, exercise (e.g. time, too much of a burden, embarrassment in the gym, do not want to 'bulk up') (21-24).

It is important to note that, in accordance with the recent literature (25), our study followed four criteria suggested as being required to demonstrate efficacy in trials like ours, including that subjects should be overweight or obese; subjects' calcium intake should be habitually low (<600 mg per day); the study should include an appropriate control group with low calcium/dairy intake; and the study should adopt and maintain a moderate caloric restriction during the intervention. With regards to the last point, a caloric restriction close to 500 kcal per day was achieved in the I.D.E.A.L. for Women study, and this was not significantly different between groups (**Table 4**, Chapter 2). Trials that did not have a study design incorporating these elements have failed to show a positive effect of dairy or calcium on body composition during weight loss (2-4, 7, 26, 27).

Although weight loss was an important endpoint in the study, the primary endpoint in our trial was body composition assessed using three-compartment DXA scans for

information on fat mass, lean mass and bone. In Chapter 2, the fat and lean mass results were highlighted, and in Chapter 3 the effect of our intervention on bone health was presented. With respect to muscle and fat mass changes, on the background of daily exercise, those consuming higher dairy, calcium and dietary protein showed a significant reduction in both total fat and visceral fat mass and a gain in lean mass. All groups lost the same amount of body weight ([mean±SE] ~4.3±0.7 kg), however, the composition of this weight loss was significantly different. The HiDairyPro group lost 4.9±0.3 kg of fat and gained 0.7±0.3 kg of muscle whereas the Control group lost 3.6±0.3 kg of fat and lost 0.7±0.3 kg of muscle. Clearly, the HiDairyPro group lost a greater amount of fat than the control but their weight loss remained equal because of the HiDairyPro groups' concomitant gain in lean mass. This begs the question of whether other studies that have assessed diets with varying macronutrient ratios and purportedly found similar body weight loss across all groups would have also demonstrated a difference in fat:lean mass ratio had it been measured (28-31). With respect to trunk and visceral fat loss assessed by DXA and MRI, respectively, the HiDairyPro group lost the greatest amount of fat from the abdominal region. Thus, the HiDairyPro group achieved what would ostensibly be an optimal body compositional change, and this would not be noticed with only the measurement of body weight. This point is further illustrated in **Figure 1** where fat and lean mass loss/gain is expressed as a percent of total weight change. The Control group lost the greatest amount of lean mass corresponding to about 15% of their total weight lost. This amount of lean tissue loss is consistent with other studies employing exercise during energy restriction (15, 32), and is 10% less than what is generally reported as the amount of weight lost as muscle if exercise

was not part of the weight loss intervention (25% of body weight lost) (32-34). Thus, we can speculate that exercise may have attenuated the lean mass loss in the Control group. On the other hand, 100% of the weight lost in the HiDairyPro group was fat, and in keeping with our study goals, we managed to achieve weight loss in this group of the highest possible quality. In addition, the greater increase in lean mass translated into greater strength gains in this group, and so we have also demonstrated here that the diet and exercise manipulations undergone by the HiDairyPro group also lead to a functional outcome that may have relevance for persons with issues of mobility. For example, populations including the elderly would stand to benefit since the preservation of muscle strength with age improves daily function and maintains autonomy (35).

While the HiDairyPro group consistently showed the greatest body compositional changes and strength benefits, the DairyPro group's changes were often somewhere between those of the other two groups. As seen in **Figure 1**, lean mass loss was only 4% (~0.2±0.2 kg) of the total weight lost in this group, and it was not significant from baseline. Given these findings, we can speculate that the lean mass retention and intermediate visceral fat loss observed in the DairyPro group versus the Control group relates to the dairy consumption since this was the only difference between these groups. It is true, however, that the DairyPro group was consuming slightly greater protein than the Control group at 18% vs. 16% of total energy, respectively, but this corresponded to a difference of only ~11 g protein per day. Although this is a small amount, it may have confounded a possible dairy effect. The difference between the DairyPro and HiDairyPro groups relates to the increased intakes of both dairy (and calcium) and dietary protein. It

is not possible to tease out the separate effects of these two nutrients with our current study design since we did not employ a fourth treatment group with high protein and lower/no dairy.

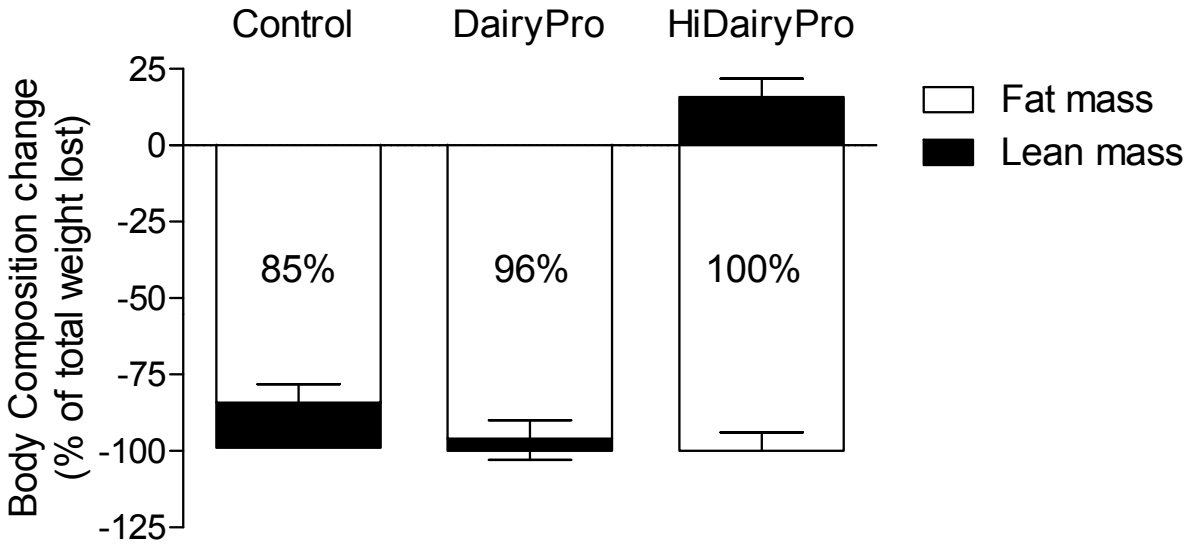

Figure 1. Change (mean±SE) in fat and lean mass in all three treatment groups expressed as % of total weight lost below the x-axis.

The pattern of weight loss achieved by the HiDairyPro group, specifically the maintenance/gain of lean mass, is not only important for short-term efficacy, but is also crucial for long term, successful weight maintenance and the prevention of weight regain following a program. **Figure 2** shows a theoretical representation of the issues that a person might face with repeated diet attempts resulting in continuous loss of muscle and increased body fat and body weight over time. Weight loss by energy restriction alone involves both fat and lean mass loss, and once weight loss efforts cease, body weight (to some degree) is usually put back on and even gained. Very rarely does this occur in the same tissue

proportion since the regain of lean mass would require the performance of resistive exercise. So it is easy to accrue body fat but nowhere near as easy to gain back the lean mass that was lost with only increased energy intake. Hence, the longer term trajectory of weight gain with accompanying weight cycling, which is a common practice in our society, without appropriate countermeasures to maintain lean mass, results in its gradual loss ultimately leading to a decreased resting metabolic rate and a concomitant increase in body weight and body fat (36). We propose this important mechanism to be, in part, a root cause of the increasing prevalence and incidence of obesity (37, 38) and its related comorbidities and complications. In fact, such a sequence of events would, particularly if accompanied with overt inactivity, likely predispose someone to develop what has been termed sarcopenic obesity (39).

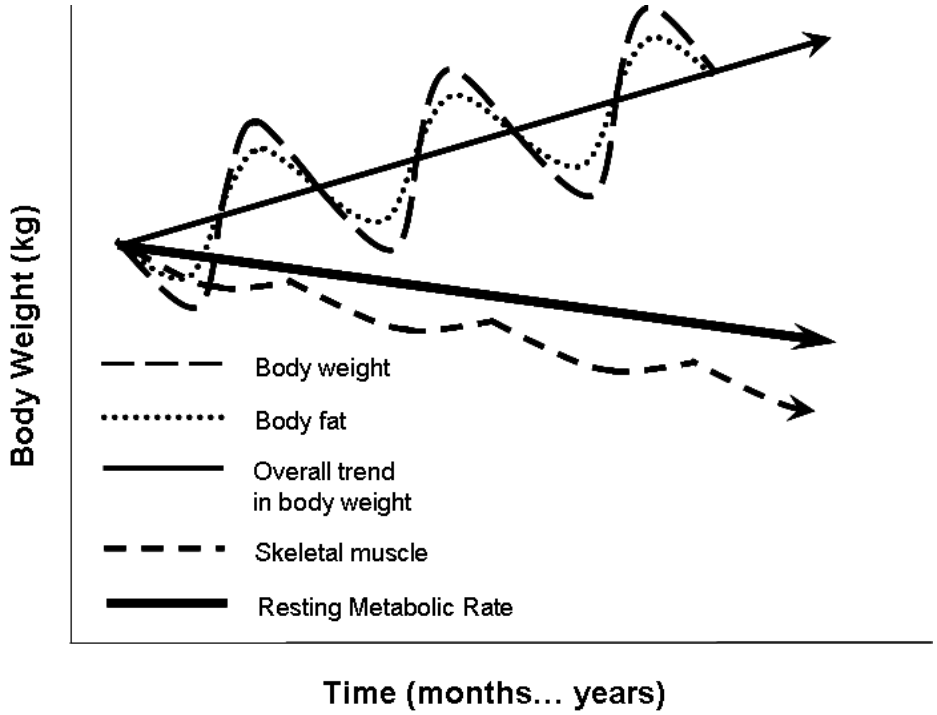

Figure 2. The long term importance of maintaining lean mass during weight loss.

5.2. The dietary intervention in the I.D.E.A.L. for Women study

The dietary intervention provided the key difference between the groups, and we succeeded, for the most part, in achieving our two main dietary goals which were to maintain a large difference in protein intakes between the HiDairyPro group (28% of daily energy) and the other two groups (18% and 16%); and to ensure consumption of all added dairy products (especially in the HiDairyPro group) in an appropriate manner (i.e. proper spacing of foods/meals (40), little to no energy overcompensation to avoid excess energy intake, protein for breakfast (41), etc.) along with minimal or no dairy consumption (0-1 serving per day) in the Control group.

Self-reported total energy intakes were slightly higher in the groups consuming dairy foods with the greatest difference in intake between the Control (1320 kcal per day) and HiDairyPro (1500 kcal per day; **Table 4**, Chapter 2) groups. The slight increase in intake in the HiDairyPro group may have to do with incomplete energy compensation. This group certainly had to make the greatest dietary change characterized by a reduction in energy intake of 500 kcal per day and the addition of 720 kcals per day of dairy foods. This left them with only 600-800 kcal per day to consume all other foods. We wonder whether our weight loss and body composition results would have been different had the HiDairyPro group consumed 150 less kcals per day. **Table 1** shows a sample meal plan for an individual with an energy requirement of ~1600 kcal per day in the HiDairyPro group. **Table 1** also shows how we advised our subjects to consume the dairy foods.

Table 1. Sample meal plan for someone in the HiDairyPro group with an energy intake level of ~1600 kcal per day.

Meal and Food	Amount	Kcals	CHO (g)	PRO (g)	FAT (g)
Breakfast					
Study drink	1 (375 ml)	150	19.5	10.5	3.75
Study Yogurt	2	70	10	8	0
Study Cheese	1 piece	90	0	5	7
Morning Snack					
Almonds (20-22)	1 oz or 28 g	162	6	6	14
Water/coffee/tea	16 oz	0	0	0	0
1% milk and sweetener	20 ml, 1 pkg	10	1	0.75	0.2
Lunch					
Salad (lettuce, tomato, onion, cucumber, carrot [any other non-starchy vegetable])	200g	60	10	1.5	1
Tuna (packed in water)	1 can (85 g)	70	0	16	0.5
Study Cheese	1 piece	90	0	5	7
Study 1% white milk	1 cup	100	12	9	2.5
balsamic vinaigrette	2.5 tbsp	104	0	0	10.4
Post workout					
Study Drink 2	1 (375 ml)	150	19.5	10.5	3.75
Dinner					
steamed brown rice (measured cooked)	3/4 cup	162	32	4	1.3
Stir-fry: chicken breast (white meat grilled)	4 oz	133	1.3	27	2.67
raw snow peas	½ cup	29	3.5	1	0
raw shredded carrots	½ cup	22	5	0.5	0
red peppers	½ cup	23	4.5	0.7	0
raw broccoli	½ cup	10	1.9	1	0
soy sauce	1 tbsp	10	0	2	0
teriyaki sauce	2 tbsp	24	5.5	0.5	0
Water with crystal light or diet pop	16 oz	0	0	0	0
Orange	1 medium	63	15.5	1.3	0
Evening Snack					
Study yogurt	2	70	10	8	0
Total (Absolute):		**1602**	**157g**	**118g**	**54g**
Total (Percent):			**~40%**	**~30%**	**~30%**

It was very important that participants spaced the foods out to provide a steady amount of high quality protein throughout the day. It was counterproductive, for a few reasons, to consume large quantities of dairy foods at once. First, our body can only utilize a certain amount of protein for protein synthesis at one time (~20 g); amino acids consumed beyond this level of use for synthesis are either oxidized or deaminated forming urea (42). Hence, if all the dairy protein was eaten together, this may have reduced the potential stimulatory effect of the amino acids on protein synthesis. Second, if the required dairy foods were not being consumed until the end of the day, this may have meant that other foods, which could add to the total caloric intake, or no foods were being consumed. The study dietitians and nutritionists worked to ensure that eating patterns of overconsumption and/or prolonged fasting (i.e., skipping breakfast or 'banking' foods to eat them all at once) were avoided. One important strategy used was to ensure that dairy foods were eaten at breakfast in both the DairyPro and HiDairyPro groups. Despite our efforts, as mentioned above, a slight caloric overcompensation occurred in the HiDairyPro group (**Table 4**, Chapter 2).

Looking at the self-reported 7-day dietary macronutrient intakes, the DairyPro group proved to be the greatest challenge with respect to keeping them within the parameters of the study. The protein intakes in the DairyPro group reached 18% which was higher than our goal of 15%. The reason for this, we propose, stems from the fact that once all the study foods were accounted for in their daily intakes, those in the DairyPro group had only 7.5% more protein per day to consume for a total intake of 15%. 7.5% protein only represents ~20-30 g protein per day that they could choose depending on the

subject's daily restricted energy requirement, so it is clearly difficult to stay within these limits when the amount of protein they could consume beyond study foods was so little. Hence many of the subjects in the DairyPro group exceeded the *a priori* protein intake. However, the increased protein intake in this group was still less than the decreased dietary fat intake, which was 24% of total energy, versus 28% and 31% in the Control and HiDairyPro groups (**Table 4**, Chapter 2). This was again for the same reason stated above, with only 7.5% of protein left to consume in a day, it was challenging to fulfill fat requirements since most foods that contain healthy fats also contain protein. We did, however, advise the use of olive oil, avocado and other healthy high fat foods lower in protein, and subjects in the DairyPro group did incorporate these fats into their diet, but fat intake was still lower than we had originally aimed for.

