EFFECTS OF PLANT STEROLS AND EXERCISE TRAINING ON APOLIPOPROTEIN A AND B, ADIPONECTIN, GROWTH HORMONE AND GHRELIN IN HYPERCHOLESTEROLEMIC SEDENTARY ADULTS

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ABSTRACT

Plant sterols (PS) lower total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and inflammatory markers, and decrease risk of atherosclerotic cardiovascular disease (CVD). Exercise increases high density lipoprotein cholesterol (HDL-C) levels and decreases triglycerides (TG) and inflammation, also reducing the risk of CVD. The study objective was to investigate the combined effects of PS and exercise on apolipoproteins (apo) A and B, adiponectin, growth hormone (GH) and ghrelin, in context of previously obtained lipid data. In an 8-wk, placebo-controlled, parallel-arm clinical trial, 84 subjects were randomly assigned to: 1) combination of PS and exercise, 2) exercise, 3) PS, or 4) control group. PS increased (P=0.04) adiponectin values by 15%. ApoA was associated with HDL and apoB with LDL values at baseline. ApoA %change was correlated to HDL %change in the exercise group. ApoB, GH and ghrelin were unchanged. The capability of PS to increase adiponectin values reinforce their role in preventing inflammation, atherosclerosis, and CVD.

RÉSUMÉ

Les stérols de plantes (SP) réduisent le cholestérol total (CT), le cholestérol de lipoprotéines de faible densité (LDL) et des cytokines inflammatoires, et diminuent le risque de l'athérosclérose et de maladie cardiovasculaire (MCV). L'exercice physique augmente le cholestérol de lipoprotéines de haute densité (HDL) et diminue les triglycérides. L'objet de cette étude était d'investiguer les effets de la combinaison des SP et de l'exercice sur les apolipoprotéines (apo) A et B, l'adiponectine, l'hormone de croissance (HC) et la ghreline, qui n'a pas encore été investigué, et de regarder ces mesures en contexte des mesures de lipides qui sont déjà obtenus. L'étude était en design de bras parallèles, et contrôlée avec un placebo de durée de 8 semaines. Quatre-vingt quatre sujets ont été mis au hasard dans un des 4 groupes d'intervention : 1) combinaison de SP et exercice, 2) exercice, 3) SP, ou 4) contrôle. Une augmentation (P=0.04) de l'adiponectine jusqu'à 15% a été attribué aux SP. L'apoA était associé au valeurs de HDL et l'apoB aux LDLs à l'état de base. Le changement dans les valeurs de apoA était relié au changement vu dans les HDL pour le groupe de l'exercice seulement. Les valeurs de apoB, HC et la ghreline n'ont pas changé avec aucun des interventions. La capacité des SP à augmenter l'adiponectine supporte un rôle anti-inflammatoire des SP, qui diminue l'athérosclérose et le risque de MCV.

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CONTRIBUTION OF AUTHORS

The manuscript included as part of this thesis is entitled "The effects of plant sterols and exercise training on apolipoproteins A and B, adiponectin, growth hormone and ghrelin in hypercholesterolemic sedentary adults" and reports the experimental part of the thesis. Contribution of authors to this manuscript follows.

As the first author, I was responsible for writing and formatting this manuscript in addition to creating tables and figures. I was responsible in part for the design of the clinical trial, which was entirely executed by Krista Varady, who also collected and analyzed all the lipid data. I was responsible for all of the laboratory analyses of adiponectin, growth hormone and ghrelin. In addition, I was responsible for all statistical analyses of apoA, apoB, adiponectin, growth hormone and ghrelin values.

Peter Jones provided editorial assistance with the manuscript. As my co-supervisor and principal investigator of the project, Peter Jones provided direction to the study.

1. INTRODUCTION

Cardiovascular disease is a leading cause of morbidity and mortality in our society with increasingly large numbers of people being affected. Certain lifestyle factors have been highlighted in the pathogenesis of the disease. A high fat diet and sedentary lifestyle are two of the main contributors and can lead directly to obesity and an unfavorably altered lipid profile, known as dyslipidemia.

Plant sterols (PS) have been shown in the literature to decrease total and LDL cholesterol (7-12). Because of their structural similarity to cholesterol, PS inhibit absorption of cholesterol at the level of the intestine. Supplementation of approximately 2g/day of plant sterols incorporated into a typical diet has been shown to decrease total and LDL cholesterol by up to 13% and 24% respectively (7) and to decrease apoB up to 14% (8). Previous studies show that plant sterols decrease inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) and increase anti-inflammatory IL-10 in an animal model (19). In humans, it has been shown that a dietary portfolio of cholesterol lowering foods are effective in lowering C reactive protein (CRP), an inflammatory marker (20). However, the effects of PS on other markers of inflammation have not been investigated in humans.

The main effects of exercise training are well established in the literature and include increases in HDL and decreased triglycerides (TG)(28,29) as well as decreasing percent body fat. Endurance training without weight loss is associated with increases in HDL of 10-21% and apoA1 9-36% as well as decreases in TG of 7% (29,30). These effects are associated with a decreased catabolic rate of HDL and apoA1 as well as an increased synthesis of apoA1 up to13% (30). Also, an increase in

pre-beta HDL therefore an up-regulation of reverse cholesterol transport (RCT) have been highlighted (21).

Adipose tissue is now recognized as an endocrine organ that secretes a variety of physiologically active peptides known as adipocytokines, including adiponectin. Adiponectin has been shown to have insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. In the unhealthy state, secretion of adiponectin is inhibited and secretion of the other inflammatory peptides including IL-6 and TNFα is favored. Adiponectin is decreased in obesity, atherosclerosis and diabetes. Adiponectin is positively associated with HDL and apoA and negatively correlated to adiposity, insulin sensitivity and IL-6 (54). Adiponectin has been shown to increase with weight loss (54) and with exercise training in association with weight loss (57, 61) and without (59). Sustained weight loss and increased physical activity causes decreased IL-6, IL-18 and CRP as well as increased adiponectin concentrations (68).

Growth hormone (GH) is inversely associated with abdominal visceral fat, which seems to be the primary determinant of its secretion (87). GH decreases with age, possibly due to increased fat mass and decreased lean body mass which may contribute to the unfavorable lipid profile seen with increasing age. The secretion of GH is regulated positively by growth hormone releasing hormone (GHRH) and insulin-like growth factor (IGF-1) and negatively by somatostatin. GH has been shown to increase with exercise training (88,89,90,91). Growth hormone also has direct effects on lipid metabolism and stimulates lipolysis and lipid oxidation (86,87). The impact of GH on substrate metabolism includes increased free fatty acid (FFA) turnover (88). Also, GH has been shown to increase the number of hepatic LDL

receptors thereby increasing the clearance of LDL and VLDL-apoB from the circulation (86).

Ghrelin is a gut-derived peptide that exerts its effects at the level of the hypothalamus, playing an important role in energy homeostasis. Ghrelin stimulates appetite and then decreases post-prandially. Ghrelin is decreased in obesity and increased in starvation. It is a potent stimulator of GH release although whether or not ghrelin is responsible for the GH increase in response to exercise is not yet clear. In most studies it has been shown that despite increases in GH, ghrelin levels did not change (88, 91). However, some trials still question the involvement of ghrelin (89, 90) in the exercise-induced increase in GH. Ghrelin has been found to increase with exercise training alone (84) or with exercise and dieting (82). The changes in ghrelin observed are strongly correlated to weight loss attained via these lifestyle interventions.

Plant sterols exert their effects mainly by decreasing TC and LDL-apoB and also play a role in decreasing inflammation and atherogenicity. Exercise exerts its effects in several different ways. Firstly, by increasing HDL-apoA1 and RCT. Secondly, by decreasing TG and adiposity, thereby potentially affecting a) the adipocyte and down-regulating secretion of inflammatory cytokines and increasing adiponectin secretion and b) secretion of GH by decreasing AVF. Thirdly, by directly increasing GH secretion which has direct effects on lipid metabolism. It is hypothesized that lifestyle modifications including plant sterols and or exercise training can favorably alter the lipid profile and the hormones aforementioned, possibly in a synergistic manner, in such a way as to decrease the risk of CVD.

2. LITERATURE REVIEW

2.1. Dyslipidemia and cholesterol metabolism

Dyslipidemia is characterized by increased total and LDL cholesterol, as well as increased triglycerides and decreased HDL-C levels. Increased circulating LDL particles, which are small enough to pass through the vascular endothelium and enter the intima of the vascular wall where, under oxidative stress, can cause the initial lesion which will later become the atherosclerotic plaque. ApoB is an apolipoprotein associated with LDL cholesterol. ApoB represents the number of LDL particles, therefore an important marker for atherogenicity.

HDL acts with apoA lipoprotein in order to transport circulating cholesterol fragments back to the liver in a process known as reverse cholesterol transport (RCT). RCT and HDL are protective against high circulating levels of cholesterol. Direct evidence of the anti-atherogenic effects of HDL has been obtained in studies of the over- or under-expression of apoA1 using genetic animal models of RCT (1). The anti-atherogenic effects of HDL are also partly due to its protective effects against LDL oxidation through antioxidant enzymes and proteins (2). High levels of LDL are predictive of coronary artery disease (CAD) and even more so when levels of HDL are low, indicating that HDL levels modify the relationship between LDL and CAD (1). HDL has also been shown to modify the endothelial reactivity and exert anti-inflammatory activity by modulating expression of adhesion molecules (2).

Therefore, the increase in LDL and decrease in HDL in dyslipidemia is highly predictive of cardiovascular disease (CVD). The modifiable risk factors associated with CVD including a high fat diet and a sedentary lifestyle affect the body via

increasing circulating lipids and creating an inflammatory state, which are the two important ingredients in creating atherosclerosis. Modification of diet, including supplementation with plant sterols can decrease the risk of CVD by decreasing circulating lipids as well as decreasing inflammation.

2.2 Plant sterols

2.2.1. Plant sterols: cholesterol lowering effects

Plant sterols are structurally similar to cholesterol and because of this fact, decrease the incorporation of dietary and biliary cholesterol into micelles which lowers cholesterol absorption (3). Plant sterols and stanols are found in all plant foods and natural dietary intake varies from about 167-437mg/d (3). It has been shown that both plant sterol and stanol esters reduce cholesterol absorption by approximately 39%, when consumed blended in butter as part of a high fat high cholesterol diet (4). Similar results have been obtained with consumption of plant sterol and stanol margarines as part of a controlled diet (5). This reduction in cholesterol absorption seems to stimulate cholesterol synthesis and increased LDL receptor activity (6). It has been shown that both plant sterol and stanol esters increase cholesterol synthesis by about 53 and 38%, respectively, compared to a control diet (5). However, this increase in synthesis does not seem to compensate completely for the decrease in absorption and hence creates the reduced circulating levels of cholesterol.

Plant sterols have consistently been shown in the literature over the past 15 years to decrease total and LDL cholesterol levels in blood. Supplementation of approximately 2g/d of plant sterols incorporated into a typical diet has been shown to

decrease total and LDL cholesterol by up to 13% and 16% respectively (7). Looking at varying doses of plant sterols, it was shown that 0.8g/d decreased LDL by only 1.6% but decreased apoB by 8.7%. Using increased doses of 1.6g/d and up to 2.4 - 3.2g/d, reductions in LDL increased to 6.1% and 10.6-11.5%, respectively, as well as further lowering of apoB up to 14% (8). A 4-week plant stanol supplementation trial of 2g/d in a Japanese population produced significant reductions in plasma TC, LDL, apoB and oxidatively modified LDL (Ox-LDL) of 6.5%, 9.6%, 8.3% and 20%, respectively (9). Similar results were found in a 4 week trial in a Belgian population consuming 2g/d of plant sterols. TC and LDL-C were lowered 7% and 10%, respectively. In the same trial, apoB was decreased by 8%, while HDL-C and apoA-1 concentrations did not change (10). ApoB concentrations lowered by phytosterol consumption (8,10) indicate that plant sterols are able to decrease the number of atherogenic particles in circulation.

A meta-analysis of 18 clinical trials showed that plant sterol consumption, either as phytosterols or hydrogenated phytostanols incorporated into spreads, led to reductions in LDL concentrations of 8-13%, which would translate into a 25% lower risk of CVD (11). All trial results agree that plant sterols, hydrogenated or not, lower TC and LDL by approximately 6 and 13%, respectively, relative to controls in short term trials (12). In a recent review looking at the efficacy and safety of plant sterols, it was concluded that 0.8-1.0g/d, which is the amount recommended by the FDA (13), is effective in lowering LDL by at least 5% and this effect is obtained mainly by inhibiting absorption of cholesterol at the brush border of the intestine (14). In humans, there is a good likelihood that a dose of 0.8–1.0g/d of free sterol equivalents,

properly solubilized, administered in 2–3 servings with a meal, will reduce LDL cholesterol by 5% or more and that this reduction in LDL cholesterol will correlate with an approximate 6–10% reduction in CHD risk at age 70 (15, 16). Nevertheless, this 6% reduction in LDL correlates with a 15% reduction in CHD risk at age 40, and a 6% reduction at age 70 (15) or a 10% reduction (16). However, at this dosage level, it is likely that not all individuals will achieve a 5% reduction in LDL cholesterol (17).

Plant sterols have been granted a conditional health claim in the U.S. regarding their effects on the prevention of cardiovascular disease and are being incorporated into functional foods in Europe and the U.S. and Australia. Although cholesterol-lowering properties have been tested mostly in spreads, due to the lipophilic nature of the plant sterols, which goes against the notion of reducing total fat intake and promoting a healthy diet, there now exist a variety of other food products fortified with sterols available for public consumption and use in clinical trials.