Another dietary challenge from the onset of the study was how to handle the random placement of subjects who stated they were sensitive to lactose and thus were labelled as 'dairy avoiders' into either of the two dairy groups. Studies have shown that young women avoid dairy products because of poor taste, a perception that dairy foods are fattening and the prevalence of adverse gastrointestinal reactions upon consumption (23, 24). Regardless of the reason why our subjects avoided dairy before the study, no subject reported any psychological issues with dairy, and we had surprisingly few gastrointestinal complaints relating to the abrupt increases in dairy consumption. Two participants took lactase enzyme supplements at the start of the study but did not require the supplement by the second month. On the other hand, since all subjects came into the study being low dairy consumers, there were no problems with maintaining these low

baseline intakes in the Control group. In fact, the control group further decreased their dairy intake because they were instructed to cut out higher fat, energy dense foods from their diet (as was the case in the other two groups), and these foods included items such as cream (in coffee), ice cream, and cheese pizza.

Every two weeks, subjects met with a study dietitian or research nutritionist for a 30-45 minute individual counselling session. In keeping with the general ethos of the study; to improve diet, exercise and lifestyle, it was important to incorporate nutrition education modules into each individual session. We wanted our subjects to leave the study with an understanding of nutrition and health and to have a basis for why we were suggesting or insisting the removal and incorporation of certain foods into their diets or the adoption of certain healthful eating patterns. **Table 2** shows a list of the topics discussed in the biweekly counselling sessions. The first session was about protein (the chief dietary manipulation in the study) and food labels. By the end of the first month, subjects had a good idea of how we wanted them to eat (as per protocol) for the remainder of the study. The actual handouts given to the subjects are in Appendix 1.

Table 2. Topics discussed in the biweekly nutrition counselling sessions.

Week	Topic of Session
0	Protein - amount and type (as per assigned group) Food Labels - what are they and why are they important
2	Carbohydrates and Fibre
4	Fats (saturated and unsaturated) and Cholesterol
6	"Get the most out of what you eat" - Eating foods that are high volume, high in nutrients and high in water content more often like vegetables and avoiding energy dense nutrient poor foods.
8	"Curb your cravings" - If you crave chocolate or ice cream, what should you eat instead?
10	"Guide to eating at restaurants" - An *eat-this-not-that* guide for local Hamilton restaurants, coffee shops and popular fast-food chains.
12	"Banishing bad habits" - Tips on how to kick bad eating habits and adopt healthier ones
14	"Two weeks to go!" - Motivation to the finish line; goal setting and a recap of things to focus on over the next 2 weeks.
16	"Maintenance for life" - More tips on how to maintain the healthier lifestyle they have worked so hard to achieve over the past 4 months in the study.

5.3. Bone health in the I.D.E.A.L. for Women study

Chapter 3 was dedicated to the bone health outcomes in the I.D.E.A.L. for Women study. We demonstrated the greatest positive effect on bone in those consuming diets higher in protein and dairy foods. Although we did not observe a change in total BMD or BMC over the course of the four month intervention, we measured six serum biomarkers of bone turnover, serum 25(OH)D, PTH and OPG/RANKL, and all showed marked changes in favour of an improvement in bone health in the HiDairyPro group. Our study is the first to

report all of these markers together especially in premenopausal overweight and obese women undergoing energy restriction. Moreover, our study is especially unique in that these markers of bone health were assessed while all subjects performed daily exercise. Only a handful of studies have assessed bone health during weight loss with exercise in young women (43-45), and none with varying levels of dairy foods or calcium.

Another novel aspect of the study with respect to bone was that we are the first to measure OPG/RANKL in response to differential dairy intakes during diet- and exercise-induced energy restriction. Although this well characterized system of osteoblast-osteoclast signalling and osteoclast differentiation is an important determinant of bone mass and skeletal integrity (46), and is well studied in relation to osteoporosis etiology and treatment (47), little information exists on this receptor-ligand system outside of the context of bone disease or in premenopausal women. Thus, we must interpret our results with some care. Nonetheless, our new OPG/RANKL findings are generally congruent with the other bone results we report in this thesis.

Dietary intakes of pertinent bone supporting nutrients; protein, calcium and vitamin D, were measured and monitored throughout the trial. Because of the dairy foods provided to the participants in the DairyPro and HiDairyPro groups, graded intakes of all three nutrients were achieved. With respect to vitamin D, the HiDairyPro group increased their dietary intake over sixteen weeks which resulted in a small but significant increase in serum 25(OH)D of 10.3 nM to 62 nM (**Figure 1**, Chapter 3). Although the quantity of dietary vitamin D consumed by this group is below the revised recommendation of 600 IU per day (48), the HiDairyPro group still managed to improve their circulating 25(OH)D level

to above that which is currently deemed as adequate according to the IOM (48). On the other hand, while the Control group started off with a 25(OH)D level around 50 nM, this decreased to 41 nM by week sixteen possibly owing to their decreased intake of vitamin D during the study. Interestingly, the DairyPro group increased their vitamin D intake from 49 IU per day to 391 IU per day, but this did not result in a serum change. This finding is inconsistent with a previous study done in our laboratory in young women where consuming 1 L per day of milk (providing 360 IU per day; similar to the intakes of the DairyPro group) increased serum 25(OH)D levels by 6.5 nM to 64.8 nM after twelve weeks (49). The inherent differences between these two studies may have accounted for the disparate results seen. The other study was carried out under anabolic conditions (resistance training only); diets (except for milk consumption) were not otherwise controlled, and dietary vitamin D (beyond what was in the milk) was not reported and thus could have been higher (49). Moreover, in the other study, unlike the I.D.E.A.L. for Women study, the control group was consuming adequate calcium levels and actually showed a small increase in 25(OH)D despite not having consumed any supplemental dietary vitamin D in their post-exercise drink (49).

5.4. Fourier-transform near infrared spectroscopy (FT-NIR) in the I.D.E.A.L. for Women study

Measuring fat mass and lean mass in weight loss trials are critical. It is clear from the results of the I.D.E.A.L. for Women study that body weight and BMI alone provide no information on weight loss quality, and these measures can miss clinically important features relating to

body composition including a true measure of chronic disease risk. FT-NIR spectroscopy is not a new technology, yet its application to human body fat determination is still relatively new. The goal of our study was to validate FT-NIR spectroscopic measures of body fat (% and kg) with DXA-measured fat mass and % body fat in the same overweight and obese young women (Chapter 4). As reported in the paper, the two measures correlated very well with each other and the agreement measurement bias was not skewed and very small (0.13 kg) (50). We are the first to report the measure of DXA and FT-NIR in the same participants, and although the console and the accompanying technology are not quite ready for mass-production, the results reported here are very strong and very promising especially for use in research and in medical clinics. Further validation is required in other populations including athletes, lean men and women, obese men, the elderly, and other more specialized populations for which DXA and/or other imaging techniques may be contraindicated including pregnant women and possibly people with spinal cord injury. If we are advocating the importance of measuring body composition during weight loss we must be able to provide a means of doing so that is accurate, portable, accessible, relatively inexpensive, and valid. Furthermore, a new method that is superior to what is currently available and comparable (i.e. bioelectric impedance analysis [BIA]) would be advantageous. Although BIA has not been directly compared to FT-NIR, BIA has been deemed less reliable at measuring % fat compared to DXA under controlled conditions with a Bland-Altman measurement bias of up to 6% (51, 52), whereas FT-NIR, according to the I.D.E.A.L. for Women study, is quite comparable.

5.5. Compliance in the I.D.E.A.L. for Women study

Compliance was monitored and measured for both the exercise and dietary components. All subjects complied very well with the daily aerobic exercise and the supervised resistance training carried out twice per week. To monitor exercise compliance, subjects recorded their aerobic exercise in daily exercise logs (Appendix 2), and on occasion, they wore a BodyMedia (SenseWear Pro 3) armband device to ensure appropriate workout energy expenditure (~250 kcal per day). These armbands have been recently validated against doubly labeled water under free living conditions (53). Weekday aerobic exercise compliance (excluding illness) was around 88%, and resistance exercise compliance was 98%. If a subject missed their scheduled resistance exercise training session, it was rescheduled for another time during the week. Weekend exercise was also recorded in a logbook and, on a random basis, a BodyMedia device was given to subjects to take home and wear during weekend workouts. Dietary compliance was more difficult to maintain as is often the case in weight loss trials (28, 54). Nonetheless, biweekly 3-day food records were handed in 95% of the time, and compliance with consumption of the study drinks in all groups and study foods in both the DairyPro and HiDairyPro groups was 97%. We monitored intake of study drinks and foods diligently (as noted on the food record templates for each group in Appendix 3), and addressed any dietary questions or concerns on a daily basis. Despite our conscientious and reliable measures of study compliance, participants had a global mean weight loss of only 4.3±0.7 kg (range: Control +1.7 kg to -11.5 kg; DairyPro +2.0 kg to -10.6 kg; HiDairyPro +0.2 kg to -11.6 kg),when they should have theoretically lost ~11 kg of body weight. Only eight subjects actually achieved a

body weight loss of ≥10 kg during the study, which is what all subjects should have theoretically lost assuming 1 kg of fat is ~7700 kcal (or 1 lb of fat is ~3500 kcal).

5.6. Study implications

The I.D.E.A.L. for Women study is unique in that it is the only research study to combine energy restriction with high protein, higher dairy and daily exercise. Other studies have assessed varying aspects of the I.D.E.A.L. for Women study separately (5, 7, 10, 43, 55, 56), but none together in one program. Moreover, ours is the only study to look at the effects of the program on all three major body compartments – lean mass (muscle), fat mass and bone. We have proven here that a lifestyle change involving weight loss, exercise, higher dairy (and calcium) and increased dietary protein favourably affected all three major structural body tissues in premenopausal women. In addition, these women experienced other favourable effects including a decrease in visceral fat accumulation, waist circumference, and inflammation, and an increase in muscular strength and cardiovascular fitness.

Although our fat mass findings were not novel, they were in agreement with results from other similar weight loss trials (5, 8, 13). The strength of our study, however, was in the many different measures employed to quantify fat mass or that relate to fat mass, including DXA, MRI, FT-NIR, waist circumference and inflammatory markers. All of these outcomes complemented each other well and the greatest favourable changes were demonstrated by the HiDairyPro group. In addition, our accurate determination of visceral fat by MRI was novel in this population, and the significant correlation between

DXA-derived trunk fat and MRI-derived visceral fat provides some useful information on the use of DXA for the determination of visceral fat changes in this population. Many research groups do not have access to MRI facilities and there are many contraindications to its use, thus employing DXA to measure trunk fat loss during weight loss would appear to yield appropriate results in a clinical setting in overweight women, according to our findings.

The increase in lean mass observed in the HiDairyPro group was a unique and unexpected finding. It is unique because no other weight loss study in young women has demonstrated this effect, and unexpected because it is thought that the catabolic state of energy restriction is not conducive to muscle anabolism (57, 58). Our research group has demonstrated on numerous occasions that the optimal condition for building muscle combines resistance exercise and amino acid/protein feeding (42, 49, 59-61), but these studies were always done under energy balance or surfeit energy provision conditions since additional calories, or in particular, additional protein/amino acid 'building blocks' are thought to be requisite for anabolism. In the I.D.E.A.L. for Women study, we did provide increased protein but in the context of energy restriction. Under these conditions, other studies of hypoenergetic diets with a higher quantity and quality of protein appear to preserve lean mass but not result in its increase (6, 62, 63), which is what we hypothesized we would observe in the HiDairyPro group. To further examine this novel finding and to demonstrate that it was not based on a few 'responsive' subjects, **Figure 3** shows the individual lean mass changes for all subjects who completed the study. It is clear that more subjects in the HiDairyPro (~70%) group gained lean mass whereas more

subjects (~70%) in the Control group lost lean mass. The DairyPro group was intermediate. Since over 2/3 of the HiDairyPro group experienced a gain in lean tissue, we are confident that this outcome was not due to chance or because of a few extreme subject responses. Therefore, we report, for the first time, that consuming higher dietary protein, with an emphasis on dairy, increased lean mass in young women during a diet- and exercise-induced energy deficit. The potential implications of this novel finding are significant for populations including the elderly in whom, during weight loss, the preservation (let alone gain) of muscle mass and muscle function are of critical importance for both metabolism and activities of daily living (64, 65). In addition, we hope that our findings will encourage the measurement of lean mass changes more frequently in research and in the clinic, and promote the design future lifestyle programs that incorporate the specific countermeasures to lean mass losses used here including low fat dairy consumption, resistance exercise and higher protein intakes, for the treatment of obesity.

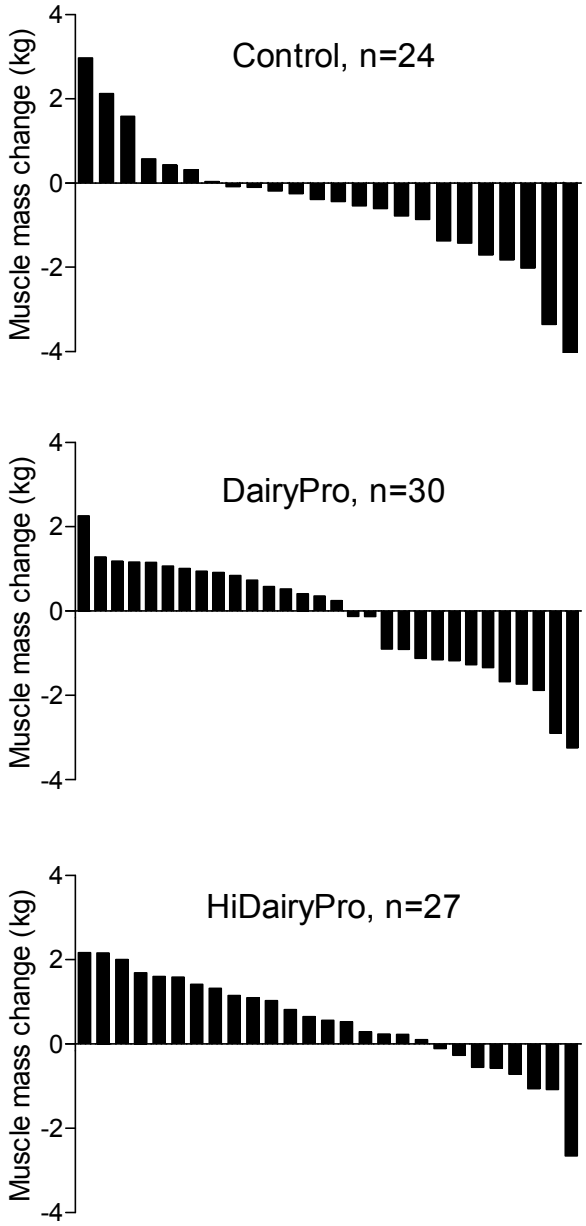

Figure 3. Lean mass changes in every subject who completed the study separated by group.

Interesting anecdotal observations were also made during the course of this trial and are discussed here since they have important implications for future study design.

Upon completion of the study, participants filled out an anonymous exit survey (Appendix 4) which asked them to comment on what specific and general aspects of the study they liked best, what they thought needed improvement and their overall experience. We received excellent feedback about the study itself, about the support provided, about the valuable knowledge gained regarding nutrition and exercise and about the sense of 'empowerment' and 'confidence' to continue along the healthful path that we had established for the participants during the study. Thus, trial results aside, the I.D.E.A.L. for Women study succeeded in also achieving one of our fundamental and overarching goals which was to create and foster change by assisting, supporting and motivating young women to improve their diet and exercise habits and to adopt healthier lifestyles for them and their families. The psychological aspects of this lifestyle change are an obvious area for further research, as understanding how people made the changes we asked of them will help with promoting the adoption of other similar healthy dietary and physical activity changes that any person may subsequently be asked to make.

5.7. Study limitations

Our trial was limited in some areas. First, our study design did not employ a fourth treatment group of higher protein without dairy. Because of this, we are unable to ascertain the individual effects of dairy and/or protein on our endpoints. Although adding the fourth group would have been interesting, other studies have shown positive and independent effects of both dairy (5, 6, 8, 14) and increased protein (30, 54, 66, 67) on weight loss and body composition. Thus, we decided to focus on other novel initiatives,

namely, the use of concurrent graded dairy and protein intakes. The second methodological limitation related to the inability to draw conclusions from our study about the specific effects of certain micronutrients. For example, although calcium intakes were different among the groups, we cannot attribute any causative result to calcium itself. Several trials and reviews have highlighted the beneficial effects of calcium on body composition (11, 25, 68-72) and bone (70, 73-75), but our trial employed the use of dairy foods (which contain calcium), and was not designed to test the individual effect calcium. Nevertheless, other data show that dairy foods have more beneficial effects on body composition than calcium alone (8, 9), probably owing to the other components contained in dairy foods (i.e. protein) which offer additional bioactivity (76, 77). Dairy foods also contain other vitamins and minerals including vitamin D, vitamin A, magnesium, potassium, phosphorus, and B-vitamins (78, 79), so increased intakes of these micronutrients may have affected outcomes between the treatment groups in the study. Vitamin D and A were consumed in different quantities between groups (**Table 4**, Chapter 2) even though all subjects were counselled to consume diets rich in nutrient dense foods (e.g. vegetables and fruits). Given that we studied the effect of dairy foods on body composition and bone health, the inability to establish the effects of individual nutrients was an inevitable outcome and one that is shared by every diet study assessing the effects of healthful whole foods and food patterns.

A missed opportunity, rather than a limitation, was that our study did not employ any formal subject follow-up. Although we did have personal anecdotal follow-up from some subjects, we did not employ, for example, a four to six month post study DXA scan. This

would have helped address the longer term utility and effectiveness of the program. We only conducted a four month intervention and it could be argued that the real measure of success is whether people could follow the changes we introduced in the longer term, and whether treatment differences can be sustained or improved upon in that time. While a follow-up DXA scan after study completion would have been interesting and could have provided beneficial information regarding the effectiveness of the advice given in the longer term, the study was not initially designed to have a proper post study follow-up as an additional endpoint, i.e. participants were not formerly informed that monitoring would continue after the study nor asked if they would come back for a follow-up DXA. Our study was designed to test the efficacy of a particular treatment and to provide a proof-of-principle that our treatment works, and it was for this reason we carried it out under tightly controlled conditions with daily coordinator-participant interaction and the provision of all necessary study foods to control dairy intakes. Having provided proof that our regimen is efficacious, it is appropriate to design a new study to test its longer term effectiveness with a similar design to other long-term weight loss studies (28, 29, 54). This idea is explored in further detailed below.

5.8. Future directions

There is great potential to build upon what we have demonstrated in the I.D.E.A.L. for Women study. As mentioned above, we have succeeded in proving that our diet and exercise protocol was efficacious over four months under controlled conditions, but we acknowledge that our design may be rigorous. The paradigm of testing a weight loss diet in the short term

with a well controlled trial and then subsequently in the long term with a more participant-driven program is not new. Popular diets and other less advertized programs of higher protein/low carbohydrate diets have been put head to head in both the short and then long term (28, 30, 31, 54, 67, 80, 81), as well as diets for cholesterol reduction (82, 83) and diets for diabetes risk reduction (84, 85) to test both their efficacy and then their effectiveness. Thus, as a future direction, a long-term trial in the same population should be undertaken. Such a trial should ideally be ≥ one year, and should feature a one to two month intensive period at the beginning of the trial where study staff would see participants three times per week to get them started on the diet and excise program. Then, for the remaining ten months, subjects would come in only once every month for an individual consultation. We would provide them with all information given to the I.D.E.A.L. for Women study participants throughout the whole study during the initial one to two month intensive phase. Aside from the study visits, communication with participants would be *via* phone, text message, twitter, or email and they will be able to call, text, or email the study team whenever they choose. As an option, monthly group sessions (by treatment group) could be held so participants can discuss their experiences, issues and successes, and gain support from others undergoing the program. Subjects would be provided with gym memberships for the whole study and would, at least initially, work individually with personal trainers twice per week to ensure they keep up with the resistance training aspect. At baseline, six and twelve months, all measures (anthropometric, body composition, blood, urine) would be made. If women can successfully sustain the HiDairyPro arm, perhaps with slightly lower dairy intakes, of a modified I.D.E.A.L. for Women study program for one year and achieve high quality weight

loss and the maintenance of bone health status in that time, than we have proven our paradigm to be effective under 'real world' conditions in this population.

It would also be interesting to assess a modified version of the I.D.E.A.L. for Women study program in different populations such as the elderly. In terms of the elderly, weight loss can still be an important outcome (39, 86), but even if weight loss was not the focus, older people would still benefit from improved bone health and better body composition, especially a maintenance or gain of lean mass. Moreover, the promotion of better nutrition (increased protein and dairy) and exercise habits in this age group are also of great importance. The risks of developing sarcopenia (39, 65) and osteoporosis (87, 88) increase with age, and come with a host of deleterious metabolic and functional consequences. Thus, strategies aimed at maintaining or increasing muscle mass, bone health and strength with age, like that of the I.D.E.A.L. for Women study, would be of benefit.

5.9. Conclusion

The papers contained in this thesis describe the outcomes of the four-month I.D.E.A.L. for Women randomized controlled parallel intervention trial. The papers highlight a significant benefit of consuming hypoenergetic diets higher in dairy foods (and calcium) and dietary protein with daily exercise on fat mass, lean mass and bone health in otherwise healthy overweight and obese young women. We have proven our initial primary hypothesis by achieving a more favourable body composition change in those consuming dairy and higher dietary protein characterized by weight loss of the highest quality versus those undergoing the identical exercise program with adequate protein and

no dairy. We have also demonstrated that the consumption of even three servings of dairy foods per day in women with previously inadequate dairy and suboptimal calcium intakes preserve bone health and maintain lean mass with weight loss. As well, even greater dairy intakes were shown to have more robust body composition changes and bone health benefits and, more positive effects on calcium metabolism.

Given the apparent relentless trend for greater rates of overweight and obesity (37, 86, 89), it would seem not only prudent but necessary to uncover the best strategies to combat obesity so that its future burden can be minimized. With this in mind, conducting high quality efficacy-based clinical studies, like the I.D.E.A.L. for Women study, provides a pivotal step forward in obesity treatment since these positive results, and the paradigms from which they ascend, will hopefully be used in the establishment and conduct of larger scale programs or clinical trials to effectively manage obesity in the long-term by lifestyle change.

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CHAPTER 6

APPENDICES

6.1. APPENDIX 1 – Bi-Weekly Nutrition Sessions

Week 0 - Understanding Food Labels

Food labels provide nutrition facts and information about the foods that you eat. The 6 components of the food label are described below. As a general guideline, choose foods that are low in fat, sodium and sugar.

http://www.fda.gov/food/labelingnutrition/consumerinformation/ucm078889.htm

1) Serving sizes are provided in familiar units, such as cups or pieces, followed by the metric amount, e.g. grams. Pay attention to the serving size, including how many servings there are in the food package, and compare it to how much you actually eat.

2) Calories provide a measure of how much energy you get from a serving of a particular food. Eating more calories than you burn causes you to store the extra energy as fat. The food label also tells you how many of the calories in one serving come from fat.

3) Eating too much fat, saturated fat, *trans* fat, cholesterol, or sodium may increase your risk of certain chronic diseases, like heart disease, some cancers, or high blood pressure. Try to limit these nutrients (% Daily Value should not exceed 100%).

4) Individuals often don't get enough dietary fibre, vitamin A, vitamin C, calcium, and iron in their diets. Eating enough of these nutrients can improve your health and help reduce the risk of certain diseases. Make sure that you get enough of these nutrients (% Daily Value should be at least 100%).

5) The % Daily Value column tells you how much fat, sodium, dietary fibre, etc. is contained in one serving of the food as a proportion of total recommended daily intake.

6) Note the * used after the heading "%Daily Value" on the Nutrition Facts panel. It refers to the Footnote in the lower part of the nutrition label, which tells you that "%DVs are based on recommendations for a 2,000 calorie diet". This statement must be on all food labels. Your %DV may be slightly higher or lower depending on your recommended calorie intake. The 2,000 calorie diet values are meant to serve as a reference point.

Taking the time to read food labels can help you make healthier diet choices!

Adapted from: http://www.fda.gov/food/labelingnutrition/consumerinformation/ucm078889.htm

http://ww2.dietitians.ca/public/content/resource_centre/feature_groups_topics.asp

Week 0 - How much protein do these foods Contain?

Beef - ~7 grams of protein per ounce
Hamburger patty, 4 oz – 28 grams protein
Steak, 6 oz – 42 grams

Chicken- ~7 grams of protein per ounce
Chicken breast, 3.5 oz - 30 grams protein
Chicken thigh – 10 grams (for average size); Drumstick – 11 grams; Wing – 6 grams
Egg, large - 6 grams protein

Fish- ~ 6 grams of protein per ounce
Most fish fillets or steaks are about 22 grams of protein for 3 ½ oz (100 grams) of cooked fish, or
Tuna, 3 oz can – 25 grams of protein

Pork
Pork chop, average - 22 grams protein
Pork loin or tenderloin, 4 oz – 29 grams
Ham, 3 oz serving – 19 grams
Ground pork, 1 oz raw – 5 grams; 3 oz cooked – 22 grams
Bacon, 1 slice – 3 grams; Canadian-style bacon (back bacon), slice – 5 – 6 grams

Dairy
Milk, 1 cup – 7-9 grams
Cottage cheese, ½ cup - 15 grams
Yogurt, 100g – 4 grams
Soft cheeses (Mozzarella, Brie, Camembert) – 6 grams per oz
Medium cheeses (Cheddar, Swiss) – 7 or 8 grams per oz
Hard cheeses (Parmesan) – 10 grams per oz

Beans (including soy)
Tofu, ½ cup 20 grams; Tofu, 1 oz, 2.3 grams
Soy beverage, 1 cup - 6 -10 grams
Most beans (black, pinto, lentils, etc) about 7-10 grams per ½ cup cooked
Soy beans, ½ cup cooked – 14 grams
Split peas, ½ cup cooked – 8 grams

Nuts and Seeds
Peanut butter, 2 Tablespoons - 8 grams
Peanuts, ¼ cup – 7 grams
Almonds, ¼ cup – 8 grams
Cashews, ¼ cup – 5 grams
Pecans, ¼ cup – 2.5 grams
Sunflower seeds, ¼ cup – 6 grams;
Pumpkin seeds, ¼ cup – 19 grams;
Flax seeds – ¼ cup – 8 grams

Week 2 - Grams of Carbohydrates in Common Foods

Breakfast Cereals	**Grams of CHO**
Granola, 1 ounce (1/4 cup)	18
Raisin Bran, 1 ounce (1/2 cup)	20
Shredded Wheat, (1/2 cup)	15
Grape nuts, 1 ounce (1/4 cup)	23
Oatmeal, ½ cup	15
Bran flakes cereal 1 oz (1/2 cup)	15
Muesli (1/2 cup)	30
Fruit	
Apple, medium	20
Orange, medium	20
Banana, medium	25
Pear, medium	25
Raisins, 15 ounce box (1/4 cup)	25
Apricots, 8 halves dried	15
Cherries, fresh, 12	15
Blueberries, ¾ cup	15
Raspberries, 1 cup	15
Strawberries, 1¼ cup whole berries	15
Grapes, 17 ea	15
Honeydew, 1 cup of cubes	15
Kiwi, 1 ea	15
Mango, 1 medium	30
Peach, 1 ea	15
Pineapple, ¾ cup	15
Plums, 2 small	15
Prunes, 3	15
Watermelon, 1¼ cup cubes	15
Vegetables	
Carrot, medium	10
Peas, 1/2 cup	15
Tomato sauce, Ragu, 1/2 cup	10
Winter squash, 1/2 cup	15
Sweet Potato/Yam, ½ cup	15
Corn, 1/2 cup	15
Potato, mashed ½ cup	15

*1 cup of raw or ½ cup cooked "non-starchy" vegetables = 5 g CHO
- Broccoli, lettuce, peppers, cucumber, celery, leek, spinach, onions, tomatoes, cabbage, zucchini, bean sprouts, eggplant, cauliflower, mushrooms

Bread-Type Foods	
Rice cake, 1	7
Graham crackers, 2 squares	10
Butter crackers (Ritz), 6 ea.	15
Saltines, 6	15
Waffle, 1 Eggo	17
English Muffin, 1	30
Small plain roll	15
Matzo, 1 sheet	28
Pancakes, 2 (4 inch)	30
Pita bread, 8-inch round	44
Flour Tortilla (10")	35
Bagel, average (3 ounces)	40
Bread (1 slice)	12
Naan (1/2 of 8"x12" piece)	30
Bran Muffin, 1 large	45
Submarine roll, 8 inch	60
Beverages	
Gatorade, 8 ounces	10
Milk, 2%, 8 ounces	13
Beer, 12-ounce can	13
Milk, chocolate, 8 ounces	25
Orange juice, 8 ounces	30
Apple juice, 8 ounces	30
Apricot nectar, 8 ounces	35
Cranberry cocktail, 8 ounces	45
Low-cal Cranberry Cocktail, 8 ounces	15
Cola, 12-ounce can	38
Grains, Pasta, Starches	
Ramen noodles, 1/2 package	25
Rice, 1 cup cooked	45
Spaghetti, 1 cup cooked	40
Penne/Rigatoni Pasta, 1 cup cooked	45
Lentils, 1/2 cup cooked	20
Baked beans, 1 cup	25
Chick Peas, ½ cup	16
Flour, dry 3 tbsp	15
Kasha (buckwheat) ½ cup	15
Bulgur (Tabouli), cooked ½ cup	15
Hummus 1/3 cup (~80g)	15

Entrees, Convenience Foods	
Split pea soup, 1 bowl	35
Pizza, cheese, 2 slices	40
Chilli, 1 cup	45
Bean Burrito, 1	50
Chow Mein noodles, ½ cup cooked	15
Sweets, Desserts, Snacks	
Oreo, 1	7
Chocolate chip cookie, 1 small	10
Fig Newton, 1	11
Strawberry jam, 1 tablespoon	13
Honey, 1 tablespoon	15
Maple Syrup, 2 tablespoons	25
Pop tart, blueberry	35
Soft-serve ice cream, 1 cup	40
Fruit yogurt, 1 cup	40
Chips – baked, 15-20 ea.	15
Melba toast, 4 rectangular pieces	15
Popcorn, 3 cups popped (no butter)	18
Croutons, 1 cup (loose)	15

Week 2 - Facts on FIBRE

Fibre: Dietary fibre is only found in plant products. It slows digestion and gives a feeling of being full which may help with portion control and weight loss.