Despite the fact that stanol and sterol ester spreads lower serum cholesterol to a similar extent in short term studies, a comparison of one year results reveals an inconsistent effect of plant sterol spread as compared with that of plant stanol spread on cholesterol concentration in both men and women (18). This favors the use of plant stanol ester spread for long-term lowering of serum cholesterol. Doses of 2g/d of plant stanols as fatty ester spread enhance fecal elimination of cholesterol, but not of bile acids, through inhibition of cholesterol absorption by about 10% and 15% as compared with control spread, respectively, and by up to 20% compared with

baseline diet. About one third of mildly hypercholesterolemic subjects reach an accepted cholesterol level. A small dose of statin should be added to treatment in individuals resistant to monotherapy with plant stanol ester spread (18). A life long consumption of plant stanol ester spread has been predicted to lower coronary events by about 20% (18).

2.2.2. Plant sterols: anti-inflammatory and anti-atherogenic effects

A study using apoE knockout mice, which have an accelerated rate of atherosclerosis, investigated how a phytosterol enriched diet affected pro-inflammatory cytokines. The mice were fed a cholesterol-supplemented diet in presence or absence of plant sterols for 14 weeks. It was shown that plant sterols decreased plasma cholesterol concentrations and decreased atherosclerotic lesions, as well as increased anti-inflammatory IL-10 and decreased pro-inflammatory IL-6 and TNFα. The anti-atherogenic effects of plant sterols were associated with improvements in lipoprotein metabolism and inflammatory pathways (19). In order to investigate the anti-inflammatory effects of plant sterols, Jenkins et al looked at a dietary portfolio of cholesterol lowering foods versus a statin drug on measures of CRP, as a biomarker of inflammation linked to increased CVD risk. Their results showed similar CRP lowering effects of both interventions (20).

2.3 Exercise

2.3.1. Effects of exercise training on lipids: mechanisms of action

The effects of exercise on modifying CVD risk factors are well established in the literature. As previously mentioned, increasing HDL by exercise training can decrease risk of CVD partly by increasing RCT, a process by which cholesterol is removed from peripheral tissues and brought to the liver where it is processed for excretion. In order to determine the mechanism by which exercise alters the lipid profile, Jafari et al studied the effects of a single bout of running in healthy adults on the lipid profile. The results were an increase in HDL:TG as well as an increase in pre-beta HDL. Pre-beta HDL is a molecular species of HDL which is especially efficient at mediating cholesterol removal from peripheral tissues and plays a pivotal role in initiating RCT (21). The increase in pre-beta HDL seen in this trial indicates that exercise induces its effects mainly by up-regulating RCT (21). In another study comparing age-matched athletes versus healthy controls, the athletes had higher measures of HDL and apoA1 and higher pre-beta HDL than controls (22) which emphasizes the effects of regular exercise training on HDL subfractions.

An important step in RCT is the efflux of cholesterol out of the peripheral tissues, which allows it to be transported by HDL back to the liver. Specific subfractions of HDL such as pre-beta1 HDL may be especially efficient at mediating cholesterol removal from peripheral cells. Regarding exercise and cholesterol efflux, the net mass of free cholesterol transport out of cultured human fibroblasts into athlete's serum is greater than that of sedentary controls (23). By increasing particles that are highly efficient in cholesterol removal such as lipoprotein-A1 and pre-beta

HDL, physical exercise may promote the RCT process by increasing HDL cycling of its apolipoprotein constituents. If pre-beta HDL is the rate-limiting acceptor in the early steps in cholesterol efflux then this offers an explanation why athletes have higher efflux (24). Findings showing that aerobic exercise can increase plasma pre-beta HDL (comprised mainly of apoA1) levels without increasing apoA1 levels (21) suggest that exercise efficiently replenishes pre-beta HDL particles by blocking hepatocyte clearance of alpha HDL particles (24). This is also consistent with studies showing that pre-beta HDL is derived from alpha HDL particles rather than de novo apoA1 synthesis.

Besides RCT, exercise-induced CVD reduction may also reflect other potentially anti-atherogenic aspects of HDL metabolism, including 1) anti-oxidant effects, 2) anti-thrombotic properties, 3) anti-inflammatory effects, 4) attenuation of endothelial dysfunction, and 5) reducing LDL retention (2).

2.3.2. Exercise training: clinical trials

Recent results suggest that regular endurance exercise may improve lipoprotein profiles in middle age as well as in older men and women. In a randomized controlled trial conducted by Welty et al, moderate intensity endurance activity in adults ages 31 through 67 was shown to decrease total cholesterol (-9.2%), LDL cholesterol (–9.3%), and triglycerides (-18.8%), while increasing HDL (2.6%) (25). In another study which examined the effect of a 12-week cardiac rehabilitation program on lipid profiles, HDL cholesterol was noted to increase (3%) while the LDL/HDL cholesterol ratios were noted to decrease significantly (-5%) (26).

Similarly, a smaller group of men and women who participated in a 12 week endurance training program had modest reductions in total cholesterol (-5%), triglycerides (-16%), LDL cholesterol (-6%), and LDL/HDL ratio (-8%), with increases in HDL cholesterol levels (6%) (27). Looking at a combination of endurance (50min at 70%) and resistance training (8exs) 3 times per week for 10 weeks in older women, similar increased HDL and decreased TG were found (28).

Endurance training (one hour 4X/wk for 1 year) without weight loss in overweight middle-aged men was associated with increased HDL of 10% and apoA1 9% as well as decreased TG 7% and decreased apoB by 10%. These effects were associated with a decreased catabolic rate of HDL and apoA1 as well as an increased synthesis of apoA1 up to 13% (29). Weight loss is not required to increase HDL-C with exercise training in overweight men, but without weight loss, even prolonged exercise training produces only modest changes in HDL-C concentrations.

Previously sedentary men on a one year training program increased HDL values 21% and apoA1 by 36% as well as a decrease in the ratio of LDL/HDL by 22% despite a lack of change in weight or BMI. However, they did show a decrease in fat mass with training. Exercise training has been shown to increase the intra-plasmic half-life of apoA1 (29), demonstrated by an increase in apoA1 with exercise training as well as a dose-response relationship of apoA1 in the different physical activity groups (30).

The optimal intensity and duration required to elicit favourable effects on lipid profiles during a single exercise session has been debated for some time now. Several studies have demonstrated that among adults who exercise regularly, those with

higher-intensity exercise regimens have significantly higher HDL cholesterol levels, lower triglyceride levels, and lower TC/HDL ratios than those with lower intensity exercise regimens (31,32,33). However, there are currently insufficient data from available training studies to establish the exact dose-response relationship between intensity and lipid response. With respect to training duration, it has been demonstrated that a minimum of 12 weeks of endurance activity is required to elicit a significant and substantial training effect on blood lipids (34). However, although a minimum of 12 weeks is ideal, favourable responses in blood lipid profiles have also been demonstrated in studies where aerobic exercise was only performed for a period of eight weeks (35,36). Thus, although improved lipid levels have been noted more consistently in training programs that last for 12 weeks or more, programs of shorter durations have also demonstrated favourable results.

Subject sex and age may also play a role in determining individual lipid and lipoprotein response to exercise. In the trials where direct comparisons between sexes were made, no consistent pattern of response was observed differentiating males and females (37,38,39,40). Although the majority of studies reported no difference in response between men and women, a few studies reported more favorable changes in HDL in men, while only one study showed more favorable changes in women. In studies where the ratio of TC to HDL (TC/HDL) was reported, no apparent consistent differences between men and women in their improvement in this ratio were noted. Furthermore, in studies where direct comparisons were made across age groups, no significant differences in responses were noted (41,42,43).

2.4 Adiponectin

2.4.1. Adiponectin: brief description

Adipose tissue is now recognized as an endocrine organ that secretes a variety of physiologically active peptides including adiponectin, resistin, leptin, IL6, and TNFα that share properties with cytokines and are therefore known as adipocytokines. The increased adiposity associated with a high fat diet and sedentary lifestyle can lead to a hypertrophy of adipocytes and distorted secretion of hormones. In a healthy state, secretion of adiponectin is favored. Adiponectin has been shown to have insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. Also in the healthy state, secretion of leptin, resistin, IL-6 and TNFα is inhibited. These molecules encourage insulin resistance and inflammation. In the unhealthy state, secretion of adiponectin is inhibited and secretion of the other peptides is favored. This is possibly one of the mechanisms involved in insulin resistance, diabetes and atherosclerotic CVD seen with obesity (44).

Other adipokines like adiponectin and leptin, at least in physiological concentrations, are insulin sparing as they stimulate beta oxidation of fatty acids in skeletal muscle (44). Reducing adipose tissue mass, through weight loss in association with exercise can lower TNF and IL6 levels and increase adiponectin concentrations, whereas drugs like thiazolinediones increase endogenous adiponectin production.

Adiponectin is present in the plasma in relatively high concentrations (5-30 μg/ml, 0.01% of total plasma protein) in two forms: A hexamer of relatively low molecular weight and a larger multimeric structure of high molecular weight (45).

Higher concentrations have been found in women than in men. It has been shown that adiponectin values undergo a diurnal variation in humans, corresponding to about 40% of variations from its 24-hr mean value (46).

2.4.2 Adiponectin in humans: cross-sectional analyses

A clear relationship exists between adiponectin values and fat mass in humans. Adiponectin release is positively correlated with fat cell size and negatively correlated with body mass index (BMI). Adiponectin values are commonly low in obesity. In a cross-sectional analysis of anorexia nervosa patients, control women and obese women, Matsubara et al. found that plasma adiponectin was negatively correlated with fat mass and BMI, fasting insulin and calculated insulin resistance (47). Further investigation by the same authors showed that adiponectin levels were inversely associated with insulin resistance in non diabetic subjects, independently of age, adiposity, blood pressure and serum lipids (48).

In a cross-sectional study of patients with normal body weight, Yamamoto et al found that adiponectin was negatively correlated with BMI, systolic and diastolic blood pressure, fasting glucose, insulin, insulin resistance, total and LDL cholesterol, triglycerides, and positively correlated with HDL cholesterol (49). Serum adiponectin was positively correlated with HDL in diabetic and non diabetic subjects. Similar findings exist in cross-sectional analyses performed at baseline in obese young women where adiponectin levels exhibited a significant negative correlation with body weight, BMI, %fat, and a significant positive correlation with VO_2max and

HDL (50). The data obtained also showed that at baseline, circulating adiponectin levels were significantly decreased in the obese young women.

In a more recent investigation of the association of adiponectin levels and the risk of coronary heart disease (CHD), Rothenbacher et al compared patients with angiographically confirmed stable CHD to healthy controls. It was shown that adiponectin concentrations were lower in CHD patients when compared with gender and age-matched controls. Also, in support of the previously mentioned findings, adiponectin was strongly correlated with HDL cholesterol. Rothenbacher et al suggest that the cardio-protective effect of high serum adiponectin may partly be mediated by its effects on the metabolism of lipoproteins, especially HDL (51).

Looking at patients with atherosclerosis and ischemic heart disease, Kawano et al measured adiponectin levels and compared them with healthy age and sex matched controls. Results showed that adiponectin concentrations were significantly lower in atherosclerosis patients then in control subjects, and further reduced in the subgroup with atherosclerosis and ischemic heart disease. Serum HDL was significantly less in subjects with atherosclerosis than controls but interestingly, there were no significant differences among the groups in blood pressure, total cholesterol, LDL or TG levels (52). This study highlights the importance of reduced adiponectin and corresponding reduced HDL values and the prevalence and magnitude of systemic atherosclerosis.

Kazumi et al looked at adiponectin values in healthy young men and found positive associations between adiponectin and HDL cholesterol, apoA1, and LDL particle size. They found negative associations of adiponectin with TG and apoB,

BMI, body fat and IR (46). After correction for BMI and multiple regression analysis, they found that adiponectin was more closely related to adiposity and dyslipidemia than IR and that adiponectin was an independent predictor of HDL, TG and LDL particle size.

The relationship of adiponectin to insulin and lipids seems to be strengthened with increasing adiposity. In a look at lean versus non-lean adolescents, Martin et al found that for TG and HDL, the relationship with adiponectin, although present in lean subjects, was strengthened in non-lean subjects. In other words, heavier adolescents had lower levels of adiponectin which were associated with higher TG and lower HDL values when compared with lean subjects. This would indicate that methods of increasing adiponectin levels (ie lifestyle changes) could be especially beneficial in heavier adolescents (53).

2.4.3. Adiponectin: clinical trials

Since it is a well known fact that weight loss, more specifically fat loss, is associated with decreased risk of CVD through changes in lipid profile, it would seem to follow that adiponectin levels, which are decreased in obesity and increased in lean-ness, would increase with weight loss. Although this is the most commonly observed outcome, the results from human trials are varied. Bruun et al reported that plasma concentrations of adiponectin in obese men increased by 51% after weight loss, were higher in lean than in obese men, and were inversely correlated with measures of adiposity, insulin sensitivity and IL-6 levels (54). Similarly, weight

reduction achieved by a low calorie diet also increased adiponectin values in diabetic and non-diabetic individuals (55).

In another study that involved weight loss with or without exercise training over a 6 month period, Ryan et al saw no changes in adiponectin values (40±16%) despite decreased body weight and body fatness (56). There was an increase in adiponectin values but possibly because of a large amount of inter-subject variation in Adiponectin values, the increase was not significant in the weight loss group compared to the other groups.

Exercise trials

Exercise has been shown to increase insulin sensitivity and decrease the risk of obesity and type 2 diabetes. It has also been shown to modify the lipid profile by increasing levels of HDL which reduces the risk of CVD. From these well-documented effects of exercise, one would expect that exercise could enhance plasma values of adiponectin. Once again, results from clinical trials are quite varied.
In two separate trials looking at glucose, insulin and adiponectin before and after 6 months of exercise training (4d/wk, for 45min at 65-85% peak oxygen consumption) with no loss of body fat or mass, these studies showed that insulin activity significantly improved but plasma adiponectin values did not change significantly (57,58). In contrast, in a separate group of patients from the Yatagai et al trial examined before and after weight loss, adiponectin increased, which was accompanied by increased insulin action (57). This suggests that adiponectin is not a

contributory factor to the exercise induced improvements in insulin sensitivity. Also, that adiponectin was increased through weight loss effects rather than exercise per se.

Kriketos et al ran an exercise trial (4-5d/wk, 40min) in overweight males for a period of 10 weeks and observed significant increases in adiponectin and insulin sensitivity despite unchanged body weight (59). These results contrast with the previously ones of Hulver et al (58) who saw no increase in adiponectin despite increased insulin sensitivity. In the study by Hulver et al their baseline results were taken after 6 wks of ramping exercise in preparation for the 6 month trial which could possibly explain why they did not observe the same initial changes in adiponectin seen by Kriketos et al who observed increases in adiponectin values of up to 260% after only two to three bouts or approximately one week of moderately intense exercise. These results suggest that short term moderate exercise training can modify regulation of adiponectin, which may provide another mechanism by which exercise reduces atherogenic risk, at least in overweight males (59).

In order to understand the regulation of the short term increase in insulin sensitivity associated with exercise, Yokoyama et al looked at the effects of only 3 weeks of diet, exercise training, or diet plus exercise intervention in type 2 diabetic males. These authors saw no significant changes in adiponectin levels in any of the groups despite increased insulin sensitivity in the exercise group. This shows that the short term improvements in insulin sensitivity are not mediated by changes in plasma adiponectin. The authors believe that restoring insulin sensitivity by aerobic exercise is mainly mediated by mechanisms other than adiponectin such as the adenosine monophosphate kinase (AMPK) activated protein kinase pathway. However, from a

review of the literature they saw that changes in plasma adiponectin are significantly correlated with anthropometrical changes induced by aerobic exercise. Marked weight reduction results in a significant increase in plasma adiponectin in obese subjects; therefore, adiponectin may play a central role in operating insulin action when an improvement of insulin sensitivity is achieved mainly by fat mass reduction. Thus, it was concluded that exercise may indirectly increase plasma adiponectin when an intervention is accompanied by a reduction in body weight or fat mass (60).

Similar conclusions were drawn from a study looking at the major factors regulating adiponectin levels and the influence of aerobic training or combined aerobic and resistance training on adiponectin levels in obese young men. After an 8 week training program, adiponectin levels were unchanged in all groups however, there was a significant negative correlation between fat mass and adiponectin levels and that the change in percent body fat was an independent predictor of adiponectin levels. These findings indicate that for increasing adiponectin levels, improvement of the body composition is more important than the way training is performed (61).

Another 8 week exercise training intervention was performed on type2 diabetic men. Although they saw improvements in insulin sensitivity (58%) and decreased abdominal fat (44%), there were no significant changes in adiponectin values. However, in the trained group changes in adiponectin were strongly correlated to changes in body weight (62) which also supports the conclusions of Yokoyama et al that is it the changes in body composition which primarily cause the changes in adiponectin values.

A seven month exercise trial in obese young women caused decreased body weight, BMI, %fat, body fat mass, lean body mass, CRP and TNFα and increased VO_2max, adiponectin and HDL (50). Exercise increased adiponectin by 42.8% and decreased TNFα level by 36.8%. This further supports the relationship between adipocytokine levels and body fat tissue. Obese and control subjects had the same meals. It is possible that loss of visceral fat resulted in increased adiponectin levels concomitant with body weight reduction in obese young women. These results suggest that suppressing excess body fat tissue is important for preventing reduction of adiponectin levels in young women. Recently, high sensitivity CRP (hs-CRP) has been reported to be a useful marker for atherosclerosis (63). Prolonged and moderate intensity exercise may prevent atherosclerotic CVD. In the study by Kondo et al (50), hs-CRP was increased in obese young women compared with controls, and hs-CRP was decreased after the exercise program. IL-6 is secreted by fat cells and induces hepatic production of CRP. Twelve weeks of aerobic training in 19 obese and overweight girls resulted in increased insulin sensitivity but no change in adiponectin, IL6 and CRP levels. There were no changes in body weight or body fat (64).

A study looking at the effects of varied intensities of exercise training in overweight inactive elderly patients showed that effects of exercise were intensity dependent. In high, medium and low intensity resistance training groups there were decreases in BMI and skin folds after 24 weeks of training. Only the high and medium intensity groups showed significant improved adiponectin values and only the HI group maintained elevated levels of adiponectin after detraining. These results indicate that the intensity of the exercise is also important (65).

In order to investigate the effects of a single bout of exercise on insulin sensitivity and adiponectin values, overweight males performed 45 minute of exercise at 65% VO_2max. There were no changes in adiponectin levels, and insulin sensitivity increased only right after exercise (66).

The varied results from the exercise trials above may reflect differences in duration, training intensity and frequency, and most importantly the differences in the sample selection including age, adiposity, insulin resistance and health status. The most consistent finding from these trials was that exercise-induced changes in adiponectin values were highly correlated to changes in body composition.

2.4.4. Synthesis and secretion of adiponectin

The fact that adiponectin levels are low in obesity despite adipose tissue being its only tissue of synthesis would suggest some sort of negative feedback action on its production. Consequently, weight loss would be expected to produce a temporary decrease in inhibition and therefore an increase in adiponectin levels, which has been shown in some of the aforementioned trials.

2.4.5. Specific effects of adiponectin and possible mechanism(s) of action

Adiponectin has been shown to have several effects on various tissues in the body, which are distinct in nature yet inter-related, and which are mediated through different mechanisms. Some of the most pronounced effects of adiponectin include insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties, as well as its effects on FFA oxidation and metabolism.

Anti-inflammatory properties of adiponectin

It has been shown in vitro that TNFα and IL6 decrease adiponectin mRNA. Since TNFα and IL6 decrease whereas adiponectin increases insulin sensitivity, it is reasonable to suggest that the balance between adiponectin versus TNFα and IL6 ultimately determines peripheral insulin action and sensitivity. Exercise decreases IL6 and TNFα but does not seem to directly alter adiponectin. In other words, exercise increases insulin sensitivity by increasing the ratio between adiponectin and TNFα and IL6, in favor of the former (67). Therefore, the anti-inflammatory effects of exercise can be attributed in part to the anti-inflammatory properties of adiponectin.

In support of this balance effect, adiponectin has been shown to reduce TNFα production and reduce TNFα effects on certain cells (55). Therefore, it may affect insulin sensitivity by interfering with TNFα production and signaling.

Sustained weight loss induced by dietary modification and increased physical activity reduced IL6, IL18 and CRP, and increased adiponectin levels significantly (68). This finding, along with the evidence that adiponectin values are increased in lean subjects compared to obese subjects, suggests that weight loss in combination with physical activity gives the most benefit in terms of lowering inflammatory markers and increasing adiponectin levels (67). This effect was seen in the lifestyle intervention trials above (50,60,61,62) where body composition was successfully changed.

Cardiovascular: anti-atherogenic and vascular effects of adiponectin

Adiponectin also plays a protective role against the development of atherosclerosis by suppressing inflammatory processes on the vascular endothelium (60). Adiponectin decreases macrophage attachment to endothelial cells by reducing the expression of adhesion molecules in endothelial cells through protein kinase A-mediated interference of nuclear factor kB signaling (69).

Matsuda et al showed that adiponectin infiltrates rapidly into the sub-endothelial space of the vascular wall when the endothelial barrier of the arterial wall is injured by balloon angioplasty. In tissue cultures they found that adiponectin attenuates monocyte attachment to endothelial cells by reducing the expression of adhesion molecules on endothelial cells. Adiponectin also suppresses lipid accumulation in monocyte derived macrophages through the suppression of macrophage scavenger receptor expression. In a recent study in adiponectin knock-out mice (70), Matsuda et al found that adiponectin deficiency increased neointimal thickening and proliferation of vascular smooth muscle cells in mechanically injured arteries. Also, by using an adenovirus-mediated supplement of adiponectin, they saw attenuation of neointimal thickening in the injured arteries (70). The postulated mechanism of adiponectin action involves evidence collected from in vitro studies that adiponectin suppresses the expression of HB-EGF which normally increases due to TNFα in injured endothelial cells and the proliferation and migration of smooth muscle cells stimulated by other growth factors such as PDGF, bFGF and EGF. The suppressive effects of adiponectin on the production and action of growth factors should explain the mechanism for the suppressive actions of adiponectin on the

vascular wall stenosis (70). Also, as previously mentioned, adiponectin has been shown to directly suppress TNFα secretion which is also true in macrophages/monocytes and foam cells (71). Adiponectin null mice have been shown in the past to show normal insulin sensitivity on a regular diet but severe insulin resistance on a high fat/sucrose diet. In the study by Matsuda et al (70), the adiponectin knock out mice showed severe neointimal hyperplasia by induced vascular wall injury despite normal lipid and glucose metabolism. This evidence indicates that neointimal thickening in vascular stenosis is not accelerated as a result of disordered glucose or lipid metabolism but that it is caused directly by adiponectin deficiency (70).

In ApoE-deficient transgenic mice, which represent a model of accelerated atherosclerosis, administration of adiponectin was shown to reduce atherosclerosis plaque formation by up to 30% (72). This correlates with the results of Matsuda et al mentioned above (70). It is suggested that adiponectin may act as an anti-atherosclerotic factor not only through direct effects on the vascular endothelial cells, but also through improving insulin resistance and lipid metabolism (50).

Adiponectin exerts its vascular actions by direct stimulation of nitric oxide (NO) production in endothelial cells (by phosphorylation of eNOS by AMPK) and stimulating new vessel growth, thereby taking part in vasodilator actions and increasing blood flow. Thus adiponectin mimics vascular as well as metabolic effects of insulin. The fact that insulin and adiponectin regulate activation of eNOS by slightly different mechanisms suggest that therapies designed to increase adiponectin

levels may be beneficial in treatment of insulin resistance, diabetes, vascular complications and atherosclerosis (73).

Effects of adiponectin on free fatty acid metabolism

Evidence to support the idea that adiponectin represents one of the hormones that mediate the cross talk between adipose tissue and skeletal muscle is accumulating. It has been speculated that adiponectin must be located on the surface of and/or inside skeletal muscles fibers for signaling. Magnetic resonance spectroscopy has demonstrated that intracellular lipid content in human muscle negatively correlates with adiponectin concentrations, potentially because of adiponectin induced FFA oxidation (74). Punyadeera et al have suggested that adiponectin regulates plasma FFA clearance by stimulating FFA uptake and/or oxidation in muscle which is what has been shown in rodents (75). These researchers measured circulating FFA levels, glycerol, whole body fat oxidation rate and adiponectin before, during and after prolonged moderate intensity exercise in two separate trials. The first trial subjects were under normal fasting conditions (HFA) and in the second trial (LFA) the subjects had received pharmacological inhibition of adipose tissue lipolysis. Although these investigators saw a pronounced decrease in FFA and glycerol concentrations as well as reduced whole body fat oxidation rates in response to exercise in the LFA trial, this did not affect adiponectin concentrations. The authors concluded that adiponectin release is unrelated to an acute temporary decline in adipose tissue lipolytic rate, plasma FFA concentration or whole body lipid oxidation rates (75). The conclusions were that there may be other more important

factors regulating adiponectin release, possibly adipocyte size and/or lipid content, circulating catecholamines, glucocorticoids, or TNFα / IL6.

Since previous research showed an important regulatory role of adiponectin on skeletal muscle metabolism, Punyadeera et al examined muscle tissue to see if adiponectin was present. Histological analyses showed adiponectin to be present in plasma and in skeletal muscle tissue. Staining on muscle cross-sections showed adiponectin presence on the sarcolemma of individual muscle fibers and within the lining of interfibrillar arterioles. This finding of adiponectin in the lining of arterioles correlates with the above mentioned evidence of adiponectin as a potent anti-inflammatory (55) and athero-protective (70,72,73) agent in vascular tissue (75).

2.5. Ghrelin

2.5.1. Ghrelin: brief description and actions

Ghrelin, a 28-amino acid peptide, is the endogenous ligand for the growth hormone secretagogue receptor located in the pituitary gland. Ghrelin is secreted mainly by the stomach from a distinct endocrine cell type, but it is also produced in small amounts by the pancreas, hypothalamus and other organs. From the ghrelin-secreting cells in the stomach, it is released directly into the plasma. Plasma concentrations normally range from 200-600ng/L. However, close to 80% of circulating ghrelin is deamidated, and biologically inactive (76). From the plasma, ghrelin is able to enter the CNS and exert its effects on the arcuate nucleus of the hypothalamus, which is the major hypothalamic site regulating food intake and body weight through the presence of neurons containing orexigenic and anorexic peptides

(77). Ghrelin is the unique gastrointestinal peptide that stimulates appetite and food intake. Ghrelin administration in humans powerfully induces a sensation of hunger in 75% of individuals tested (76) and has been shown to increase food intake (78). Clinically, it has profound orexigenic, adipogenic, and somatotropic properties, increasing food intake and body weight (77). The brain-gut axis is the effector of anabolism by regulating growth, feeding, and metabolism via vagal afferent-mediating ghrelin signaling.