There are 2 types of dietary fibre: **Soluble and Insoluble**
Soluble: helps to lower LDL-C and total Cholesterol, it also helps to control blood sugar levels.

- Psyllium – Metamucil, All Bran Buds®
- Legumes – beans, lentils, chickpeas
- Oat products - oatbran and oatmeal
- Barley
- Pectin-rich fruits - apples, berries, pears, citrus fruits
- Some vegetables – eggplant, okra, artichoke, squash, broccoli, peppers
- Soybeans

Insoluble: helps to relieve and prevent constipation, and helps to keep your bowels healthy (colonic health). *Only works with the consumption of a lot of fluids.

- Wheat bran (shredded wheat)
- Whole **grain or multigrain** cereals and breads
- Whole **grain** products – brown rice, couscous, multigrain pasta, buckwheat, bulgur
- Colourful fruits and vegetables – dark leafy greens, brussel sprouts, cabbage, yellow orange and red vegetables/fruits (especially in the skin!)

Try to eat 25-30 g of fibre per day!!
*Increase fluid intake accordingly

"All natural, no fat, no cholesterol, no sodium, no calories,high fiber, no chemical additives, and we spiced it up so you'd never know it was sawdust!"

http://www.cartoonstock.com/newscartoons/cartoonists/jko/lowres/jkon62l.jpg

Week 2 - Grams of Fibre in Common Foods

Breakfast Cereals	Grams of Fibre
Fibre 1 Cereal, ½ cup	13
All Bran Buds, 1/3 cup	12
All Bran Original Cereal, ½ cup	10
Raisin Bran, ¾ cup	4.5
Cheerio's, 1 cup	3
Oatmeal, ½ cup cooked	2
Shredded Wheat, 1 biscuit	1
Fruit	
Dried figs, 5	3
Large prunes, ¼ cup	3
Raspberries, ½ cup	4
Blueberries, ½ cup	2.5
Blackberries, ½ cup	3.5
Strawberries, 1 cup	4
Apple, 1 medium	3.5
Applesauce, ½ cup	3
Apricots, 3	2
Kiwi, 1	3
Nectarine, 1 medium	3
Orange, 1 medium	3
Pear, 1 medium	3
Raisins, ½ cup	3
Banana, 1 medium	2
Plum, 1 medium	1
Vegetables	
Artichoke, 1 cup	5
Green peas, ½ cup	3.5
Yam, 1 medium	3.5
Broccoli raw, ½ cup	1.5
Corn, ½ cup	2
Potato (with skin), 1 medium	3
Spinach, ½ cup cooked	2
Spinach, 1 cup raw	2
Green pepper, medium	2
Brussels sprouts, ½ cup	2
Cabbage, ½ cup cooked	2
Green beans, ½ cup	2
Tomato, 1 medium	2
Baby carrots, ½ cup	2
Asparagus, 5 spears	1
Lettuce, 1 cup	1

Bread-Type Foods	
Bran muffin, small	3
Whole wheat bread, 1 slice	2
Pumpernickel bread, 1 slice	2
Bagel, medium	2.5
Pita bread, 6½"	2
Flour Tortilla, 10"	2.5
Grains, Pasta, Starches	
Pinto Beans, ½ cup	7
Kidney Beans, ½ cup	6
Whole wheat pasta, ½ cup cooked	3
Baked beans, ½ cup	7
Soybeans (fresh), ½ cup	5
Edamame (frozen), ½ cup	5
Chickpeas, ½ cup	5
Lentils (cooked), ½ cup	7
Split peas (cooked), ½ cup	7
Brown rice (cooked), ½ cup	2
White rice (cooked), ½ cup	0.5
Snacks, Seeds, Nuts	
Almonds, ¼ cup	4.3
Peanuts, ¼ cup	3
Sunflower seeds, ¼ cup	3.5
Natural peanut butter, 2 Tbsp	2
Pecans, ¼ cup	2.5
Walnuts, ¼ cup	1.7
Cashews, ¼ cup	1

Week 4 - Facts About Dietary Fats

http://theactivecubby.files.wordpress.com/2011/07/oils.jpg

✓Lipids (scientific name for fats) have an important role in our body.
- Make up the phospholipid bi-layer in cell membranes
- Lipids are a good fuel-source for the body
- Cholesterol is also a key structural component in our cell membranes and it is used to build hormones.

✓But at the same time, too much of certain fats can increase the risk for chronic disease, by increasing circulating blood lipid levels (triglycerides) and possibly clogging our arteries (plaque). The fat we consume in our diet is also very high in energy, and if we don't burn it, it can be easily packed away in our adipose, muscle and liver tissue and our arteries.

✓Generally speaking, you have to be especially careful of saturated fat and Trans fat because they both have been shown to raise 'bad' cholesterol levels (LDL) and increase the risk of heart disease. You find some saturated fat in all animal products, and Trans fat in many fast foods and baked goods.

The recommended intake per day is:
- Cholesterol intake: less than 300 mg
- Saturated fat intake: less than 10% of your total calories
- Total fat intake: ~ 30% of your total calories

Some general tips to help you eat the right type and amount of fat per day:

✓ Use added fats very sparingly in your diet. These fats include: oil for cooking, salad dressings, margarine/butter on toast, cream in coffee/tea and recipes, shortening/lard for cakes

✓ Use oils that are liquid at room temperature such as canola (vegetable), olive, and soybean. Also, use calorie reduced non creamy salad dressing. 1 tbsp oil = 15 g fat

Nutrition Facts

Serving Size: 1/2 cup (124g)

Amount Per Serving	
Calories 80	Calories from Fat 3
	% Daily Value*
Total Fat 0.36 g	**1%**
Saturated Fat 0.08 g	**0%**
Trans Fat	
Cholesterol 0 mg	**0%**
Sodium 512.48 mg	**13%**
Potassium 353.4 mg	**10%**
Total Carbohydrate 16.53 g	**6%**
Dietary Fiber 4.46 g	**18%**
Sugars	
Sugar Alcohols	
Protein 5.05 g	
Vitamin A 186 IU	4%
Vitamin C 9.05 mg	15%
Calcium 34.72 mg	3%
Iron 2 mg	11%

http://quitehealthy.com/nutrition-facts/food-labels/label110331.gif

✓ Choose soft margarine instead of butter. These are generally lower in saturated fats and trans fats.

- ✓ Avoid frying foods (deep fry and pan-fry*) – bake, broil, steam, poach, BBQ, microwave
  * Pan-frying is ok if you use "PAM" cooking spray or water or very little oil in the process.

- ✓ Keep sauces and dressings "on the side" instead of pouring into foods (e.g. salads) – dip your fork into the sauce or dressing instead and then into food

- ✓ Choose lean meats, poultry and fish – trim away all visible fat and skin, choose canned fish packed in water (not oil), avoid sausages, bacon, hamburgers, fried fish sandwiches, chicken fingers and other high fat processed meats (e.g. salami). Have a vegetarian meal including legumes and soy products at least 1x per week.

- ✓ Watch for "hidden fats" – anything made with hydrogenated oils or shortening.

- ✓ Limit the consumption of these snack foods: chips, cheezies, tortilla chips, snack crackers, cookies, store bought muffins, croissants, donuts, pastries, cheese cake, creamy soups, ice cream, chocolate, frozen convenience meals/foods, processed cheese and meats.

A little bit on Nuts:

- Nuts are high in unsaturated fatty acids omega-3, 6 and 9
- Nuts also contain: protein, fibre, vitamins, minerals, plant sterols, vitamin E and other polyphenols (important antioxidants).
- Be careful → Nuts are high in GOOD fat: 1 oz almonds (20-22 nuts) = 14 g fat. Some nuts are lower in fat than others. Lower fat nuts: almonds, walnuts, pistachios, peanuts; Medium fat: hazelnuts, cashews; higher fat nuts: pecans, macadamia nuts.
-Adding nuts to your day: 1 oz* per day is recommended → try eating them as snacks, in salads, in yogurt, in a stir-fry, have nut butter (e.g. almond or peanut) on celery or high fibre breads.

  * 1 oz is: 20-22 almonds, 18 pecan halves, 3 tbsp peanuts, 10-12 macadamia nuts, 45-50 pistachios, 8-10 walnut halves, 8-10 hazelnuts

http://www.webdoctoradvice.com/wp-content/uploads/2011/01/almonds.jpg

Table on Fats

Type of Fat	Sources	Recommendations
Saturated - Animal products - Solids at room temp*	Butter, lard, meat, poultry (esp. skin), higher fat milk and milk products (cream, ice cream, cheese), chocolate. *Liquids: Palm oil, coconut oil, cocoa butter	Use lower fat dairy products (1% or skim milk and lower fat cheese), choose lean meat, skinless poultry, fish, legumes, and soy products
Trans (formed mostly by hydrogenation) "Double Whammy" -Raise BAD and Lower GOOD	Hard margarine, foods make with hydrogenated vegetable oils, lard or shortenings (cookies, cakes, pie crust, crackers, pastries, donuts), fast food items (French fries, burgers, etc.), sometimes from deep frying foods	Use "non-hydrogenated" margarines, look at food labels and avoid eating foods with Trans fat (they may be disguised as "partially Hydrogenated oil" or "Shortening").
Cholesterol - Animal products	All animal foods (highest concentration in organ meats and egg yolks)	Limit your intake of cholesterol-rich foods
Unsaturated - Plant foods - Liquid at room temp - May lower LDL and increase HDL	**Monounsaturated** (MUFA) – olives and olive oil, canola oil, nuts (almonds, peanuts, cashews), avocados **Polyunsaturated** (PUFA) – Safflower oil, sunflower oil and seeds, canola oil, corn oil, soybean oil, sesame oil and seeds	Substitute these fats for Saturated fats. -Salad dressings that are olive oil based are usually better than the creamy ones.
Unsaturated (more specifically….) **-Omega-3 PUFA** - reduce TG, reduce blood clotting, anti-inflammatory	-FISH: Salmon, trout, tuna, mackerel, herring, sardines (DHA and EPA), cod liver oil, -Plants: flax seeds, walnuts, pumpkin seeds, wheat germ, soybean products, canola oil, Salba, (ALA)	Include fish in your diet 2-3 times per week. Have 1 oz of nuts (unsalted, dry roasted or raw) every day *Remember that some fat is GOOD for you!

Week 6 - Get the most out of what you eat

Adapted from: http://www.weightwatchers.ca/util/art/index_art.aspx?tabnum=1&art_id=34441

To get more satisfaction out of food, the idea is to fill up on foods that give you a lot of **volume** for relatively few calories. For example, 1/3 cup of raisins and 1.7 cups of red grapes has the same number of calories. With the fresh fruit, you obviously get to eat much **more**, and you're more likely to feel satisfied when you're through.

Besides fresh fruit, other satiating high-volume foods include complex carbohydrates that are high in water, air and/or fibre, such as air-popped popcorn, non-starchy vegetables and whole-grains like brown rice. Also, lean protein-rich foods such as a skinless chicken breast or other skinless poultry, fish, legumes (beans) and lean meats can also contribute to satiety. That's why, for maximum satisfaction, you might want to make sure all your meals also contain some lean protein.

How to feel full - Other mealtime tricks for increasing the volume of food consumed to maximize your satisfaction on fewer calories:

- **Start meals with a first course:** broth-based or puréed vegetable soup, vegetable juice or a salad with low-fat or fat-free dressing. One caveat: Make sure that first course isn't more than 100 calories. Otherwise, you could end up eating too many calories at that particular meal.

- **Eat more non-starchy vegetables:** spinach, lettuce, cabbage, tomatoes, carrots, green beans, broccoli, cauliflower, cucumber, celery, zucchini and onions. In fact, put them in stews, soups, pasta sauces, stir-fry's, and pizza. They're high-volume, high-satisfaction and low-calorie because they're loaded with water and fibre.

- **Limit dry foods:** dried fruit, pretzels, crackers and chips. Dry foods lack water and thus are low in volume. Dry foods pack a lot of calories into a small portion and are easy to overeat.

Eventually you may find yourself gravitating towards these high volume, more satisfying, less caloric choices - make your healthy eating habits a way of life, not a chore!

http://www.eating-in.com/wp-content/uploads/2008/03/salad1.jpg
http://www.roasterscoffee.co.uk/images/fruit.jpg

LESS Filling Choice (Lots of Calories in a small volume)	**VS**	**MORE Filling Choice** (lots of volume with similar Calories)
¼ cup Trail Mix	175 kcal	An apple & 4 celery stalks with 1.5 tsp peanut butter
4 slices of bacon	175 kcal	2 Egg omelet with ¾ cup mixed vegetables (e.g. peppers, spinach, mushrooms, broccoli, onion) ★with 6 egg whites + ¾ cup veggies – only 125 kcal!★
Mr. Noodles Chicken soup in a cup	380 kcal	1 cup mixed green salad with peppers, cucumber and 2 tbsp low fat balsamic vinaigrette dressing; 6 oz of skinless chicken breasts; 2/3 cup steamed broccoli & cauliflower and a medium apple for dessert! ★with one chicken breast – only 280 kcal!★
Tim Horton's Blueberry Bran Muffin	300 kcal	1 package Quaker Instant cinnamon oatmeal (with water), ¼ cup of bran buds, ½ cup blueberries, 6 almonds (crushed)
Oh Henry! Bar	263 kcal	Medium apple, 1 tbsp light peanut butter, 5 1" whole wheat crackers

Week 8 - Curbing Your Cravings

Adapted from: http://www.weightwatchers.ca/util/art/index_art.aspx?tabnum=1&art_id=32121

What is a craving?? A craving is defined as an intense desire for a particular thing. Food cravings can focus on a specific food, such as cookies, or they may be more general, such as anything sweet or anything chocolate.

- Whether or not there is a physical need that triggers a craving is unclear, but they tend to hit us hardest when we are over-tired, stressed-out, or just feeling blue.

- Focusing on proper nutrition may be a long-term solution to reducing the frequency of cravings; a balanced diet that includes all the food groups, plenty of water, and a moderate exercise program has been reported to help. *Good thing we are doing ALL of these things!*

Craving	The Definitely DON'TS	The Smart Substitutions (in moderation!)
Salty	- potato chips, salted nuts/trail mix,	- air popped or light microwave popcorn, pretzels, light whole grain crackers
Sweet	-cookies, cakes, ice cream, dried fruits, donuts, pastries, candy	- fruit filled granola bar, fig Newton, low fat hot chocolate, fruit (pineapple, grapes, watermelon), sugar free candies
Spicy	- Spicy (Jalapeño/Mexican) flavoured tortilla chips, nacho cheese	- baked Tostitos scoops with Salsa, add hot flavour spice to your meals
Fatty	- hamburgers, fried fish, French fries, nachos	- Same food but baked (chicken fingers, fish fillet, French fries), make your own LEAN burgers (red meat, chicken, turkey…)
Creamy	- ice cream, custard, rice pudding, regular cream cheese, salad dressings (Caesar/ranch)	- fat free pudding, fat free sugar free fudgsicle or creamsicle (they are out there!), low fat cream cheese (less of it!), frozen yogurt, sorbet, make fat free onion dip (fat free sour cream + Lipton's onion mix
Chocolaty	-chocolate bars, white chocolate, truffles	- fat-free chocolate pudding, low sugar chocolate sauce (Brown Cow), *a piece of* rich dark chocolate (high cocoa).
Crunchy	- chips, nachos, trail mix	- cereal (Bran Buds, bran flakes, cheerio's), baked chips, rice cakes/rice crackers

Strategies for combating cravings before they start

1) Eat regularly. Plan for plenty of smaller healthy snacks to keep you going.
   - Skipping meals and starving yourself often only intensifies cravings.