The stimulus for ghrelin production in the stomach is related to glucose and insulin metabolism. In a study by Fagerberg et al, the authors investigated whether insulin sensitivity measured by the gold standard clamp technique was associated with plasma ghrelin concentrations in a sample of men obtained from the general population. The simple correlation analyses showed that ghrelin was related to many factors in the metabolic syndrome apart from plasma insulin concentrations, such as blood pressure, HDL-C, and small LDL particles. However, after adjustment for body fat, these associations did not remain, pointing to the strong relationship between obesity and ghrelin (79).

Ghrelin is also a potent stimulator of GH secretion and it is able to release GH when administered IV and ICV. Since it is able to enter the CNS from the periphery , it is possible that stomach-derived ghrelin may physiologically participate in GH regulation (76). Ghrelin mediated-GH secretion is partially insensitive to somatostatin inhibition and of metabolic compounds such as glucose or FFA. Since ghrelin anticipates the initiation of meals and releases GH, it may be seen that ghrelin integrates anabolic changes in the body. In catabolic situations, raised ghrelin levels

may induce a combination of enhanced food intake, increased gastric emptying and food assimilation coupled with GH levels which promote a prompt nutrient incorporation into muscles and to fat (76).

Ghrelin exhibits a diurnal rhythm, gradually rising throughout the day until reaching a peak between 1 and 2 and a meal response, rising 1-2 h before the initiation of a meal and falling to trough levels 1-2 h after a meal (80). This is suggestive of the involvement of ghrelin in short-term energy homeostasis. Investigation into the possible involvement of ghrelin in long-term energy balance and body weight regulation revealed that ghrelin levels are elevated in anorexia nervosa, decreased in obesity, and normalized with weight gain or weight loss.

Continuous or repeated ghrelin administration in animals significantly increases food intake and decreases energy expenditure in animals (81) leading to weight gain. Similarly, blockade of endogenous ghrelin signaling leads to decreased food intake and weight loss (81). These findings suggest that ghrelin may participate in a negative feedback loop regulating body weight. From this, it follows that weight loss should trigger an increase in ghrelin levels as part of the known adaptive mechanism to energy deficit. Plasma ghrelin levels have been shown to increase in response to weight loss resulting from hypocaloric diets, cancer, cachexia, anorexia nervosa, and chronic failure of the heart, liver or kidneys (82). The hypothesized role of ghrelin in the adaptive response to weight loss would be better supported if ghrelin levels were found to increase in the setting of weight loss that is not associated with decreased food intake, such as that resulting from chronic aerobic exercise.

2.5.2. Exercise, weight loss and ghrelin levels: clinical trials

Energy balance is a determinant of plasma ghrelin concentration. Circulating ghrelin levels rise during fasting and hypoglycemia (83) and decline upon refeeding. A six month weight reduction diet was associated with a significant increase in plasma ghrelin secretion (80). Because ingested nutrients suppress ghrelin, increased ghrelin levels in hypophagic weight loss may result from decreased inhibitory input by ingested food, rather than from lost weight (82). Foster-Schubert et al investigated whether ghrelin levels increase in response to exercise-induced weight loss without decreased caloric intake. Moderate intensity aerobic exercise of 45mins, 5d/wk or stretching control was performed for a 12 month period in 168 previously sedentary post-menopausal women. At baseline, ghrelin levels in all study participants correlated negatively with all measurements of body size and adiposity. After the trial, exercisers lost 1.4 ± 0.4kg over the one year intervention and manifested a significant progressive increase in ghrelin levels. When subjects were divided into groups according to weight lost, ghrelin levels increased commensurately with the amount of weight lost. Ghrelin levels increased 18% in exercisers who lost more than 3kg. Among the exercisers, plasma ghrelin levels did not change in the group without weight loss. On further analysis, they determined that the magnitude of increase in plasma ghrelin correlated significantly with the magnitude of decreases in body weight, BMI, waist circumference and total fat mass. There was no change in caloric intake and no effect on ghrelin of exercise per se independent of its effect on body weight. There was no association between changes in fitness level resulting from the exercise intervention and plasma ghrelin levels. These findings suggest that ghrelin

can respond in a compensatory manner to loss of body weight, not simply hypophagia (82). The data obtained indicate that ghrelin increases in response to modest weight loss without a concomitant decrease in caloric intake or a direct effect of exercise itself. The mechanisms by which weight loss signals an increase in ghrelin levels are not yet understood.

A 3 month energy-deficit-imposing diet was looked at in a population of healthy young women 1) who did not exercise, 2) who exercised and remained weight-stable or 3) who exercised and lost weight. Contrary to the above-mentioned study, at baseline, there were no correlations observed between ghrelin concentrations and body weight, BMI or fat mass. Subjects were fed a specific diet and exercise was performed 5 days per week at 70-80% of max HR. Ghrelin significantly increased over time (770 ± 296 to 1322 ± 664 pmol/L) in the weight loss group compared to the controls and the weight-stable group ($P<0.05$). Similar to previously mentioned findings, changes in ghrelin were negatively correlated to changes in body weight, as well as to caloric intake, fat mass and fat-free mass in the exercising subjects. When the time course of changes in ghrelin and other energy balance variables was examined, changes in body weight, body composition, and resting metabolic rate were found to precede changes in ghrelin, suggesting that alterations in circulating ghrelin may be mediated by changes in these energy balance parameters. These findings suggest that ghrelin responds in a compensatory manner to changes in energy homeostasis in healthy young women, and that ghrelin exhibits particular sensitivity to changes in body weight (84). Since ghrelin values at baseline were not related to body composition variables, in contrast to previous findings in obese or anorexic

subjects, it was suggested that ghrelin may play an important role in returning the body to a prior setpoint after weight loss (or gain). The absence of changes in ghrelin in the weight stable exercisers indicates that exercise alone has little impact on at least one powerful modulator of food intake. This finding also reinforces the concept that the increase in ghrelin in the weight loss group was in response to the overall energy deficit created by the combination of reduced food intake and exercise, and not due to the endocrine and/or metabolic effects of exercise itself.

2.6. Growth Hormone

2.6.1. Growth hormone: description and actions

Growth hormone is secreted by the anterior pituitary in response mainly to hypothalamic stimuli. The secretion of growth hormone is regulated positively by growth hormone releasing hormone (GHRH) from the hypothalamus and insulin like growth factor-1 (IGF-1) from the liver, and negatively by somatostatin from the hypothalamus. Growth hormone's actions are implicated in a dual action on somatic growth and in the regulation of general metabolism, and which is in turn, regulated by the energetic homeostasis of the individual (76). Secretion declines with age due to a decline in function of the IGF-1/GH axis due to age-related variations in hypothalamic control of somatotroph function (85). From cross-sectional analyses, abdominal visceral fat is a major negative determinant of both stimulated and spontaneous growth hormone secretion in healthy adults (86,87). The hyperinsulinemia, hyperlipidemia, and elevated levels of free IGF-1 seen in visceral obesity all contribute to the inhibition of GH secretion. It seems that several

metabolic feedback mechanisms are involved in this regulation between adiposity and GH status (86). In older age, decreased GH secretion associated with increased body fat and decreased lean body mass may contribute to the unfavorable lipid profile in older adults (87). Severe growth hormone deficiency in hypopituitary adults and ageing are associated with an increase in fat mass, dyslipidemia and CVD (86).

2.6.2. The effects of growth hormone on lipid metabolism

The effects of endogenous GH on circulating lipoproteins was evaluated by Vahl et al. and they found that there are beneficial effects of GH on body composition, physical fitness, and lipoproteins. Their data strongly suggested that GH also has a direct effect on the metabolism of lipids and lipoproteins. One of the mechanisms behind this direct effect of GH could be the ability of GH to induce hepatic LDL receptor expression which has been shown in both rats and humans. This would lead to increased clearance of LDL as well as increased clearance of VLDL-ApoB since the LDL receptor is involved in hepatic removal of partially depleted VLDL (86). Administration of GH to GH-deficient adults has been shown to result in favorable alterations in TC, LDL, HDL and apoB (87).

2.6.3. The effects of exercise on growth hormone

Exercise is a potent, dose-dependent stimulus of growth hormone secretion. The hypothalamic peptides, GHRH and somatostatin are regarded as major regulators of this stimulation. The role of the stomach-derived peptide ghrelin, which has been shown to exert strong GH releasing effects, has not been fully characterized yet and

the neurotransmitters involved in the GH secretion in response to exercise remain uncertain.

In a study by Jorgensen et al (88), the effects of GH administration in GH-deficient subjects on lipids was compared to untreated healthy subjects during exercise. Ghrelin was measured before, during and after submaximal exercise in healthy subjects and GH-deficient subjects. Ghrelin levels were found to be unchanged during and after exercise. GH stimulates lipolysis and lipid oxidation during basal and fasting conditions in all subjects. GH predominantly stimulated turnover of free fatty acids in the recovery phase after exercise. It was concluded that 1) the increase in GH release during exercise is associated with concomitant increase in body temp, 2) GH stimulates sweat secretion and heat evaporation during exercise, 3) ghrelin is not involved in exercise-induced GH release, 4) the impact of GH on substrate metabolism during exercise includes increased FFA turnover (88). It has been shown previously that the concomitant increase in core temperature is essential for the exercise-induced GH release (88).

In order to investigate whether GH release during exercise is due to complete inhibition of hypothalamic somatostatin activity, eight healthy males performed strenuous exercise on a cycle ergometer. This was compared to administration of pyridostigmine (PD) which inhibits all somatostatin activity. The levels of GH attained in the exercise group, measured by area under the curve (AUC), were significantly higher than those in the PD group, suggesting that other mechanisms involving release of GHRH or ghrelin must be operative (89). In a follow-up trial, looking at the involvement of endogenous growth hormone-releasing hormone

(GHRH) in the growth hormone release during strenuous exercise, de Vries et al found that there was a potentiating effect between GHRH and exercise, ie the GH measured in the GHRH+EX group was higher than either intervention alone or additively. It was concluded that GH responses to strenuous exercise are only partially due to maximal GHRH activation and that next to complete inhibition of hypothalamic somatostatin activity, which is achieved by strenuous exercise, activation of endogenous GH-releasing peptides, such as ghrelin, must be operative (90).

In an investigation of GH release in response to graded exercise, GH serum concentrations increased at all three exercise intensities (50% for 40mins, 70% and 90% for 20mins) however ghrelin plasma concentrations remained unchanged at all three intensities (91). Assuming the sensitivity of the GH neuroendocrine/metabolic regulation of GH is unaltered, ghrelin does not seem to participate in the regulation of the GH response to exercise in healthy males.

In another study looking at similar parameters, in well-trained young males, GH increased over time with progressive running exercise, measured by area-under-the-curve total GH. Blood samples were collected before exercise, after each exercise intensity, and at 15 and 30mins following the exercise protocol. Though running produced substantial GH increases, peripheral ghrelin levels were not affected therefore no relationship between ghrelin and GH was found. However, significant relationships were found between ghrelin and both IGF-1 and IGFBP-3 during intense running and recovery (92).

Similarly, looking at the effects of training in eight healthy males and eight GH-deficient males, it was found that submaximal aerobic exercise of an intensity sufficient to stimulate GH release was not associated with significant alterations in plasma ghrelin concentrations, which indicated that systemic ghrelin is not involved in the exercise induced stimulation of GH secretion (93). With a dose of GH replacement, the ghrelin levels were lower suggests that GH may feedback-inhibit systemic ghrelin release.

From the above results, it appears that ghrelin may not be involved in the acute exercise-induced release of GH. However, some authors still question its involvement (89,90). It is clear that ghrelin is involved in both short term and long term regulation of energy homeostasis, through feeding behavior and weight gain. How potential changes in energy balance achieved through exercise over a period of time, without dieting, affect circulating GH and ghrelin levels is not yet clear in the literature.

3. RATIONALE

Plant sterols exert their effects mainly by decreasing TC and LDL-apoB and also play a role in decreasing inflammation and atherogenicity. Exercise exerts its effects in several different ways. Firstly, by increasing HDL-apoA1 and RCT. Secondly, by decreasing TG and adiposity, thereby potentially affecting a) the adipocyte and down-regulating secretion of inflammatory cytokines and increasing adiponectin secretion and b)secretion of GH by decreasing AVF. Thirdly, by directly increasing GH secretion which has direct effects on lipid metabolism including an increase in LDL receptors and LDL clearance. It is hypothesized that lifestyle modifications including plant sterols and or exercise training can favorably alter the lipid profile and the hormones aforementioned, possibly in a synergistic manner, in such a way as to decrease the risk of CVD.

4. HYPOTHESES AND OBJECTIVES

4.1. HYPOTHESES

4.1.1 Ho: There will be no effect of feeding plant sterols or performing endurance exercise training on plasma lipoprotein A and B levels, or adiponectin, growth hormone or ghrelin hormone levels, over the 8 wk intervention period.

4.1.2 Ho: There will be no correlation of the values of apolipoproteins and hormones to the previously obtained lipid data at baseline or regarding percent change over the 8 wk intervention period.

4.2. OBJECTIVES

4.2.1. To determine the values of apoA, apoB, adiponectin, growth hormone and ghrelin at baseline and following 8 weeks intervention with plant sterol supplementation and/or exercise training.

4.2.2. To compare the obtained values to previously obtained lipid data from this trial (95) including TC, LDL, HDL, TG levels and particle size.

5. MANUSCRIPT

This paper will be submitted shortly for journal publication.

The Effects of Plant Sterols and Endurance Training on Apolipoprotein A and B, Adiponectin, Ghrelin and Growth Hormone

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5.1 ABSTRACT

Background: Plant sterols lower total cholesterol, LDL, and inflammatory markers such as IL-6 and TNFα, and decrease risk of atherosclerotic cardiovascular disease. Exercise increases HDL and decreases circulating triglycerides, percent body fat, and inflammatory mediators thereby also reducing the risk of CVD. However the combined effects of plant sterols and exercise training on apoA, apoB, adiponectin growth hormone and ghrelin, has not been investigated previously.

Objective: The objective was to examine the effects of plant sterol supplementation and exercise training on apolipoproteins A and B, adiponectin, growth hormone and ghrelin and to look at these values in the context of previously obtained lipid data.

Design: In an 8-wk, placebo-controlled, parallel-arm clinical trial, 84 subjects were randomly assigned to receive 1 of 4 interventions: 1) combination of sterols and exercise, 2) exercise, 3) sterols, or 4) control treatment

Results: Plant sterol supplementation increased ($P<0.05$) adiponectin values by up to 16%. ApoA was associated with HDL and %change in apoA was correlated to %change in HDL for the exercise group only. ApoB was associated with LDL values and particle size at baseline but did not change significantly with either intervention. Growth hormone and ghrelin values were not altered by either intervention.

Conclusions: Besides the well-known effects of plant sterols on reducing total and LDL cholesterol, the capability of plant sterols to increase adiponectin values suggest a role in preventing inflammation and atherosclerotic lesions, thereby reducing the risk of CVD. Also, the combination of lifestyle interventions including plant sterols

and exercise can favorably alter inflammatory and other markers for CVD as an alternative or adjunct to drug therapy.

5.2 INTRODUCTION

Cardiovascular disease is a leading cause of morbidity and mortality in our society with increasingly large numbers of people being affected. Certain lifestyle factors have been highlighted in the pathogenesis of the disease. A high fat diet and sedentary lifestyle are two of the main contributors and can lead directly to obesity and an unfavorably altered lipid profile, known as dyslipidemia.

Plant sterols (PS) have been shown in the literature (7,8,9,10,11,12) to decrease total and LDL cholesterol. Because of their structural similarity to cholesterol, PS inhibit absorption of cholesterol at the level of the intestine. Supplementation of approximately 2g/day of plant sterols incorporated into a typical diet has been shown to decrease total and LDL cholesterol by up to 13% and 24% respectively (7) and to decrease apoB up to 14% (8). Previous studies show that plant sterols decrease inflammatory mediators such as TNF and IL-6 and increase anti-inflammatory IL-10 in an animal model (19). In humans, it has been shown that a dietary portfolio of cholesterol lowering foods are effective in lowering C reactive protein, an inflammatory marker (20). However, the effects of plant sterols on other markers of inflammation have not been investigated in humans.

The main effects of exercise training are well established in the literature and include increases in HDL and decreased triglycerides (28,29) as well as decreasing percent body fat. Endurance training without weight loss is associated with increases

in HDL of 10-21% and apoA1 9-36% as well as decreases in TG of 7% (29,30). These effects are associated with a decreased catabolic rate of HDL and apoA1 as well as an increased synthesis of apoA1 up to13% (30). Also, an increase in pre-beta HDL therefore an up-regulation of RCT have been highlighted (21).

Adipose tissue is now recognized as an endocrine organ that secretes a variety of physiologically active peptides known as adipocytokines, including adiponectin. Adiponectin has been shown to have insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. In the unhealthy state, secretion of adiponectin is inhibited and secretion of the other inflammatory peptides including IL-6 and TNFα is favored. Adiponectin is decreased in obesity, atherosclerosis and diabetes. Adiponectin is positively associated with HDL and apoA and negatively correlated to adiposity, insulin sensitivity and IL-6 (54). Adiponectin has been shown to increase with weight loss (54) and with exercise training in association with weight loss (57, 61) and without (59). Sustained weight loss and increased physical activity causes decreased IL-6, IL-18 and CRP as well as increased adiponectin concentrations (68).

Growth hormone is inversely associated with abdominal visceral fat, which seems to be the primary determinant of its secretion (87). Growth hormone decreases with age, possibly due to increased fat mass and decreased lean body mass which may contribute to the unfavorable lipid profile seen with increasing age. The secretion of growth hormone is regulated positively by GHRH and IGF-1 and negatively by somatostatin. Growth hormone has been shown to increase with exercise training (88,89,90,91). Growth hormone also has direct effects on lipid metabolism and stimulates lipolysis and lipid oxidation (86,87).The impact of GH on substrate

metabolism includes increased FFA turnover (88). Also, GH has been shown to increase the number of hepatic LDL receptors thereby increasing the clearance of LDL and VLDL-apoB from the circulation (86).

Ghrelin is a gut-derived peptide that exerts its effects at the level of the hypothalamus, playing an important role in energy homeostasis. Ghrelin stimulates appetite and then decreases post-prandially. Ghrelin is decreased in obesity and increased in starvation. It is a potent stimulator of GH release although whether or not ghrelin is responsible for the GH increase in response to exercise is not yet clear. In most studies it has been shown that despite increases in GH, ghrelin levels did not change (88, 91) however, some trials still question its involvement (89,90) in the exercise-induced increase in GH. Ghrelin has been found to increase with exercise training alone (84) or with exercise and dieting (82) however, the changes in ghrelin are strongly correlated to weight loss attained via these lifestyle interventions.

Plant sterols exert their effects mainly by decreasing TC and LDL-apoB and also play a role in decreasing inflammation and atherogenicity. Exercise exerts its effects in several different ways. Firstly, by increasing HDL-apoA1 and RCT. Secondly, by decreasing TG and adiposity, thereby potentially affecting a) the adipocyte and down-regulating secretion of inflammatory cytokines and increasing adiponectin secretion and b) increasing secretion of GH by decreasing AVF. Thirdly, by directly increasing GH secretion which has direct effects on lipid metabolism. It is hypothesized that lifestyle modifications including plant sterols and or exercise training can favorably alter the lipid profile and the hormones aforementioned in such a way as to decrease the risk of CVD.

5.3 SUBJECTS AND METHODS

Human subjects

Subjects were recruited from the greater Montreal area by means of advertisements placed in local newspapers. A total of 142 persons expressed interest in the study, but only 84 were deemed eligible after the preliminary questionnaire, blood screening, and physical examination. Key inclusion criteria were as follows: age, 40–70 y; previously sedentary, defined as <1 h/wk of light intensity exercise at 2.5–4.0 metabolic equivalents for the 3 months before the study (12); total cholesterol concentrations > 4.5 mmol/L; nonsmoking; free of CVD; nondiabetic; body mass index (in kg/m^2) between 18 and 40; not taking lipid- or glucose-lowering medications; normotensive or hypertensive controlled by medications not affecting lipid or glucose metabolism; free of other medical conditions that would preclude subjects from participating in a moderate-intensity endurance exercise program. In addition, women of menopausal age were either premenopausal or postmenopausal (absence of menses for >2 y) and were required to maintain their current hormone replacement therapy regimen for the duration of the study.

The experimental protocol was approved by the Human Ethical Review Committee of the Faculty of Agricultural and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University. All volunteers gave their written informed consent to participate in the trial before the commencement of the study.

Experimental design

An 8-wk, randomized, single-blind, placebo-controlled, parallel-arm clinical intervention trial was implemented as a means of testing the study hypotheses. Subjects were divided into strata according to total cholesterol concentrations and age. Subjects from each stratum were then randomly assigned into the following 4 intervention groups: 1) combination group (administered sterol-enriched margarine with exercise intervention), 2) exercise group (administered placebo margarine with exercise intervention), 3) sterol group (administered sterol-enriched margarine with no exercise intervention), and 4) control group (administered placebo margarine with no exercise intervention).

Exercise protocol

Subjects assigned to the exercise intervention groups trained at a moderate intensity (94) 3 times/wk under supervised conditions in the research laboratory. Control subjects were asked to maintain their regular level of activity throughout the course of the 8-wk trial. Endurance training was performed with the use of stair-stepping machines and stationary bicycles. Training intensity was estimated for each subject with the use of an age-predicted heart rate maximum (HRmax) equation [209 - (0.7 x age)] (96). Initial exercise sessions consisted of 25 min of exercise corresponding to 60% of each subject's HRmax. Training duration and intensity increased incrementally at week 2, week 4, and week 6, by 5 min and 5% HRmax. Thus, at week 6, the participants trained for a 40-min duration at an intensity of 75% HRmax.

Subjects wore Polar Heart Rate Monitors (Polar USA Inc, Woodbury, NY) while training to estimate their training intensity. Heart rates were assessed every 5 min throughout the training session to ensure that the subjects were exercising within safe limits. Compliance was assessed by recording the subject's attendance at each session. If a training session was missed, the subject was required to make up for the missed session during that same week.

Plant sterol protocol

Throughout the study, subjects were asked to replace their habitual margarine intake with the experimental margarine provided. On day 0 of the trial, subjects were given a 1500-g container of unlabeled margarine along with a standardized utensil that measured 5.5 g margarine per scoop. The subjects were instructed to consume 4 level scoops of the margarine/d on a bread product of their choice. Subjects randomly assigned to the sterol supplement groups consumed daily 22 g Proactive margarine (Unilever BestFoods, Purfleet, United Kingdom), corresponding to an intake of 1.8 g plant sterols/d. Subjects randomly assigned to receive the control margarine consumed daily 22 g Flora Light (VandenBergh Foods, Crawley, United Kingdom), a spread not fortified with sterols. The nutrient distribution of the control and sterol-enriched margarines were similar with respect to total energy, fat, carbohydrate, protein, and fiber (Table 1). The study was single-blinded such that the subjects did not know whether they were receiving the control or sterol-enriched margarine. Compliance with the margarine protocol was assessed by weighing the containers on days 0 and 56, and

the calculated difference was taken to represent the amount of margarine consumed. In addition, subjects were required to complete a "Daily Margarine Diary," indicating the number of scoops consumed per day. Subjects were asked to maintain their regular diet regimens throughout the course of the trial.

Blood collection protocol

Twelve-hour fasting blood samples were collected on the mornings of days 0, 53, 54, and 55 of the trial. Blood was centrifuged for 15 min at 520 x g and 4 °C to separate plasma from red blood cells and was stored at –20 °C until analyzed.

Analyses

Plasma lipoprotein determination

ApoA and apoB were measured using Dade Behring N Antisera to Apo A-1 and Apo B assay (Dade Behring Diagnostics, Marburg, Germany) on the BN ProSpec Nephelometer (Dade Behring Diagnostics).

Plasma hormones

Adiponectin and total ghrelin were measured using radioimmunoassay technique (Linco Research Inc., St. Charles, MO). RIA for adiponectin has a limit of detection of 1microg/L and a linear range of 0.78-200microg/L (intraassay CV = 3.6% low and 1.8% high; interassay CV = 9.3%). The sensitivity of the assay is 30pmol/L for samples of 100microL. All samples from a given subject were analyzed in duplicate and in the same assay. The total ghrelin kit had a sensitivity of 93 pg/mL

when using a 100microL sample size. The intraassay CV was 10% for low and 4.4% for high and the interassay CV was 14.7% for low and 16.7% for high. Growth hormone was measured using a high-sensitivity H-hGH enzyme linked immunosorbant assay (ELISA) kit that employed a quantitative sandwich immunoassay method (Anogen, Mississauga, Ontario). The sensitivity of the kit was 0.1ng/mL.

Statistics

Results are presented as means ± SEMs. Differences between groups at baseline were analyzed with the use of a one-way analysis of variance (ANOVA) model. When a significant difference was found between groups, a Tukey post hoc test was performed to determine the differences between group means. When baseline differences were noted for a specific variable, analysis of covariance was performed with the baseline value as a covariate. Differences between group post-treatment values and percentage of change from the beginning to the end of the trial were analyzed with the use of a two-factor ANOVA model, which identified sterol and exercise effects and their interactions. A level of statistical significance at $P < 0.05$ was used in all analyses. Tests for normality were included in the model.

Sample size was calculated with the assumption of a 10% change in LDL-cholesterol concentrations, with a power of 80% and an risk of 5%. Data were analyzed by using Graph Pad Prism (version 4.0) and SAS software (version 8.0; SAS Institute Inc, Cary, NC).

5.4 RESULTS

Subject dropout and compliance

Eighty-four subjects commenced the study, with 74 completing the entire 8-wk trial. Eight subjects dropped out because of time constraints, and 2 others dropped out because of injuries not resulting from participation in the study. After loss because of dropouts, the remaining subjects in each intervention group were as follows: combination group (n = 18), exercise group (n = 18), sterol group (n = 18), and control group (n = 20). The mean attendance at the 24 exercise sessions was 23.4 and 23.2 sessions attended for the combination and exercise groups, respectively. The mean daily margarine consumption for the combination, exercise, sterol, and control groups was 21.7, 21.7, 21.6, and 21.9 g/d, respectively. With respect to blinding, subjects were not able to identify which margarine they were consuming. Furthermore, during the study, no changes were reported with regard to diet or lifestyle habits.

Subject baseline characteristics

Baseline characteristics of the subjects who completed the 8-wk trial are presented in **Table 1**. Lipid concentrations denoted in the table are based on the values obtained from the initial blood screen. On average, the subjects within each intervention group were hypercholesterolemic (total cholesterol concentrations >5.2 mmol/L). No significant difference was noted at the beginning of the study between the groups with regard to age, body mass index, plasma lipid concentrations, exercise level, apoA, apoB, adiponectin and growth hormone. Only ghrelin values were different at baseline measurement. Furthermore,

no differences were noted between those participants who completed the trial and those participants who did not.