2) Eat more complex-carbohydrates, aim for those high in fibre and whole grains.
   - Avoid simple sugars and refined products like cakes, cookies, sweets and white foods—rice, bread, pasta. They'll take your blood sugar for a ride and leave you feeling unsatisfied, hungry shortly thereafter, sluggish and craving more sugar.

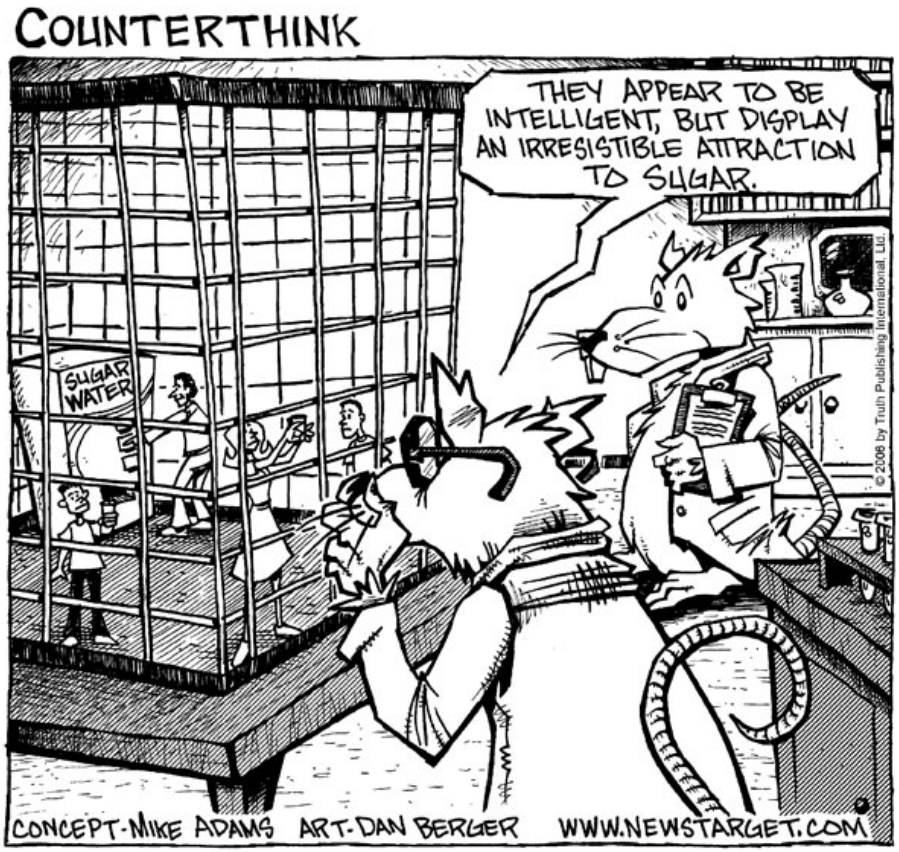
3) Include a good source of protein with every meal.
- Protein has been shown to provide eating satisfaction and to help with satiety so you'll feel full longer.

4) Drink lots of Fluids
- Keep the water coming! Use low-calorie flavourings (Crystal Light) to spice it up. Drink herbal tea often.
- Listen to your body. Sometimes it is hard to tell if you are truly hungry or just thirsty, so grab a drink first before eating!

5) Focus on flavour
- Flavour can satisfy the senses quickly so you may be less likely to overeat. Try adding some zest to your meals: roasted red or yellow peppers, sun-dried tomatoes, fresh ginger, mint, lemon or orange zest, fresh herbs, spicy salsa, or simply just garlic and onion!

6) Try to "Wait it out" before hitting the fridge/pantry.
- Most cravings will dissipate in about 20 minutes. Cravings start in the brain so they must end there too!

7) Exercise - Moderate exercise helps keep our bodies function optimally!



http://conben.typepad.com/photos/uncategorized/2007/12/30/cartoon_sugar_addiction_news_targ_2.jpg

Week 10 - 8 Tips on How to Successfully Eat at Restaurants

Adapted from : http://www.weightwatchers.ca/util/art/index_art.aspx?tabnum=1&art_id=6321&sc=3030

http://www.cartoonstock.com/newscartoons/cartoonists/rma/lowres/rman2867l.jpg

1) Determine how much you're willing to eat before looking at the menu.

2) Decide on some general guidelines before you go to restaurants, and stick to them. For example:

- Skip the all-inclusive menu or the all-you-can-eat buffet and opt for à la carte selections. Doing so might not be as economical, but you'll probably eat less.

3) Make special requests and ask questions! Don't be afraid to make special requests or slight modifications to your meals or to ask how dishes are cooked. Ask things like:

- Can I get that without butter?
- Can you please put the sauce/dressing on the side?
- I'd like mixed greens instead of fries with my sandwich.
- Can you please decrease the amount of oil used to cook my meal?
- Can you put less cheese on my pizza?

4) Practice portion control. Some restaurant portions can be two, three, even four times the normal size – especially super-sized fast food meals. Try to keep proper portion sizes by:

- Ordering a salad or a non-creamy soup as a starter and then splitting a main entrée with a friend.
- Creating your own scaled-down meal to share from a couple of appetizers and/or side dishes.
- Know you will only eat half and take the rest home for the next day
- Never “supersize” anything!

5) If you don't know what a preparation term means, ask. In general, the following words translate into high-fat, high-calorie dishes:

- Au gratin, scalloped, hollandaise, parmigiana, scampi, bolognese

6) Watch out for "extras". A homemade lean burger with ketchup, lettuce and tomato isn't so bad. But one from a fast-food restaurant with "the works" is usually a nightmare. Skip over the:
- Bacon and cheese
- Double-burger patties and extra pieces of bread
- Fried fish and chicken sandwiches
- Mayonnaise and "special sauces"

7) Salad bars and garden salads are very popular and can be a very healthy choice, but the extra toppings can sabotage your diet-conscious choices:
- Go light on croutons, grated cheese, nuts/trial mix and bacon.
- Take small amounts of low-fat or nonfat dressing on the side.
- Add dried fruit (raisins, cranberries) and nuts/seeds (almonds, sunflower seeds) sparingly.

8) You don't have to be a member of the "clean plate club". Downsize by:
- Eating half of a larger meal and doggie-bagging the rest.
- Pushing your plate away when you're full. And remember to eat slowly and drink lots of fluids with a meal. It takes 20 minutes for your body to recognize that it's full!

Some MORE Tips on how to choose your meals at restaurants

At the Salad Bar
- Walk around the entire salad bar and look at all the selections before making your choices.
- Try to select as much as you want of the fresh, low-fat, non-starchy items such as lettuce, spinach, broccoli, carrots, bell peppers, mushrooms, cauliflower, celery, cucumber, radishes and onions.
- Avoid high-fat items, such as processed meats, (excess) cheese, (excess) nuts and prepared salads (like three-bean, coleslaw, macaroni or potato). Also avoid regular salad dressings, which contain high amounts of fat.
- Select a small amount of fresh fruit; avoid canned fruit, which is usually packed in syrup.
- Stick with the broth-based soups, not the creamy ones.
- Remember to include as carbohydrates the croutons, crackers and bread you may eat with your salad.

At a Chinese Restaurant
- Avoid Chinese restaurants if you are on a low-sodium diet. They often use soy sauce as a main ingredient, and it is loaded with sodium.
- Read the menu carefully. Avoid battered and fried meats. If you can't figure it out, ask the server.
- Avoid foods with "sweet-and-sour" or high-fat egg foo yong dishes.
- Pay attention to the kind and portion size of rice. Avoid fried rice, ask for the steamed rice. One-half cup portion has about 20 grams of carbohydrates.
- Same thing for noodles – fried noodle dishes with a lot of sauce is not a healthy choice.
- Order stir-fried items instead of combination plates with fried rice and egg rolls (especially at fast-food Chinese restaurants).
- Remember, each fortune cookie has about 5 grams of carbohydrate.

At a Mexican Restaurant

- Skip the tortilla chips. Twelve chips have about 20 grams of carbohydrate and 9 grams of fat.
- Choose black beans over the refried beans, which are usually cooked with lard.
- Order individual items and side dishes instead of combination plates, which usually have loads of refried beans and rice.
- Skip the guacamole and sour cream (or use a little bit) and request more salsa.
- Select soft flour tortillas, which are usually a better choice than the crispy fried corn tortillas
- Choose to have chicken, beef or vegetarian fajitas instead of burritos, tacos, enchiladas or quesadillas. Try to share an order of fajitas because the order is usually enough for two people.
- Skip the margaritas; they're loaded with sugar and alcohol.

At an Italian Restaurant

- Watch for better menu choices that include the words marinara (tomato sauce), pomodoro (tomato) and primavera (indicates the dish has vegetables).
- Look for sauces labeled "light" and ask if they have reduced calories (or if they are referring to the colour!)
- Choose something lightly sautéed in olive oil ("Swimming in oil" is also not good).
- Avoid choices that are generally high in fat such as alfredo and Rosé sauce, parmigiano (lightly breaded, fried and usually topped with cheese), cream sauces, anything sautéed in butter or that is cheese-stuffed or filled.
- Lean meats, chicken or seafood sautéed or grilled are better choices and are great for sharing.
- If you want a coffee after the meal, have a decaf espresso or a regular brew. Avoid the cappuccinos since they are usually made with cream or homo milk.
- When Eating Pizza:
    - Stick with thin crust pizza; it has fewer calories and carbohydrates.
    - Load it up with vegetables and go light on the cheese.
    - Choose a leaner meat topping such as chicken or lean ham (if any meat).
    - Skip the extra breadsticks or rolls.
    - Split a pizza with someone and get a salad to share too

Week 10 - Making Smart ***Fast Food*** Choices

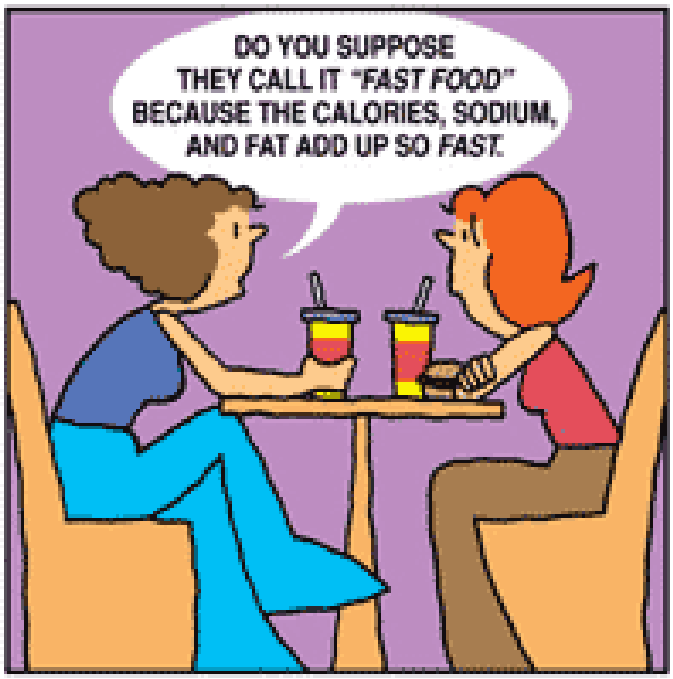
Adapted from: http://www.helpguide.org/life/fast_food_nutrition.htm

Ordering take-out and going out to eat at restaurants is usually worse for you than making meals at home as far as nutritional intake is concerned.

Here are some tips on what to choose if you go out to eat at one of the following restaurants. For the I.D.E.A.L. study, please limit restaurant eating and take-out meals as much as you can (at most, one or two times per week). You can see how even "home-cooked" varieties of your favourite dishes are usually healthier than those at restaurants. Put yourself in control!

Here are a few quick tips to get you started:

- If you must, order plain hamburgers with mustard, pickles and ketchup without the extra fat and calories from cheese, bacon and mayo.
- Choose broiled or BBQ, non-breaded chicken, turkey (or fish) sandwiches, which are lower in fat and calories than their fried counterparts.
- Try the chili -- it's usually a better alternative to a hamburger because it's lower in fat and the beans are a great source of fiber. Make sure to go easy on the sour cream and cheese!
- Keep in mind that English muffins and whole wheat breads have less fat, less carbohydrates and fewer calories than biscuits and croissants.
- Choose low-fat milk, diet pop, or water (+ your favourite calorie-free flavouring!) as opposed to regular pop, fruit juice and milkshakes.
- For something sweet, select fruit, frozen yogurt or a smoothie instead of ice cream or a milkshake.