Plasma lipids

Plasma lipids, including TC, LDL-C, HDL-C, and TG, as measured by KA Varady and presented in the subsequent journal article (95) are presented in **Table 2.** Particle size data, referred to in certain correlations in this study, was also measured by KA Varady, and is presented in the respective journal article (96).

Plasma hormone levels

Mean plasma hormone levels over the 8-wk trial are presented in **Table 3**. No significant difference was observed between groups in mean apoA concentrations at baseline. Baseline apoA concentrations showed a weak correlation (r^2=0.054, P=0.05) to HDL values. When these data were analyzed with the use of two-factor ANOVA, sterol-by-exercise interactions were non significant. Also, no significant main effect was observed for sterols or exercise on post-treatment absolute values or on percent change for ApoA. After correction for changes in the control group, ApoA concentrations for the combination, exercise and plant sterol group were -1.7%, 15.3%, and -6.7%, respectively. Percent change in ApoA was correlated to percent change in HDL (r^2=0.328, P=0.01) for the exercise group only.

No significant difference was noted for ApoB concentrations at baseline. There were no significant sterol-by-exercise interactions. ApoB values at baseline were correlated (r^2=0.129, P=0.0018) to LDL values, particle size (r^2=0.143, P=0.001) and number of small particles (r^2=0.259, P<0.0001). There were no significant main effects of sterols or exercise in post-treatment values or in percent change in ApoB.

After correcting for changes in the control group, ApoB concentrations for the combination, exercise and plant sterol groups were 6.4%, 13.6%, and 1.2%, respectively.

Mean adiponectin concentrations did not differ significantly at baseline between groups and there were no significant sterol-by-exercise interactions. At baseline, adiponectin values were positively correlated to BMI (r^2=0.104, P=0.006) and HDL concentrations (r^2=0.167, P=0.0003). No significant main effect of sterols or exercise was noted for absolute adiponectin values post-treatment. When adiponectin concentrations were expressed as the difference between pretreatment and post-treatment values, a significant (P=0.04) main effect of sterols was noted. After correction for the changes in the control group, adiponectin concentrations in the combination, exercise and plant sterol groups were 9.0%, 2.2%, and 15.3% respectively.

Ghrelin concentrations were shown to be significantly (P<0.01) different between groups at baseline. After further analysis, it was shown that the mean baseline exercise group ghrelin concentrations were higher than those of the combination, sterol and control groups. Results of the two-factor ANOVA showed a significant (P<0.05) sterol-by-exercise interaction. Using an ANCOVA analysis, no significant main effects of sterols or exercise on absolute post-treatment values or percent change were shown. After correction for the changes in the control group, the ghrelin concentrations of the combination, exercise and sterol groups were 7.5%, 4.0%, and -4.6%, respectively.

Growth hormone concentrations were not significantly different between groups at baseline. There was a significant ($P<0.05$) sterol-by-exercise interaction when groups were compared with two-way ANOVA at baseline. No significant main effect of exercise or sterols was shown on absolute post-treatment values or percent change in growth hormone values. After correction for the changes in the control group, mean concentrations of growth hormone were -0.77%, 0.74%, and 0.46%, respectively.

5.5 DISCUSSION

This study showed that plant sterol supplementation can favorably alter markers for cardiovascular disease such as adiponectin. Adiponectin has been shown to have anti-inflammatory properties as well as cardio-protective effects in reducing vascular stenosis. Increased adiponectin values have been associated with less risk of cardiovascular disease. In previous studies, increased adiponectin levels have been attained through exercise and weight loss regimes. This is the first study to show a significant increase of adiponectin through the use of plant sterols. Plant sterols have been shown in previous studies to exert anti-inflammatory effects, by decreasing CRP, IL-1 and IL-6, and increasing IL-10 (20,21). Similar findings are true for adiponectin, which has been shown to be inhibited by TNFα and in turn decreases TNFα, IL-6 actions (55,67). Possibly by altering the ratio of anti-inflammatory to pro-inflammatory mediators, supplementation with plant sterols may increase adiponectin values, and highlight the anti-inflammatory nature of adiponectin. This in turn can exert anti-atherogenic effects at the level of the vessels. Exercise training alone showed no significant increase in adiponectin values (2.2%), which is similar to most of the previous findings in exercise-only trials without significant weight loss (57,60, 61,62).

Ghrelin levels at baseline were not correlated to body weight, BMI or body fat which supports previous findings (84). Although there was a significant decrease in percent body fat in the exercise group, the average increase of 4% in ghrelin values for this group was not significant. In previous studies, changes in ghrelin values have been mostly obtained through dieting-induced weight loss (82, 84). Since diet was not

controlled in this trial, it is possible that the energy deficit caused by exercising, if compensated by increasing food intake and thereby neutralizing an energy deficit, would not cause significant changes in ghrelin. The changes in body composition caused by exercise including decreased fat mass and increased lean body mass don't necessarily cause changes in body weight and therefore may explain why exercise alone does not seem to alter ghrelin values. However, there was a trend of increased ghrelin with decreased body fat (r^2=0.08, P=0.05) which is similar to previous results showing changes in ghrelin are sensitive to changes in body weight.

Regarding the large diurnal variation in ghrelin values, it has previously been shown that a single fasting ghrelin measurement is representative of 24hr ghrelin values. Also, in terms of sample storage, it has been shown that plasma can be stored from 3 months to 3 years in minus 80°C with no effects on ghrelin values (82). However, precaution is needed in interpreting these ghrelin values as it has been shown that bioactive ghrelin doesn't have a fixed ratio to total ghrelin values, and that up to 80% of total ghrelin is deaminated and inactive (76).

ApoA values increased by 16.8% in the exercise group, which was correlated to the changes previously found in HDL in this group. ApoA showed a mean decrease in the plant sterols group similar to the small decrease in HDL values.

ApoB lipoprotein is associated with LDL cholesterol and the number of small dense LDL particles. ApoB decreased in the plant sterol group similar to decreases previously observed in LDL values. The small decrease in apoB in the control group was similar to a small decrease in LDL in controls and may be due to the fact that the diet was not controlled. ApoB values increased in the exercise and combination

groups. There was also an increase in LDL in the exercise group and it was concluded that exercise may decrease the cholesterol lowering effects of the plant sterols. Therefore, the combination group showed less lowering of LDL and an increase in ApoB. Exercise in previous trials has been shown to exert its effects mainly by increasing HDL, and thereby increasing RCT (21,29,30). According to a previously mentioned trial by Thompson et al, exercise has been shown to decrease apoB (29), but for the most part, LDL and apoB have not been shown to vary significantly with exercise alone (30).

Growth hormone values did not change significantly in any of the three intervention groups. In previous studies where growth hormone has been shown to increase with exercise, the blood samples were obtained at intervals before, during and directly after exercise training in order to observe the exercise-induced changes (89,90,91,92). Other studies have used 24hr GH sampling (86,87) in order to estimate total GH secretion. Diurnal variations in growth hormone are quite large, and it has not been shown that a single fasting sample is representative of total growth hormone variation. It was concluded from these results that the lack of variation between the groups and in the pre to post treatment measures was due to inadequate sampling. In order to observe the changes associated with exercise in particular, it may be necessary to take more specifically timed blood samples.

In summary, this study showed that adiponectin was correlated at baseline with HDL values and inversely with BMI, as had previously been shown in the literature. Adiponectin was increased significantly with plant sterol supplementation which is a novel finding for research in nutrition and CVD. Ghrelin and growth

hormone values were not changed with either exercise training or plant sterol supplementation, possibly due to experimental design or other confounding factors as mentioned above. ApoA and ApoB were correlated at baseline with HDL and LDL values, respectively, and changes in apoA from exercise training were correlated to the changes previously observed in HDL values.

TABLE 1

Baseline characteristics of the subjects in the four intervention groups who completed the 8 wk trial[1, 2]

Variables	Combination group (n = 4 M, 14F)	Exercise group (n = 4 M, 14F)	Sterol group (n = 8 M, 10F)	Control group (n = 4 M, 16F)	P between groups[3]
Age (y)	50.9 ± 1.9	53.3 ± 1.9	58.6 ± 2.2	54.8 ± 2.4	0.10
BMI (kg/m^2)	30.0 ± 1.4	29.8 ± 1.1	26.6 ± 1.4	26.1 ± 1.1	0.07
Lipids (mmol/L)					
Total cholesterol	5.64 ± 0.29	5.92 ± 0.36	5.90 ± 0.23	5.88 ± 0.26	0.89
LDL cholesterol	3.72 ± 0.26	3.80 ± 0.27	3.63 ± 0.20	3.93 ± 0.31	0.88
HDL cholesterol	1.26 ± 0.07	1.39 ± 0.07	1.33 ± 0.10	1.44 ± 0.09	0.48
Triacylglycerol	1.45 ± 0.15	1.57 ± 0.19	1.78 ± 0.17	1.64 ± 0.27	0.73
Exercise level (h/wk)[3]	0.81 ± 0.05	0.88 ± 0.06	0.78 ± 0.06	0.78 ± 0.05	0.54

1 Baseline characteristics from KA Varady (95)
2 All values are expressed as mean ± SEM
3 P between groups measured using two-way ANOVA

TABLE 2

Plasma lipid concentrations at baseline and after treatment[1]

	Concentration				Change		
	Value		*P*			*P*	
	Baseline	After treatment	Main effect of sterols	Main effect of exercise	% value	Main effect of sterols	Main effect of exercise
	mmol/L				%		
Total cholesterol							
Combination	5.44±0.33	5.02±0.31	0.26	0.45	-7.7±1.24	0.01	0.21
Exercise group	5.51±0.29	5.50±0.27			-0.2±2.11		
Sterol group	5.94±0.35	5.39±0.29			-9.4±1.24		
Control group	5.61±0.27	5.48±0.25			-2.3±0.79		
LDL cholesterol							
Combination	3.60±0.33	3.30±0.31	0.01	0.92	-8.3±1.85	0.48	0.96
Exercise group	3.61±0.22	3.77±0.21			4.5 ±332		
Sterol group	3.55±0.37	3.06±0.28			-13.7±2.17		
Control group	4.04±0.25	3.95±0.24			-2.4 ± 0.92		
HDL cholesterol							
Combination	1.09±0.06	1.18±0.06	0.61	0.76	7.5±1.29	0.43	0.01
Exercise group	1.19±0.08	1.31±0.09			9.5 ± 1.82		
Sterol group	1.31±0.12	1.29±0.12			4.1 ± 0.97		
Control group	1.28±0.10	1.25±0.10			-1.7 ± 1.09		
Triacylglycerol							
Combination	1.34±0.14	1.19±0.12	0.03	0.06	-11.8±1.63	0.52	0.01
Exercise group	1.35±0.14	1.13±0.11			-16.6±2.15		
Sterol group	1.80±0.25	1.74±0.20			-3.4 ± 1.76		
Control group	1.33±0.12	1.31±0.11			-2.1 ± 1.40		

1 Values are expressed as mean ± SEM

2 After-treatment values are an average end-point of day 53 and day 54

TABLE 3
Plasma apolipoproteins and hormone values at baseline and after interventions[1]

	Baseline	Post-treatment[2]	Effect of PS	Effect of EX	%value	Effect of PS	Effect of EX
					%		
APOA (g/L)							
Combination	1.05±0.054	1.04±0.31	0.58	0.07	-0.11±3.8	0.05	0.09
Exercise group	1.2± 0.093	1.10±0.085			16.8± 9.1		
Sterol group	1.09±0.085	1.01±0.068			-5.07±3.9		
Control group	0.88±0.082	0.87±0.068			1.59±5.43		
APOB (g/L)							
Combination	0.66±0.042	0.67±0.043	1.00	0.3	4.99± 6.7	0.66	0.17
Exercise group	0.69±0.051	0.74±0.044			12.2±7.6		
Sterol group	0.71±0.055	0.69±0.048			-3.82±5.1		
Control group	0.65±0.054	0.62±0.058			-1.41±7.3		
Adiponectin [3]							
Combination	23.4 ± 3.27	26.0 ± 3.87	0.72	0.90	9.29±4.35	0.04	0.69
Exercise group	20.9 ± 2.34	21.6 ± 2.88			2.55±6.21		
Sterol group	19.3 ± 2.23	22.4 ± 2.60			15.7±6.09		
Control group	24.0 ± 3.59	24.4 ± 3.80			0.33±4.32		
Ghrelin (pg/mL)							
Combination	783 ± 82	823.8±88.7	0.44	0.44	10.4±15.0	0.95	0.35
Exercise group	1257±121	1358±110			10.6±6.15		
Sterol group	759 ± 131	760±131			2.07±6.51		
Control group	816 ± 73.8	870± 88.2			6.64±5.16		
GH (ng/mL)							
Combination	4.49±0.41	4.32±0.32	0.67	0.97	-1.37±3.7	0.79	0.88
Exercise group	3.75±0.30	3.74±0.27			0.14±3.8		
Sterol group	3.79±0.23	3.62±0.32			-0.14±2.8		
Control group	4.52±0.37	4.46±0.30			-0.60±3.8		

1 Values are expressed as mean ± SEM
2 Post-treatment values are an average end-point of day 53 and day 54
3 Adiponectin values are expressed in μg/mL

Figure 1

ApoA and HDL-C at baseline correlation (r^2=0.328, P=0.01)

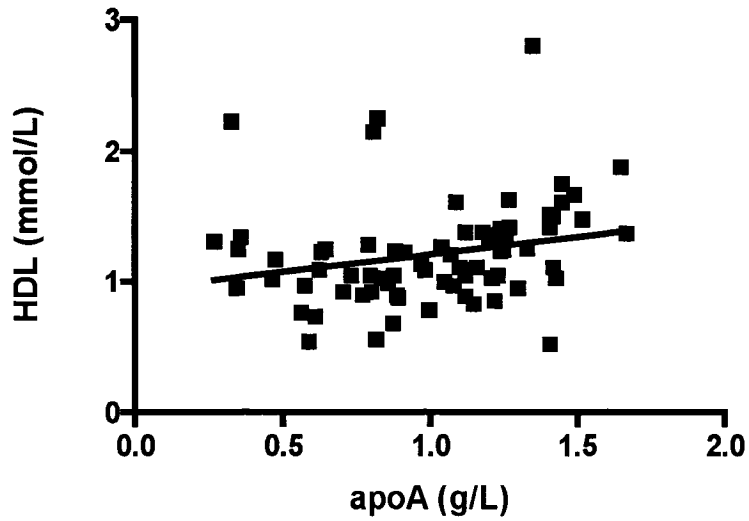


Figure 2

ApoA and HDL percent change

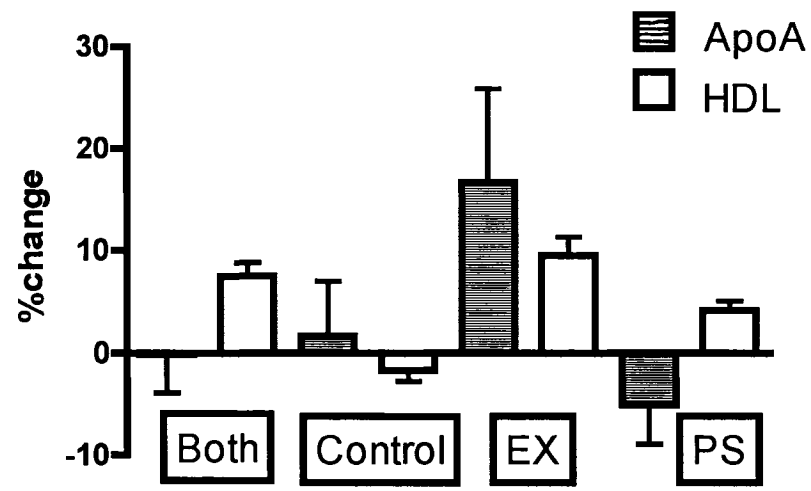


Figure 3

ApoB and LDL at baseline correlation (r^2=0.129, P=0.0018)

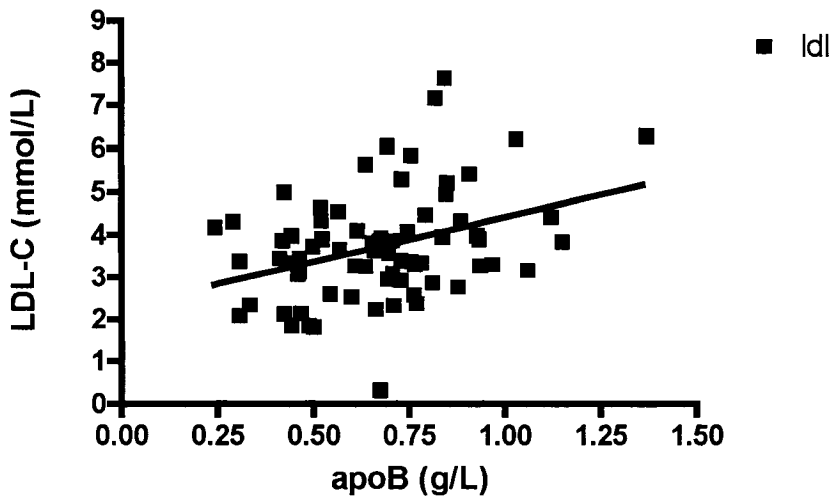
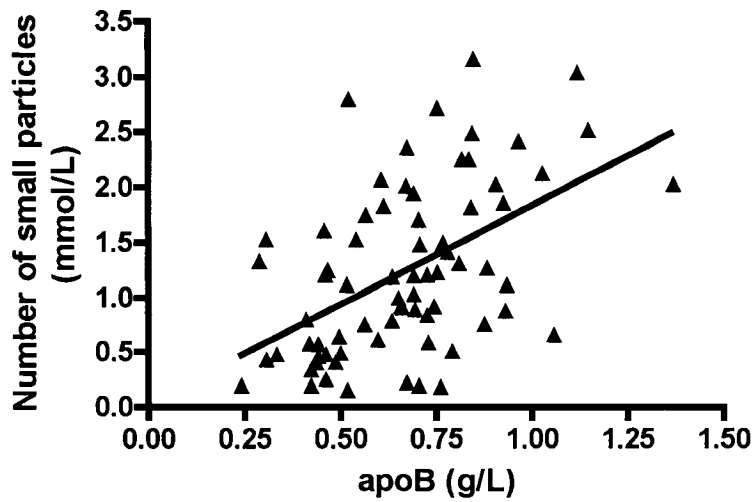


Figure 4

ApoB and #small particles[1] **at baseline correlation** (r^2=0.259, P<0.0001)



[1]Particle size and number data obtained from KA Varady (96)

Figure 5

ApoB and LDL percent change

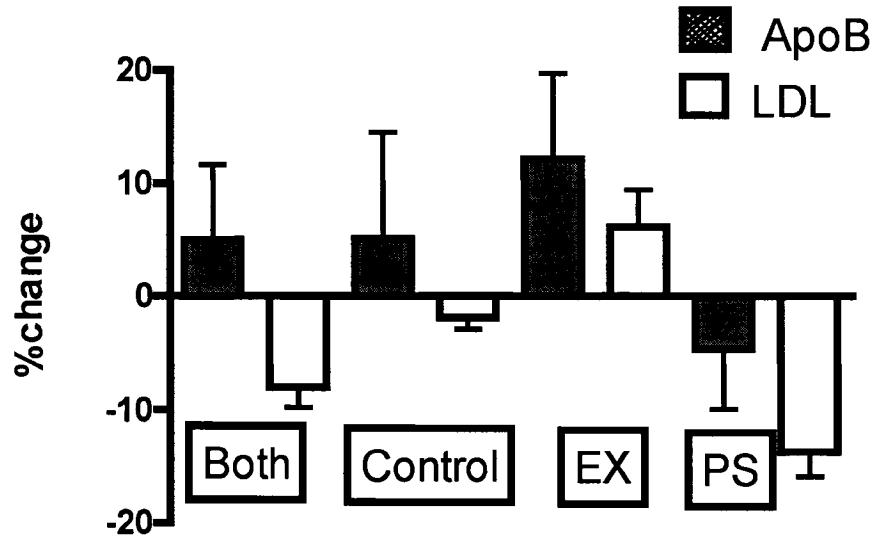


Figure 6- Adiponectin and HDL at baseline correlation (r^2=0.167, P=0.0003)

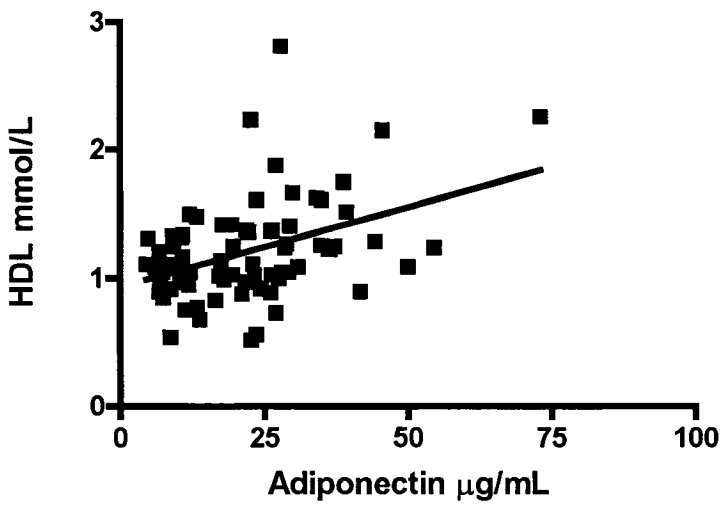


Figure7

Adiponectin and BMI at baseline correlation (r^2=0.104, P=0.006)

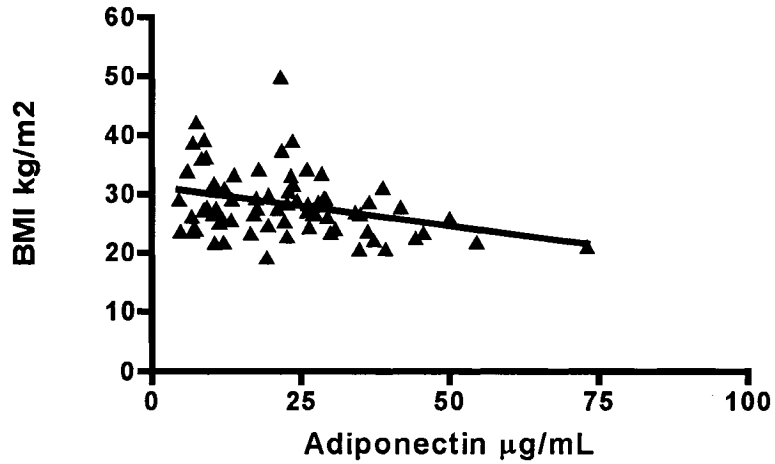
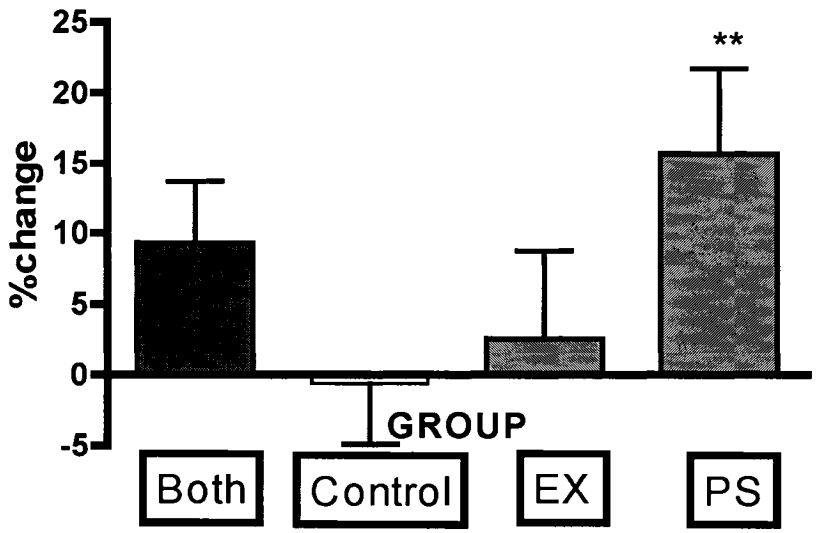


Figure 8

Adiponectin percent change for all 4 groups



**** significant change in adiponectin values (P<0.05)**

7. FINAL CONCLUSIONS

7.1 Summary of Results

Plant sterol supplementation in hypercholesterolemic adults led to an increase in adiponectin values of 15.3% compared to controls. Exercise training alone had no effect on adiponectin values (2.2%) nor did the combination of both exercise and plant sterols (9% compared to controls). Adiponectin values at baseline were correlated to HDL and BMI.

ApoA values increased with exercise training by 16.8%, which was correlated to the changes previously seen in HDL values. ApoB values were correlated with LDL values, particle size and with the number of small particles in plasma at baseline and were not altered with either intervention.

Growth hormone values were not altered substantially with either exercise training or plant sterol supplementation. Ghrelin values increased slightly in all intervention groups, and the small changes were negatively correlated to the changes in body weight. Due to the lack of changes in growth hormone values, possibly due to inadequate timing of blood sampling, we were unable to determine whether the changes in growth hormone that have been shown to be associated with exercise, were related to or caused by, changes in ghrelin.

In conclusion, consumption of plant sterols can increase adiponectin levels and offer anti-inflammatory and anti-atherosclerotic protection against cardiovascular disease. The combination of lifestyle modifications including plant sterols and endurance training has been shown in previous work to favorably alter lipid profiles. This study further shows that modifying diet to include plant sterols can alter markers for cardiovascular disease including adiponectin.

7.2 Future Research

There are few studies that have looked at the effects of plant sterols on inflammatory markers in humans (20,21). There is evidence from animal studies and the current trial that support the role of plant sterols as an anti-inflammatory agent in the prevention of CVD. A similar study to this one, involving plant sterols and exercise training, including measures of CRP, IL-1, IL-6 or TNFα may reveal interesting results. By measuring other markers of inflammation as well as adiponectin, the mechanism by which plant sterols affect adiponectin levels either directly or indirectly through the ratio involving IL-6 and TNFα, may be clearer.

In contrast to other adipocytokines, adiponectin levels are low in obesity, diabetes and CVD. This makes adiponectin an excellent therapeutic target for pharmacologic treatment. It has been suggested from animal experiments that increasing plasma adiponectin might be useful in preventing vascular re-stenosis after vascular intervention (70) and that adiponectin supplementation may reduce atherosclerotic plaque formation (72).

The therapeutic use of adiponectin could be quite beneficial in humans, although human experiments have yet to be performed. In humans, it has already been shown that increased coronary artery atherosclerosis and ischemic heart disease are associated with low adiponectin levels (52). Investigating the effects of increasing adiponectin levels in humans with regards to progression of atherosclerotic coronary artery disease would provide more substantial evidence for targeting adiponectin in the prevention of CVD.