Common Fast Food restaurants around McMaster

Choose This:	Not That:
Salads: o Entrée Garden Salad- 290 kcal, 23 g fat, less if you go light with the dressing o Tuscan Chicken Salad (Lifestyle Menu)- 330 kcal, 12 g fat o Chicken Garden Salad- 420 kcal, 25 g fat	Salads: o Entrée Caesar Salad- 560 kcal, 52 g fat
Entrees: o Seafood Linguine (Lifestyle Menu)- 420 kcal, 9 g fat o Hell's Kitchen Chicken- 530 kcal, 20 g fat o Lasagna Alforno- 590 kcal, 21 g fat o Gnocchi Napolitana- 640 kcal, 16 g fat	Entrees: o Spaghetti with Meatballs (Grande)- 1340 kcal, 55 g fat o Sausage Al Forno- 1280 kcal, 63 g fat o New York Steak- 830 kcal, 58 g fat o Braised Beef Short Ribs- 770 kcal, 44 g fat
Side Servings: o Cup of Italian Wedding Soup- 60 kcal, 2.5 g fat o Vegetables- 60 kcal, 3.5 g fat o Cup of Vegetable Soup- 70 kcal, 0 g fat	Side Servings: o French Fries- 380 kcal, 16 g fat o Mushrooms- 220 kcal, 16 g fat

Choose This:	Not That:
Salads: o Toasted Sesame Salad with Asian Sesame Dressing- 450 kcal, 34 g fat o Choose light dressings for all salads (dressings can be killer!). Go with Italian,	Salads: o Taco Salad- 1120 kcal, 75 g fat o Cajun Chicken Caesar Salad- 760 kcal, 55 g fat

or Fat-Free Raspberry	
Entrees: o Fire-Grilled 11 oz. Striploin Steak- 430 kcal, 18 g fat o Roasted Vegetable Wrap- 580 kcal, 24 g fat o Balsamic Chicken in Sauce- 630 kcal, 33 g fat	Entrees: o Prime Rib Philly Sandwich- 1140 kcal, 74 g fat o Tomato Vodka Penne- 1100 kcal, 61 g fat o Backyard Pork Side-Ribs (Half Rack)- 770 kcal, 46 g fat
Side Servings: o Seasonal Vegetables- 60 kcal, 3 g fat o Chicken Noodle Soup- 100 kcal, 3 g fat	Side Servings: o Loaded Baked Potato- 410 kcal, 12 g fat o Broccoli Cheddar Soup- 265 kcal, 10 g fat

Choose This:	Not That:
o Garden Entrée Salad with Grilled Chicken and Low-Fat Italian Dressing, Apple Slices with Caramel Dip and a Diet Coke- 310 kcal, 6 g fat o Chicken McNuggets with Ketchup, Side Garden Salad with Low-Fat Italian Dressing and a Diet Coke- 405 kcal, 23 g fat o Grilled Chicken Classic Sandwich, Side Garden Salad with Low-Fat Italian Dressing and a Diet Coke- 440 kcal, 14 g fat o Hamburger, Small Fries and a Diet Coke- 470 kcal, 19 g fat	o Quarter Pounder with Cheese, Medium Fries and a Coke- 1100 kcal, 45 g fat o Big Mac, Medium Fries and a Coke- 1110 kcal, 47 g fat o Southwest Crispy Chicken Sandwich, Medium Fries and a Coke- 1130 kcal, 46 g fat

Choose This:	Not That:
Pitas: o Hummus on a Whole Wheat Pita- 255 kcal, 4.5 g fat o Chicken Breast, Turkey Breast or Roast Beef on a Whole Wheat Pita- each approximately 305 kcal, all under 5 g fat	Pitas: o Rib on a White Pita- 429 kcal, 14 g fat o Philly Steak on a White Pita- 426 kcal, 11 g fat
Sauces: o Dijon Mustard- 5 kcal, 0 g fat o BBQ Sauce- 17 kcal, 0 g fat	Sauces: o Mayonnaise- 45 kcal, 4.6 g fat o 'Secret Sauce'- 40 kcal, 4.7 g fat

Choose This:	Not That:
o Cheese, large walk-in slice- 570 kcal, 14 g fat o Garden Veggie, large walk-in slice- 620 kcal, 16 g fat o Spicy Barbeque Chicken, large walk-in slice- 620 kcal, 15 g fat	o Big Bacon Bonanza, large walk-in slice - 710 kcal, 26 g fat o Canadian Eh, large walk-in slice - 680 kcal, 22 g fat o New York Pepperoni, large walk-in slice- 680 kcal, 24 g fat

- Try opting for whole wheat or multigrain thin-crust whenever possible! Also, try to avoid using dipping sauces; they contain a lot of empty calories and fat. Use hot sauce or chili flakes instead for that extra kick!
- When ordering, ask for a little less cheese and load on the vegetables. Try to avoid having meat on pizza.
- Remember, making your own pizza at home is always the best choice! (and, it is fun too…)

Choose This:	Not That:
Subs: o Veggie Delight Sub (hold the cheese)- 230 calories o Turkey Breast Sub with Veggies- 280 calories, 4.5 g fat o Roast Beef Sub with Veggies- 280 kcal, 5 g fat	Subs: o Meatball Marinara Sub with Veggies and Cheese- 560 kcal, 24 g fat o Tuna Sub with Veggies and Cheese- 530 kcal, 30 g fat o Chicken and Bacon Ranch Sub with Veggies and Cheese- 510 calories, 25 g fat
Salads: o Veggie Delite - 60 kcal, 1 g fat; No cheese = less fat but no protein o Oven Roasted Chicken Breast Salad- 140 kcal, 2.5 g fat o Fat-Free Italian Dressing- 35 kcal, 0 g fat	
Sub Sauces: o Fat-Free Honey Mustard- 30 kcal, 0 g fat o Fat-Free Sweet Onion- 40 kcal, 0 g fat o Mustard- 5 kcal, 0 g fat	Sub Sauces: o Ranch Salad Dressing- 320 kcal, 35 g fat o Mayonnaise- 110 kcal, 12 g fat o Chipotle Southwest Sauce- 100 kcal, 10 g fat

- Stick with 6-inch subs to keep portions in check and always load up the veggies.
- Choose whole wheat breads and wraps.
- If you choose to get a combo, go with baked chips (as opposed to cookies or regular chips) and diet pop (instead of regular pop or juice).

Choose This:	Not That:
Sandwiches: o Smoked turkey on whole wheat roll with	Sandwiches: o BLT + mayo- 450 kcal, 18 g fat

mustard, lettuce and tomato – 300 kcals, 4 g fat o Chicken Salad with Lettuce and Tomato- 380 kcal, 9 g fat o Roast Beef with lettuce and tomato- 390 kcal, 8 g fat o Toasted chicken club- 7 g fat o Ask for no butter on sandwiches	o Tuna and Egg sandwiches have 13g fat o Breakfast sandwich with sausage, cheese and egg – 530 kcals, 33 g fat
Soups (10 oz): o Hearty Vegetable- 70 kcal, 0.4 g fat o Chicken Noodle- 120 kcal, 2 g fat o Chili, although high in fat, 19 g, is also high in protein and fiber	Soups (10 oz): o Hearty Potato Bacon- 250 kcal, 13 g fat o Cream of Broccoli- 160 kcal, 9 g fat
Bagels: o Plain Bagel- 260 kcal, 1.5 g fat o Cinnamon Raisin- 270 kcal, 1 g fat o Sesame, Poppy Seed and Everything Bagel- 270 kcal, 2 g fat	Bagels: o Twelve Grain- 330 kcal, 9 g fat o Wheat 'n' Honey- 300 kcal, 3 g fat
Cream cheese: o Plain Light- 100 kcal, 8 g fat	Cream cheese: o Plain- 144 kcal, 14 g fat
Treats: o Low-fat Strawberry Yogurt with Berries- 140 kcal, 2.5 g fat o Honey Dip Donut- 210 kcal, 8 g fat o Low-Fat Blueberry or Cranberry Muffin- 290 kcal, 2.5 g fat o Timbit (go for only one or two… if you must!)- 70 kcal, 4 g fat each	Treats: o Chocolate Chips Muffin- 430 kcal, 16 g fat o Walnut Crunch Donut- 360 kcal, 23 g fat o Honey Cruller- 320 kcal, 19 g fat o Peanut Butter Cookie- 280 kcal, 16 g fat o Limit Iced Cappuccino drinks, or order them with chocolate milk instead of cream

- Choose sandwiches on whole wheat buns and soups with whole wheat rolls.

Choose This:	Not That:
o Jr. Hamburger Deluxe (no mayo), Side Salad with Low-Fat Ranch Dressing and a Diet Coke- 360 kcal, 16 g fat o Mandarin Chicken Salad with Balsamic Vinaigrette Dressing (hold the crispy noodles) and a Diet Coke- 390 kcal, 19 g fat *or try Fat-Free French Dressing o Ultimate Chicken Grill Sandwich, Side Garden Salad with Low-Fat Honey Mustard Dressing and a Diet Coke- 450 kcal, 10 g fat o Small Chili, Baked Potato (no sour cream) and a Diet Coke- 460 kcal, 7 g fat	o Baconator Burger with Medium Fries and a Coke- **1460 kcal**, 71 g fat o Spicy (breaded) Chicken Sandwich with Medium Fries and a Coke- 1070 kcal, 36 g fat o Southwest Taco Salad with Ancho Chipotle Ranch Dressing and a Coke- 810 kcal, 40 g fat

Choose This:	Not That:
Original Items: o Spicy Chicken Soft Taco- 170 kcal, 6 g fat	Original Items: o Grilled Stuff Burrito (Beef)- 680 kcal, 30 g fat
"Fresco Menu": o Grilled Steak Soft Taco- 160 kcal, 4.5 g fat o Ranchero Chicken Soft Taco- 170 kcal, 4 g fat	"Fresco Menu": o Fiesta Taco Salad- 840 kcal, 45 g fat o Zesty Chicken Border Bowl- 640 kcal, 35 g fat
Sides: o Mexican Rice- 110 kcal, 3 g fat	Sides: o Nachos Supreme- 440 kcal, 26 g fat

Frozen Treats and Sweets

With the start of spring and hotter weather, it is hard to resist all of the frozen treats available at ice cream stores, coffee shops and in the frozen food section of the grocery store!

Here are some tips on what to select if you want to have a cool or frozen treat. For the I.D.E.A.L. study, please limit special frozen treats and other decadent desserts as much as you can (one or two times per week). Here are some general guidelines when selecting frozen desserts:

- Size matters! Portion ½ cup of ice cream into a dish or order a small portion of ice cream
- Keep it plain! Extra toppings, sauces and whip cream add up calories, fat and sugar fast!
- Plan in advance! Check out online nutrition information before you go out *(Most of the popular ice cream/coffee shops have their information online)*.
- Compare frozen treats at the grocery store using the nutrition facts label.
- Instead of going out for a treat, try making your own treat, e.g. a yummy smoothie, at home… a cheaper and more nutritious alternative!!

Choose This:	Not That:
o **Vanilla cone (small)** - 260 kcal, 7 g fat, 40 g CHO o **Strawberry or chocolate sundae (small)** – 300 kcal, 7 g fat, 58 g CHO o **Frozen sandwich** – 180 kcal, 5 g fat, 31 g CHO	o **Cookie Dough Blizzard (medium)** – 980 kcal, 36 g fat, 157 g CHO o **Peanut Buster Parfait** – 710 kcal, 31 g fat, 97 g CHO

Choose This:	Not That:
o **Iced sugar-free syrup flavoured Espresso (grande, 2% milk)** – 110 kcal, 4 g fat, 12 g CHO o **Iced Café Americano** – 15 kcal, 0 g fat, 3 g CHO	o **Iced Café Mocha with whip (grande, 2% milk)** – 330 kcal, 19 g fat, 38 g CHO o **Iced Tazo Green Tea Latte (grande, 2% milk)** – 290 kcal, 9 g fat, 44 g CHO

Choose This:	Not That:
o **Original Iced Cappuccino (regular)** – 90 kcal, 4.5 g fat, 10 g CHO	o **Blended Ice Cappuccino (regular)** – 340 kcal, 13 g fat, 50 g CHO

Choose This:	Not That:
o **Matcha Green Tea Chiller (no whip)** – 170 kcal, 3.5 g fat, 30 g CHO o **Mixed Berry Smoothie** – 140 kcal, 0 g fat, 36 g CHO	o **Frozen Hot Chocolate (no whip)** – 410 kcal, 5 g fat, 81 g CHO

Choose This:	Not That:
o **Vanilla cone** – 230 kcal, 6 g fat, 39 g CHO	o **Oreo McFlurry (small)** – 500 kcal, 17 g fat, 79 g CHO o **Triple Thick Chocolate Milkshake (small)** – 560 kcal, 14 g fat, 99 g CHO

Choose This:	Not That:
o **Original Chocolate Frosty (small)** – 320 kcal, 8 g fat, 52 g CHO o **Junior Original Chocolate Frosty (*just a tiny little treat!)*** – 160 kcal, 4 g fat, 26 g CHO	o **M&Ms Twisted Frosty** – 550 kcal, 19 g fat, 86 g CHO

A few more helpful notes:

- Read the nutrition facts table to compare similar products; compare calories, grams of fat and grams of sugar
- Select lower fat products and ones with less sugar (frozen yogurts and lower-fat ice cream)
- Portion size matters! Limit yourself to ½ cup of ice cream/frozen yogurt and place it in a dish instead of eating out of the container.
- Craving ice cream but none in the house? Make a delicious smoothie with frozen fruit, and/or yogurt/milk/real juice and ice cubes. Blend and enjoy!!!

Week 12 - 5 Tips on how to Banish Bad Habits

Adapted from: http://www.weightwatchers.com/util/art/index_art.aspx?tabnum=1&art_id=103781&sc=66

"I haven't conquered my procrastination problem yet, but just you wait."

http://www.cartoonstock.com/newscartoons/cartoonists/jha/lowres/jhan154l.jpg

1) ***Take it slowly.***
Old habits die hard. And the more years you've had yours, the more time you may need to give it up. So try not to do anything too suddenly. Though the cold turkey approach can sometimes work, it's usually only successful for a minority of people. For the majority, it's better to learn to live without your habit in stages.

Solution: Instead of trying to get rid of your habit, aim to indulge in it less often. Once you feel like you have more control, try cutting back even more. This habit-reduction method can be successfully applied to many kinds of habits. With time you'll be able to cut back more and more, and, eventually, stop altogether.

> e.g. *Eating too much after dinner and before bed* → try to minimize this to 3 times per week (at first) and consume foods that are not "bad for you" like fruit and vegetables, rice cakes or air popped popcorn.

2) ***Identify why you're hooked.***
What's driving your habit? Anxiety, boredom, frustration and/or depression are often at the heart of our most deep-seeded habits. If one particular emotion seems to chronically overwhelm you, maybe you should consider exploring why it's an issue and discussing some counter strategies.

Solution: Identify your triggers. These are the situations, places or feelings that cause you to eat poorly. Write them down in order from strongest to mild. Once you know why you are hooked, you can work on strategies to counter those triggers.

> e.g. *The WHY behind it all* → Need to get to the root of the problem. Try to give yourself "therapy" in other ways…. Shopping perhaps? Or better yet…. Exercise???

3) ***Avoid temptation.***
You know you have to avoid unhealthy foods. But every time you go for coffee with a friend or to the cafeteria for lunch, you can't seem to control yourself.

Solution: For the first few weeks, it is a good idea to avoid situations that you know will trigger your craving. This will give you a few weeks to build your confidence before you expose yourself to potentially risky situations.

> e.g. *When you just cant resist* → If you know that you can't resist eating donuts, croissants or ice cream when they are around, don't bring them home from the grocery store! Also, you should avoid all-you-can-eat buffets, dessert places, clubs and bars (for excess alcohol consumption). If you are going to a place where "bad foods" are, think ahead and plan what you are going to get (e.g. sneak a granola bar or a fruit [something healthier] to have with your coffee!).

4) ***Create new behaviour patterns***
Do you chew on your nails or reach for a cookie whenever you're feeling stressed? How about whenever you're bored?

Solution: The next time you catch yourself in the middle of this knee-jerk response, stop and do something else—count to 50, stretch your arms or flex your fingers. Have a small repertoire of behaviours you can use to replace the habit.

> - *Train your Brain!* → It is a challenge for sure. These are lifestyle changes that we are working towards. It is not going to happen overnight. As a first step, we must replace bad behaviour patterns with good ones (e.g. going for a walk, choosing vegetables and fruit instead of a cookie).

5) ***Be prepared for Relapse.***
Everything was going so well, but then you had a horrible day so you cut yourself some slack. Now you're slipping back into old patterns.

Solution: Don't go down the self-defeatist road of thinking, Oh well, I just can't kick it. Regard your relapse as one small step back and focus on the many forward steps you've taken. Now switch gears a bit and go into damage control. A relapse can be a momentary lapse or can be a return to your habit. As a strategy, we must be constructive and analyze the relapse, identifying why it happened, in what situation, how you were feeling and what you can do to prevent the same thing from happening again. Then renew your commitment – you are back on track!

- Relapses will happen from time to time. If they do, be prepared to accept the consequences (e.g. burning more calories at the gym the next day…). Be true to yourself – give yourself a fair chance to succeed!

WE BELIEVE IN YOU!!!!

The I.D.E.A.L. for Women Study Team

Week 14 - 2 weeks to go … YEY!!!!

http://www.offthemark.com

Throughout the study, you have been given a great deal of information to facilitate you in making healthy nutrition choices.

In these last 2 weeks, we want you to utilize what you've learned in the previous nutrition sessions. Take what you already know and put it all into action!!! It is important to us that you finish off with results that are in line with your expectations and ones that you can be proud of! ☺

Take a minute to write down **2-3 weakness points** you've encountered with regards to your diet, or even just **specific aspects** of your diet that you would like to pay particular attention to. Try to work extra hard on these as we approach the end of the study.

E.g. Situations that trigger food cravings for you, meeting recommended macronutrient ratios, reducing total calories, consuming adequate dietary fibre, avoiding high sugar and high fat foods

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As far as exercise is concerned, we really want you to push yourself for these last 2 weeks! You will feel an even greater sense of accomplishment if you put your mind to crossing that finish line with all you've got. Write down **2 or 3 goals** that you will work towards in the final two weeks of the study.

E.g. Reach at least 20 minutes of vigorous exercise every day, increase the weight you are lifting in the resistance exercises, do 15 EXTRA minutes of a cardio exercise that you don't normally do (running on the track, stairs, treadmill, etc.)

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You've come so far over the course of the study and should be **proud** of everything that **you've accomplished**. If you have any questions or if we can do anything to help motivate you in these final weeks, please don't hesitate to ask!!

2 weeks to go!!!!

Week 16 - Maintaining a Healthier Lifestyle

Adapted from: http://caloriecount.about.com/favorite-tips-weight-loss-coming-back-ft30986; http://www.guelphmercury.com/living/healthfitness/article/560307--keeping-those-pounds-off-forever;

Now that you've changed your diet and exercise habits, your efforts must **not** stop here! A healthy lifestyle requires daily physical activity, a nutritious diet, long-term commitment, and constant vigilance. Here are some helpful tips:

http://www.unicef.org/influenzaresources/files/cartoon_chicken(2).jpg

1. **Keep a brief food record of what you eat.** It is easy to slip back into old patterns without realizing it. If you get into the habit of recording what you eat, it forces you to be accountable and in control of the situation.

2. **Take the time to eat a healthy breakfast.** Skipping breakfast can easily lead to weight gain, since it leaves you hungry and makes you more likely to eat throughout the day.

3. **Don't keep comfort foods in the house.** Know your weaknesses and avoid situations that can trigger out-of-control eating for you. If you tend to eat high-fat, high-calorie foods when you're upset or bored, don't keep them around. Availability of food is one of the strongest factors in determining how much a person eats. If you do have these foods in the house, create obstacles to avoid consuming them (e.g. wrap and freeze them, making them less quickly accessible).

4. **Respect your appetite.** Eat slowly. It takes time for your body to register the feeling of fullness, so eating too quickly will make you eat more than your appetite desires. Also, try eating smaller portions, more often (i.e. five small meals a day rather than 3 large ones). This will keep your metabolism going and help you avoid unhealthy snacking between meals.

5. **Eat healthy foods first.** Eat foods that are healthy and low in calories first so that when it comes time to enjoy your favourite sweets or junk food, you won't be so hungry. Also, make an effort to try new foods. Healthy doesn't have to mean boring when it comes to food. Treat yourself to new cookbooks regularly and experiment with recipes that will make healthy eating more enjoyable and fun.

6. **Eat at home.** People eat more food in restaurants than at home. Limit how often you eat at restaurants. If you do eat out, decide what and how much you're going to eat before you start and have the rest boxed to go.

7. **Be realistic.** The important thing is to be true to yourself. Look at your weight in relation to your health and well being. Healthy weight is about eating sensibly, being physically active and feeling good – not about monitoring every bite. There is no such thing as the ideal weight, and healthy weight varies from one person to the next depending on body size and bone structure.

8. **If you do slip, don't waste time with self-blame.** Learn from the experience and move on. If you've gained a couple of pounds or have skipped out on a few exercise sessions, make the changes today that will motivate you to get back on track.

9. **Reward yourself.** Losing weight and adopting a healthier lifestyle is a major accomplishment. Celebrate your success with non-food rewards, such as: shopping for new clothes, attending a sporting event, going to a movie, exercising, listening to music, taking a bubble bath, or vacationing.

10. **Continue to make exercise part of your everyday routine**. One of the most important things you can do for weight maintenance is to continue a vigorous exercise program. Studies suggest that it takes 30 to 60 minutes of daily moderate intensity physical activity to maintain weight loss. If you are short on time, try taking the stairs, getting off the bus one stop early, or parking your car far from the entrance. Mix it up, but make sure to keep exercise in your daily routine.

11. **Get enough sleep**. Adequate sleep is vital to good health. While researchers are studying the effects that sleep has on hormones -- and consequently on hunger and metabolism -- the reasoning is more or less common sense. When you're tired, you're more likely to reach for quick sources of energy (sugar and other refined carbohydrates) and make unhealthy eating decisions, like choosing the drive-through over a home-cooked meal. You are also less likely to exercise because you don't have enough energy. Make sure to get enough sleep and you'll be helping your health in more ways than one.

If you really want to adopt a healthy lifestyle that can be maintained, the best approach is to focus on small changes. Make a conscious effort to exercise regularly (include both cardio and resistance training) and develop an eating plan that's enjoyable, yet healthy and low in calories. This approach will result in changes that you can live with!

CONGRATULATIONS and GOOD LUCK!!!!

6.2. APPENDIX 2 – Aerobic and Resistance Exercise Logs

Aerobic Exercise Training Log

Subject Code: _____

Date and Time	What was done, for how long?
e.g. Thursday Jan. 1, 2010	Elliptical machine – 20 min Walking on track - 25 min
Monday _____, 2010	
Tuesday _____, 2010	
Wednesday _____, 2010	
Thursday _____, 2010	
Friday _____, 2010	
Saturday _____, 2010	
Sunday _____, 2010	
Monday _____, 2010	
Tuesday _____, 2010	
Wednesday _____, 2010	
Thursday _____, 2010	
Friday _____, 2010	
Saturday _____, 2010	
Sunday _____, 2010	

Resistance Exercise Training Log

Subject Code:	**Weeks 1/5/9/12**	**Weeks 2/6/10/13**	**Weeks 3/7/11/15**	**Weeks 4/8/12/16**
Lower Body Exercises	Once every week in The Pulse			
Date				
Name of Trainer				
Leg Extension *Back: ___ Legs: ___*				
Hamstring Curl *Back: ___ Legs: ___*				
Glute Isolator *Chest: ___*				
Leg Press *Seat: ___*				
Abductor				
Adductor *Seat: ___*				
Upper Body Exercises	Once every week in room 230			
Date				
Name of Trainer				
Seated Row *Seat: ___ Chest: ___*				
Chest Press *Bench: ___*				
Lat Pull-Down *Seat: ___ Legs: ___*				
Tricep Pull-Down *Height: ___*				
Bicep Curl (free weights)				

6.3. APPENDIX 3 – Food Record Templates (Control[1], DairyPro[2] and HiDairyPro[3])

Please return to
I.D.E.A.L. office by:
_____

RECORD OF FOOD INTAKE [1]

Subject Code: _____ Date: _____ (Study week ____)

Day of Week (circle): Mon Tue Wed Thu Fri Sat Sun

Meal/Time	Food consumed	Amount	Notes

❑ ❑ 2 Study Drinks

For Office Use Only

Initials of who Entered FR ____

Initials who Proofread FR ____

Please return to
I.D.E.A.L. office by:
_____

RECORD OF FOOD INTAKE [2]

Name: _____ Date: _____ (week ____)

Day of Week (circle): Mon Tue Wed Thu Fri Sat Sun

Meal/Time	Food consumed	Amount	Notes

❑ ❑ 2 Study Drinks
❑ ❑ 2 Yogurts

For Office Use Only

Initials of who Entered FR ____

Initials who Proofread FR ____

Please return to
I.D.E.A.L. office by:
_____

RECORD OF FOOD INTAKE [3]

Name: _____ Date: _____ (week ____)

Day of Week (circle): Mon Tue Wed Thu Fri Sat Sun

Meal/Time	Food consumed	Amount	Notes

❑ ❑ 2 Study Drinks

❑ ❑ ❑ ❑ 4 Yogurts

❑ ❑ 2 Study Cheeses

❑ 1 White Milk

For Office Use Only

Initials of who Entered FR ____

Initials who Proofread FR ____

6.4. APPENDIX 4 – End-of-Study Questionnaire

Dear I.D.E.A.L. for Women study Participant,

Congratulations! You have just completed the I.D.E.A.L. (Improving Diet, Exercise and Lifestyle) for Women study. We hope you are pleased with your efforts in making a true lifestyle change over the past 4 months. We commend you on a job well done!

We hope that through this intensive diet and exercise research study we have equipped you with knowledge, motivation to continue and the means for application of daily exercise routines and healthy nutritional strategies. In terms of your exercise, you have had the chance to use different cardio and weight machines, the great outdoors and participate in team sports, and have learned appropriate strategies with respect to intensity, duration and frequency of activity that will help you achieve your goals. With nutrition, we believe education is of utmost importance. Now that you know more about what to eat and what foods to avoid, and you understand macronutrient (protein, carbohydrate and fat) balance and meal timing, we are confident that you can continue these healthy eating patterns to produce the long-term results you are looking for. We strongly encourage you to continue with these positive changes and to continue to find out what works best for you. Furthermore, use your participation in this study as a stepping stone to making your health and well-being a top priority from now on.

We enjoyed having you in our study and hope that this has been a positive experience for you. As you embark on your own healthy lifestyle journey, please do not hesitate to call/email us with any questions you may have – remember, you have been a tremendous help to us through your dedication and hard work over the last 4 months, so it is the least we can do to help you!

Sincerely,

The I.D.E.A.L. for Women Study Team

End-of-Study Questionnaire

This questionnaire is intended to be **completely anonymous.** We ask that you please be as honest and truthful as possible with your answers to the following questions. We also ask for you to please give us any general feedback you may have about the study. We certainly value your opinion and we hope to implement your suggestions to improve the study for future participants.

Thank you, we really appreciate you taking the time to do this for us.

Please **return the survey only** in the envelope provided to the I.D.E.A.L. For Women study drop-box located in the Ivor Wynne Centre next to the Study Food Room.

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1) For the questions below, please rank your **compliance** or **satisfaction** with the advice given on a scale from 1-9; 1 being non-compliant or not satisfied and 9 being 100% compliant or satisfied. Also, please feel free to add any comment you may have regarding the questions.

(0%) Never	Almost Never	Seldom		50%		usually	almost always	(100%) always
1	2	3	4	5	6	7	8	9

a) *Please rank your **compliance** with the OVERALL dietary advice.* I.e. how well did you follow the nutrition advice provided to you? (While answering this question, please think about your macronutrient intakes (protein, carbohydrate and fat); your overall calorie intake; your intake of higher fat higher sugar foods or high fibre lower fat foods; eating out at restaurants or cooking healthily at home; eating vegetables and fruits or less healthy snacks [pastries, donuts, etc.]).

1 2 3 4 5 6 7 8 9

b) *Please rank your **compliance** with the daily intake of your respective study supplement(s).* Did you have all (drinks and/or food) you were supposed to have every day?

1 2 3 4 5 6 7 8 9

c) *Please rank your overall **satisfaction** with the dietary advice provided to you.* Were you pleased with what was suggested? Did you learn some valuable nutrition information?

1 2 3 4 5 6 7 8 9

d) *Please rank your overall **compliance** with the exercise portion (resistance and aerobic) of the study*. I.e. did you exercise 5 d/wk with us and on the weekends on your own? Did you do resistance training 2x/wk? Did you record your exercise on a daily basis?

1 2 3 4 5 6 7 8 9

e) *Please rank your overall **satisfaction** with the aerobic (cardio) exercise portion of the study.* Were you pleased with the daily exercise? Were you satisfied with the choice of exercise modalities available (Pulse, Track, rm 230 [I.D.E.A.L. office gym])?

1 2 3 4 5 6 7 8 9

f) *Please rank your overall **satisfaction** with the resistance exercise portion of the study.* Did you like the resistance exercise component?

1 2 3 4 5 6 7 8 9

2) Please rank the **effectiveness** of the following equipment and materials used or provided to you during the study:

	Poor				Excellent
a) The biweekly nutrition handouts	NA 1	2	3	4	5
b) The use of the biweekly food records	NA 1	2	3	4	5
c) The BodyMedia devices	NA 1	2	3	4	5
c) IPOD Shuffle (if received one)	NA 1	2	3	4	5
d) Gym membership (at the Pulse)	NA 1	2	3	4	5
e) Use of equipment in rm. 230	NA 1	2	3	4	5

3) We know that recording food intake (i.e. filling out food records) can be tedious, but we believe that it facilitates "better" and more "conscious" eating. Please comment on whether you welcome the idea of recording your food intake during the study every day or for a whole week (every other week) as opposed to biweekly for 3-days. In terms of analysis, we would choose 3 days at random. Do you think this would help improve dietary compliance?

_____

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_____

4) Please provide us with any additional comments regarding your time in the I.D.E.A.L. Study and/or suggestions to implement for future cohorts? We really appreciate your input.

_____
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_____

Please put the survey pages in the envelope provided and drop it off in the **study drop-box** outside the I.D.E.A.L. for Women study food room on the Mezzanine level of the Ivor Wynne Centre.

Thank you for taking the time to fill out this survey.

Yours in Health and lifestyle,
The I.D.E.A.L. For Women Study Team

6.5. APPENDIX 5 – Poster presentations of I.D.E.A.L. for Women study data

2 posters:

1) **Multimodal Measurement of Body Composition Change with Diet- and Exercise-Induced Weight Loss in Obese Women.** Presented at the Canadian Obesity Network's Biannual meeting: The National Obesity Summit, Montreal QC, May 2011.

2) **Diets higher in dairy and total dietary protein during diet- and exercise-induced weight loss preserve bone health in overweight and obese young women.** Presented at the Canadian Nutrition Society (CNS) Annual Meeting, Guelph ON, June 2011.

Multimodal Measurement of Body Composition Change with Diet- and Exercise-Induced Weight Loss in Obese Women

Andrea R Josse[1], Stephanie A Atkinson[2], Mark A Tarnopolsky[3], Hormoz Azizian[4], John KG Kramer[5] and Stuart M Phillips[1]

[1]Department of Kinesiology, McMaster University, Hamilton, ON; [2]Pediatrics and [3]Medicine, McMaster University Medical Centre, Hamilton, ON; [4]NIR Technologies, Oakville, ON; [5]Agriculture and Agri-Food Canada (retired), Guelph, ON.

ABSTRACT

INTRODUCTION: Using changes in BMI or bodyweight to classify the success of any anti-obesity intervention has inherent flaws. Measuring changes in body composition would be more clinically meaningful since a weight loss profile characterized by the loss of fat mass and maintenance/gain of muscle mass would be associated with greater reductions in disease risk. **METHODS:** We assessed how daily exercise and different hypoenergetic diets varying in dairy (0-1, 2-4, 6-7 servings/d), total protein (% energy intake: 15, 15, 30) and dairy source calcium (~300, ~1000, ~1700 mg), affected changes in the tissue composition of weight loss in 90 premenopausal, obese women over 16 wk. We compared 3 different modalities: DXA, MRI, and Fourier Transform Near-Infrared Spectroscopy (FT-NIR), to assess body composition changes before and after a diet- and exercise-based intervention. **RESULTS:** We observed comparable weight loss across all 3 study groups, however, subjects who consumed higher protein (30%) and high calcium showed the greatest reduction in total and visceral/abdominal fat mass, and a gain in lean mass. FT-NIR and DXA measures of body fat (kg) were highly correlated (r=0.95, p<0.001), as were direct measures of visceral fat volume by MRI and total abdominal fat by DXA (r=0.76, P<0.001). **CONCLUSIONS:** All three modalities provided comparable results in tracking changes in various components of body fat in young, obese women on a weight loss program. DXA is a more practical and accessible modality than the gold-standard MRI for measuring visceral fat mass, and FT-NIR, while still fairly new, is a cheaper and far more accessible method of assessing fat mass loss that is comparable to DXA. Thus, measuring body composition to monitor progress during weight loss interventions can be done using a variety of modalities, but FT-NIR is particularly suited to large scale clinical trials.

INTRODUCTION

- Body mass index (BMI) is the most common variable calculated and reported as an estimate of overweight and obesity (1–3). however, it is body fat, and not body weight that is the primary physiological risk factor for morbidity and mortality (4).
- Reductions in body weight or BMI following weight loss is the standard measure of success. It provides a certain indication of the program's effectiveness, but no details about the composition of the weight lost (fat vs. muscle loss), which should be of great clinical concern.
- Thus, it is important to find other quick, easy and economical ways to accurately assess fat mass loss and hence also disease risk reduction in both the research and clinical setting.

Several methods are available for measuring body composition (5):

- Dual energy x-ray absorptiometry (DXA), used most often in research, is accurate, reliable and easy to use, but its disadvantages relate to low-level radiation exposure, cost and non-portability (6).
- Magnetic resonance imaging (MRI) is considered the gold standard, however it is costly, non-portable, relatively inaccessible for research and requires trained technicians to conduct scans (6).
- Fourier-Transform Near Infrared Spectroscopy (FT-NIR) is a new modality to rapidly and non-invasively measure body fat content (7, 8). It is low cost and portable, but its measures have yet to be evaluated against DXA or MRI in the same subjects.

OBJECTIVES

To measure changes in body composition (fat mass) after a 16-week, hypoenergetic, diet and exercise-induced weight loss intervention using different gold-standard and new methodologies, and to assess how these measures relate to each other.

To validate the accuracy of FT-NIR measures of fat mass by comparing them with fat mass measures from DXA taken in the same subjects at the same time.

METHODS

***I.D.E.A.L. for Women* Study**: 16-week hypoenergetic diet- and exercise-induced weight loss intervention in 90 overweight and obese premenopausal women.

✓BMI 27-40 kg/m²; age 19-45 years; non-smokers; infrequent exercisers; low dairy consumers; otherwise healthy

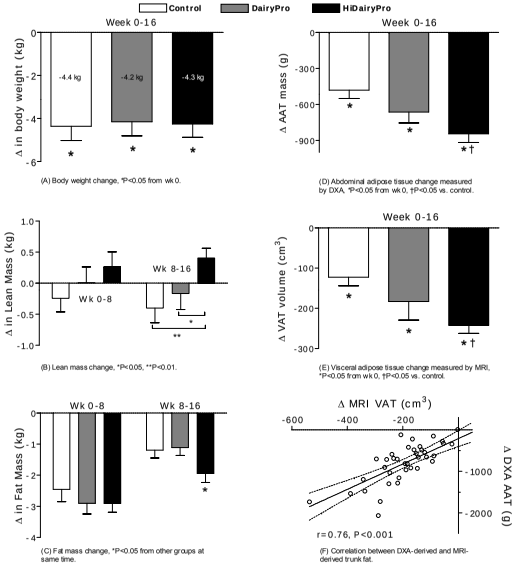
✓3 treatment groups: **Control** (0-1 svg dairy/d, 15% E from protein, 300 mg Ca^{2+}/d); **DairyPro** (2-4 svgs dairy/d, 15% E from protein, 1000 mg Ca^{2+}/d); **HiDairyPro** (6-7 svgs dairy/d, 30% E from protein, 1700 mg Ca^{2+}/d).

✓**DXA (Discovery QDR-4500A, Hologic Inc., Waltham, MA):** Whole body scans at wks 0, 8, 16; regional trunk fat analysis (Region of Interest: L1-L4), wks 0 and 16.

✓**MRI (3T Signa Scanner, GE Healthcare, Waukesha, WI):** Torso regional scans only (from top of the liver to the iliac crest) wks 0 and 16. 3 separate scans of 11 slices each.

✓**FT-NIR (matrix-F Spectrophotometer, Fibre-Optic Probe by Bruker Optics, Milton, ON):** Measurements of fat mass taken from the back of the upper ear (cartilaginous part) with laser light from a fibre-optic probe. Timing paralleled that of the DXA scans (wks 0, 8, 16).

RESULTS: Body Composition; DXA vs. MRI



(A) Body weight change, *P<0.05 from wk 0.

(D) Abdominal adipose tissue change measured by DXA, *P<0.05 from wk 0, †P<0.05 vs. control.

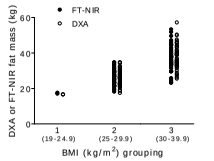
(B) Lean mass change, *P<0.05, **P<0.01.

(E) Visceral adipose tissue change measured by MRI, *P<0.05 from wk 0, †P<0.05 vs. control.

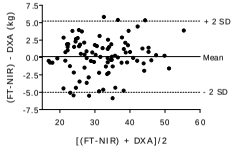
(C) Fat mass change, *P<0.05 from other groups at same time.

(F) Correlation between DXA-derived and MRI-derived trunk fat.

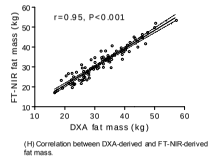
RESULTS: DXA vs. FT-NIR



(G) Relationship between body weight and body fat content measured by DXA and FT-NIR based on BMI subgroups.



(I) Bland-Altman analysis showing the mean ± 2 SD of the two methods used to measure body fat; DXA and FT-NIR spectroscopy. Measurement bias was low expressed as the mean difference between the two measures ((FT-NIR) – DXA) = 0.13 ± 2.6 kg.



(H) Correlation between DXA-derived and FT-NIR-derived fat mass.

(J) Picture of someone being scanned with the probe at the upper ear. Each scan takes ~5 seconds to complete.

CONCLUSIONS

✓ All treatment groups lost similar amounts of body weight. The HiDairyPro lost more total and visceral/trunk fat and gained lean mass, whereas the control group lost the least amount of visceral/trunk fat and the most lean mass.

✓ DXA-measured trunk fat (g) correlated significantly with gold-standard MRI-measured visceral fat (cm^3). Therefore, in this population, DXA, a more accessible and less costly modality, provided a reasonable proxy measure for changes in visceral fat.

✓ FT-NIR provided an accurate measure of body fat content that correlated significantly with DXA-measured body fat (kg). The agreement between the two methods was good with little measurement bias. Thus, FT-NIR spectroscopy can be used to provide safe and accurate single or repeated measures of body fat content to help monitor obesity in a clinical setting or for those participating in weight loss programs.

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- Data was collected in collaboration with NIR Technologies Inc.

Diets higher in dairy and total dietary protein during diet- and exercise-induced weight loss preserve bone health in overweight and obese young women

Andrea R Josse[1], Stephanie A Atkinson[2], Mark A Tarnopolsky[1,3] and Stuart M Phillips[1]

[1]Department of Kinesiology, McMaster University, Hamilton, ON; [2]Pediatrics and [3]Medicine, McMaster University Medical Centre, Hamilton, ON.

ABSTRACT

Improving and maintaining bone mass in young adulthood is important; however, many lifestyle practices including inactivity, low dairy, calcium and protein consumption, and weight loss through energy restriction alone have been shown to negatively affect bone health. The purpose of this study was to determine how increasing intakes of dairy and dietary protein on a background of diet- and exercise-induced weight loss for 16wk affects bone health and vitamin D status in otherwise healthy, pre-menopausal, overweight and obese women. Ninety subjects were randomized to 3 groups (n=30/group): HiDairyPro (HDP), DairyPro (DP) and Control (CON), differing in the quantity of total protein consumed (30%, 15% or 15% of energy, respectively) and the amount of dairy (high, moderate or low, respectively). All groups performed daily (7d/wk) aerobic exercise and 2d/wk resistance exercise during the 16wk intervention. BMD and BMC were measured by DXA at 0, 8 and 16wk. Serum and urine bone turnover biomarkers, serum parathyroid hormone (PTH) and 25-hydroxyvitamin D were measured at 0 and 16wk. All treatment groups lost similar amounts of body weight (P<0.05). NTX, CTX, uDPD and osteocalcin increased in CON (P<0.01), whereas in HDP, only osteocalcin increased (P<0.05), and all resorption markers remained unchanged. NTX was slightly elevated in DP (P<0.05). PTH was significantly decreased in HDP and DP versus CON (P<0.005), and 25-hydroxyvitamin D increased in HDP (P<0.05), remained unchanged in DP and decreased in CON (P<0.05). No changes were detected in BMD or BMC. In summary, these data show that hypoenergetic diets higher in dairy and total dietary protein with daily exercise, favourably affected markers of bone turnover, PTH and 25-hydroxyvitamin D levels versus diets without these bone-supporting nutrients.

INTRODUCTION

- Peak bone mass is accrued by the end of adolescence with final consolidation achieved between the 2nd and 3rd decade of life (1, 2).
- Efforts to maximize the accretion of bone by early adulthood are crucial since a higher peak bone mass may help to delay the decline in bone health status with age and lessen the burden of diseases like osteoporosis later in life (3, 4).
- Dairy foods are excellent sources of calcium, vitamin D and high quality protein. These key nutrients impact bone structure and strength either by influencing bone mineralization or collagen formation (2, 5).
- Bone turnover is assessed by measuring markers of bone formation and resorption. It is a necessary process that, when balanced, acts to maintain the structural integrity and strength of bone during adulthood (6). Weight loss is associated with increased turnover, however, several studies have shown this not to be the case if calcium intakes during energy restriction are sufficient (7, 8).
- Bone biomarkers such as the osteoprotegerin and receptor activator of nuclear factor kappa-B ligand (OPG/RANKL) system (9) may add new insights into how bone responds to diet- and exercise-induced weight loss with and without dairy foods.

OBJECTIVE

To assess whether higher intakes of dietary protein, calcium and vitamin D (mostly from dairy foods) would positively affect bone health status (i.e. markers of bone turnover, parathyroid hormone levels, vitamin D status and a proxy marker of osteoclast activity) in overweight and obese young women participating in a 16 week, hypoenergetic diet- and exercise-induced weight loss program.

METHODS

***I.D.E.A.L. for Women* Study**: 16 week hypoenergetic diet- and exercise-induced weight loss intervention in 90 overweight and obese premenopausal women.

✓BMI 27-40 kg/m^2; age 19-45 years; non-smokers; infrequent exercisers; low dairy consumers; otherwise healthy

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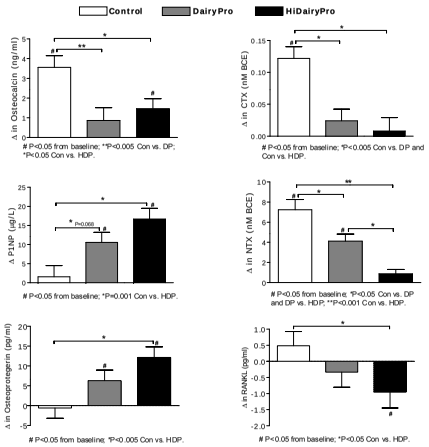
✓Aerobic exercise 5d/wk with us, 2d/wk on own and supervised resistance exercise 2d/wk.

RESULTS

Table 1: Dietary intakes during the study

Dietary Variable	Control	DairyPro	HiDairyPro
Energy intake	1322±197^a	1425±229^{ab}	1497±185^b
Energy restriction achieved	-498±203^a	-477±231^a	-435±194^a
% Protein	16±3^a	18±2^b	28±2^c
Protein (g)	55±7^{a*}	66±12^b	108±16^c
Protein (g/kg/d)	0.72±0.1^a	0.84±0.1^b	1.33±0.2^c
% Fat	28±5^a	24±4^b	31±3^c
% Carbohydrate	56±6^a	58±4^a	41±4^b
Dietary Fibre (g)	20.5±7^a	17.5±6^a	16.2±5^a
Calcium (mg)	299±109^a	1199±103^b	1835±70^c
Vitamin D (IU)	26±21^a	391±54^b	529±38^c

Means with different letters are different from each other, P<0.05.



# P<0.05 from baseline; *P<0.005 Con vs. DP; *P<0.05 Con vs. HDP.

# P<0.05 from baseline; *P<0.005 Con vs. DP and Con vs. HDP.

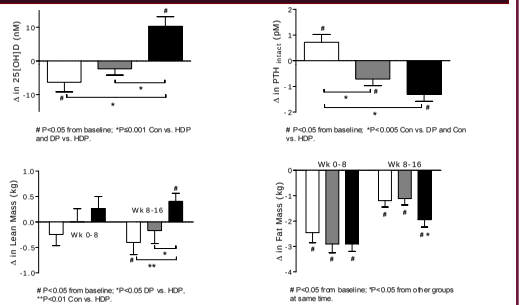
# P<0.05 from baseline; *P<0.001 Con vs. HDP.

# P<0.05 from baseline; *P<0.05 Con vs. DP and DP vs. HDP; **P<0.001 Con vs. HDP.

# P<0.05 from baseline; *P<0.005 Con vs. HDP.

# P<0.05 from baseline; *P<0.05 Con vs. HDP.

RESULTS



# P<0.05 from baseline; *P≤0.001 Con vs. HDP and DP vs. HDP.

# P<0.05 from baseline; *P<0.005 Con vs. DP and Con vs. HDP.

# P<0.05 from baseline; *P<0.05 DP vs. HDP, **P<0.01 Con vs. HDP.

# P<0.05 from baseline; *P<0.05 from other groups at same time.

CONCLUSIONS

✓ This study demonstrates the importance of diet composition to the maintenance of bone health status during diet- and exercise-induced weight loss.

✓ Consuming diets adequate or higher in bone supporting nutrients (calcium, vitamin D and protein) primarily from dairy positively affected markers of bone turnover favouring formation, decreased circulating PTH, increased 25(OH)D, and potentially reduced osteoclast formation and function in young women who lost weight through a hypoenergetic diet- and exercise-induced weight loss program.

✓ The higher OPG (and OPG:RANKL ratio; not shown) in the HiDairyPro group reflects a decrease in the differentiation and action of osteoclasts, indicating a protective effect for bone (9).

✓ Our data provide good reason to recommend the consumption of dairy foods to not only to aid in high-quality weight loss but also for the promotion of bone health in women who are at the age of peak bone mass accrual and consolidation (1, 2) and in whom adequate dairy consumption would offset sub-optimal intakes of calcium and vitamin D as is often observed (10, 11).

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