With regards to studying the potential involvement of ghrelin in regulating the growth hormone response to exercise, there are several details that would be important for future study. Firstly, the growth hormone measurements need to be taken before during and after exercise training in order to monitor the small changes. Also, active ghrelin might be a more accurate measurement of the involved ghrelin in the plasma, and for that measurement the samples need to be stored appropriately.

7.3 Significance

This is the first study to show that plant sterol supplementation can increase adiponectin values in a group of sedentary hypercholesterolemic adults.

The anti-inflammatory effects of adiponectin indicate that it is an interesting protective factor for atherosclerosis development, particularly in clinical situations associated with low plasma concentrations of adiponectin (77).

From the evidence presented in the review of the literature, it is clear that hypoadiponectinemia can be interpreted as an important marker for CVD. It may be suggested that adiponectin be used as a measure in routine clinical screening for populations at risk for atherosclerotic heart disease. Unlike conventional lipid profile measurements such as LDL, HDL, TG and TC/LDL, adiponectin may serve as a more specific indicator of the risk that a hypercholesterolemic state may actually cause inflammatory artery disease. It is clear that high levels of circulating cholesterol impose a greater risk of artery disease however, the reaction of the arteries ie. number of circulating pro-inflammatory versus anti-inflammatory mediators, is also very important in determining the risk of atherosclerosis. From previous research it has

been shown that adiponectin and HDL were significantly lower in patients with confirmed atherosclerosis then in controls, and further reduced in the subgroup with atherosclerosis and ischemic heart disease although LDL, TC and TG levels were unchanged (52). This study highlights the importance of reduced adiponectin and corresponding reduced HDL values and the prevalence and magnitude of systemic atherosclerosis.

It is evident that lifestyle modifications that may increase adiponectin levels in individuals at risk, including plant sterol supplementation, may be protective against atherosclerotic CVD. The target population who would benefit from 1) adiponectin testing as part of routine clinical screening as well as 2) plant sterol supplementation as part of daily diet include all those with risk factors of CVD including: obesity or sedentary lifestyle and high fat diet in people with age over 40, diabetes, and previous personal or family history of CVD.

Bibliography

1. Zhang B, Bai H, Liu R, Kumagai K, Itabe H, Takano T & Saku K. (2004) Serum high-density lipoprotein-cholesterol levels modify the association between plasma levels of oxidatively modified low-density lipoprotein and coronary artery disease in men. *Metabolism* 53: 423-429.

2. Lupattelli G, Marchesi S, Lombardini R, Siepi D, Bagaglia F, Pirro M, Ciuffetti G, Schillaci G & Mannarino E. (2003) Mechanisms of high-density lipoprotein cholesterol effects on the endothelial function in hyperlipemia. *Metabolism* 52: 1191-1195.

3. Ostlund RE Jr. (2002) Phytosterols in human nutrition. *Annual Review Nutrition* 22: 533-549.

4. Normen L, Dutta P, Lia A & Anderssen H. (2000) Soy sterol esters and B-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. *American Journal of Clinical Nutrition* 71: 908-913.

5. Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY & Parsons WE. (2000) Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *Journal of Lipid Research* 41: 697-705.

6. DeJong A, Plat J & Mensink RP. (2003) Metabolic effects of plant sterols and stanols (Review). *Journal of Nutrition Biochemistry* 14: 362-369.

7. Vanstone CA, Raeini-Sarjaz M, Parsons WE & Jones PJ. (2002) Unesterified plant sterols and stanols lower low-density lipoprotein cholesterol levels equivalently in hypercholesterolemic individuals. *American Journal of Clinical Nutrition* 76: 1271-1278.

8. Hallikainen MA, Sarkkinen ES & Uusitupa MI. (2000) Plant stanol ester affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependant manner. *Journal of Nutrition* 130: 767-776.

9. Homma Y, Ikeda I, Ishikawa T, Tateno M, Sugano M & Nakamura H. (2003) Decrease in plasma low-density lipoprotein cholesterol, apolipoprotein B, cholesteryl ester transfer protein, and oxidized low-density lipoprotein by plant stanol ester-containing spread: a randomized, placebo-controlled trial. *Nutrition* 19: 369-374.

10. Temme EH, Van Hoydonck PG, Schouten EG & Kesteloot H. (2002) Effects of a plant sterol-enriched spread on serum lipids and lipoproteins in mildly hypercholesterolemic subjects. *Acta Cardiologica* 57: 111-115.

11. Law M. (2000) Plant sterol and stanol margarines and health. *British Medical Journal* 320: 861-864.

12. St-Onge MP & Jones PJ. (2003) Phytosterols and human lipid metabolism: Efficacy, safety, and novel foods. *Lipids* 38: 367-375.

13. FDA: Food labeling: health claims; plant sterol/stanol esters and coronary heart disease. [http://www.access.gpo.gov/su_docs/fedreg/a000908c.html]Food and Drug Admin Fed Reg 2000, 65: 54686-54739.

14. Berger A, Gremaud G, Baumgartner M, Rein D, Monnard I, Kratky E, Geiger W, Burri J, Dionisi F, Allan M & Lambelet P. (2003) Cholesterol-lowering properties of amaranth grain and oil in hamsters. *International Journal of Vitamin Nutrition Research* 73: 39-47.

15. Law MR, Wald NJ & Thompson SG. (1994) By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischemic heart disease? *British Medical Journal* 308: 367-372.

16. Stampfer MJ, Sacks FM, Salvini S, Willett WC & Hennekens CH. (1991) A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *New England Journal of Medicine* 325: 373-381.

17. Sierksma A, Weststate JA & Meijer GW. (1999) Spreads enriched with plant sterols, either esterified 4,4 dimethylsterols or free 4 desmethylsterols, and plasma total and LDL cholesterol concentrations. *British Journal of Nutrition* 82: 273–282.

18. Miettinen TA & Gylling H. (2004) Plant stanol and sterol esters in prevention of cardiovascular diseases. *Annals of Medicine* 36: 126-134.

19. Nashed B, Yeganeh B, HayGlass KT & Moghadasian MH. (2005) Antiatherogenic effects of dietary plant sterols are associated with inhibition of proinflammatory cytokine production in Apo E-KO mice. *Journal of Nutrition* 135: 2438-2444.

20. Jenkins DJ, Kendall CW, Faulkner DA, Nguyen T, Kemp T, Marchie A, et al. (2005) Direct comparison of dietary portfolio vs statin on C-reactive protein. *European Journal of Clinical Nutrition* 59: 851-860.

21. Jafari M, Leaf DA, MacRae H, Kasem J, O'Connor P, Pullinger C, Malloy M & Kane JP. (2003) The effects of physical exercise on plasma prebeta-1 high-density lipoprotein. *Metabolism* 52: 437-442.

22. Olchawa B, Kingwell BA, Hoang A, Schneider L, Miyazaki O, Nestel P & Sviridov D. (2004) Physical fitness and reverse cholesterol transport. *Arteriosclerosis Thombosis Vascular Biology* 24: 1087-1091.

23. Gupta AK, Ross EA, Myers JN & Kashyap ML. (1993) Increased reverse cholesterol transport in athletes. *Metabolism* 42: 684-90.

24. Leaf DA, Parker DL & Schaad D. (1997) Changes in VO2max, physical activity, and body fat with chronic exercise: effects of plasma lipids. *Medicine Science Sports and Exercise* 29: 1152-1159.

25. Welty FK, Stuart E, O'Meara M & Huddleston J. (2002) Effect of addition of exercise to therapeutic lifestyle changes diet in enabling women and men with coronary heart disease to reach Adult Treatment Panel III low-density lipoprotein cholesterol goal without lowering high-density lipoprotein cholesterol. *American Journal of Cardiology* 89: 1201-1204.

26. Lavie CJ & Milani RV. (1996) Effects of cardiac rehabilitation and exercise training programs in patients >or = 75 years of age. *American Journal of Cardiology* 78: 675-677.

27. Lavie CJ & Milani RV. (1997) Benefits of cardiac rehabilitation and exercise training in elderly women. *American Journal of Cardiology* 79: 664-666.

28. Fahlman MM, Boardley D, Lambert CP & Flynn MG. (2002) Effects of endurance training and resistance training on plasma lipoprotein profiles in elderly women. *Journal of Gerontology A Biological Science Medical Science* 57: B54-60.

29. Thompson PD, Yurgalevitch SM, Flynn MM, Zmuda JM, Spannaus-Martin D, Saritelli A, Bausserman L & Herbert PN. (1997) Effect of prolonged exercise training without weight loss on high-density lipoprotein metabolism in overweight men. *Metabolism* 46: 217-223.

30. Tikkanen HO, Hamalainen E & Harkonen M. (1999) Significance of skeletal muscle properties on fitness, long term physical training and serum lipids. *Atherosclerosis* 142: 367-378.

31. Kim JR, Oberman A, Fletcher GF & Lee JY. (2001) Effect of exercise intensity and frequency on lipid levels in men with coronary heart disease: Training Level Comparison Trial. *American Journal of Cardiology* 87: 942-946; A3.

32. Talbot LA, Morrell CH, Metter EJ & Fleg JL. (2002) Comparison of cardiorespiratory fitness versus leisure time physical activity as predictors of coronary events in men aged < or = 65 years and > 65 years. *Amercian Journal of Cardiology* 89: 1187-1192.

33. Neuhouser ML, Miller DL, Kristal AR, Barnett MJ & Cheskin LJ. (2002) Diet and exercise habits of patients with diabetes, dyslipidemia, cardiovascular disease or hypertension. *Journal of the American College of Nutrition* 21: 394-401.

34. Leon AS & Sanchez OA. (2001) Response of blood lipids to exercise training alone or combined with dietary intervention. *Medicine Science Sports Exercise* 33: 502-515 (Suppl).

35. Kin Isler A, Kosar SN & Korkusuz F.(2001) Effects of step aerobics and aerobic dancing on serum lipids and lipoproteins. *Journal of Sports Medicine and Physical Fitness*. 41: 380-385.

36. Farrell PA & Barboriak J. (1980) The time course of alterations in plasma lipid and lipoprotein concentrations during eight weeks of endurance training. *Atherosclerosis* 37: 231-238.

37. Woolf-May K, Kearney EM, Owen A, Jones DW, Davison RC & Bird SR. (1999) The efficacy of accumulated short bouts versus single daily bouts of brisk walking in improving aerobic fitness and blood lipid profiles. *Health Education and Research* 14: 803-815.

38. King AC, Haskell WL, Taylor CB, Kraemer HC & DeBusk RF. (1991) Group- versus home-based exercise training in healthy older men and women. A community-based clinical trial. *Journal of the American Medical Association* 266: 1535-1542.

39. Schuit AJ, Schouten EG, Miles TP, Evans WJ, Saris WH & Kok FJ. (1998) The effect of six months training on weight, body fatness and serum lipids inapparently healthy elderly Dutch men and women. *International Journal of Obesity Related Metabolic Disorders* 22: 847-853.

40. Woolf-May K, Jones W, Kearney EM, Davison RC & Bird S. (2000) Factor XIIa and triacylglycerol rich lipoproteins: responses to exercise intervention. *British Journal of Sports Medicine* 34: 289-292.

41. Giada F, Vigna GB, Vitale E, Baldo-Enzi G, Bertaglia M, Crecca R & Fellin R. (1995) Effect of age on the response of blood lipids, body composition, and aerobic power to physical conditioning and deconditioning. *Metabolism* 44: 161-165.

42. Leon AS, Rice T, Mandel S, Despres JP, Bergeron J, Gagnon J, Rao DC, SkinnerJS, Wilmore JH & Bouchard C. (2000) Blood lipid response to 20 weeks of supervised exercise in a large biracialpopulation: the HERITAGE Family Study. *Metabolism* 49: 513-520.

43. Schwartz RS, Cain KC, Shuman WP, Larson V, Stratton JR, Beard JC, Kahn SE,Cerqueira MD & Abrass IB. (1992) Effect of intensive endurance training on lipoprotein profiles in young andolder men. *Metabolism* 41: 649-654.

44. Ronti T, Lupattelli G & Mannarino E. (2006) The endocrine function of adipose tissue: an update. *Clinical Endocrinology (Oxford)* 64: 355-365.

45. Pajvani UB, Hawkins M, Combs TP, Rajala LW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SF, Olefsky M, Buchanan TA & Scherer PE. (2002) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidine-mediated improvement in insulin sensitivity. *Journal of Biological Chemistry* 279: 12152-12158.

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

47. Matsubara M, Maruoka S & Katayose S. (2002) Decreased plasma adiponectin concentrations in women with dyslipidemia. *Journal of Clinical Endocrinology and Metabolism* 87: 2764-2768.

48. Matsubara M, Katyose S & Maruoka A. (2003) Decreased plasma adiponectin concentrations in nondiabetic women with elevated homeostasis model assessment ratios. *European Journal of Endocrinology* 148: 343-348.

49. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama N, Matsubara K, Okazaki Y, Ishii T, Nishikai K& Saruta T. (2002) Correlation of the adipocyte derived protein adiponectin with insulin resistance index serum high-density lipoprotein-cholesterol in the Japanese population. *Clinical Science* 103: 137-142.

50. Kondo T, Kobayashi I & Murakami M. (2006) Effect of exercise on circulating adipokine levels in obese young women. *Endocrine Journal* 53: 189-195.

51. Rothenbacher D, Brenner H, Marz W & Koenig W. (2005) Adiponectin, risk of coronary heart disease and correlations with cardiovascular risk markers. *European Heart Journal* 26: 1579-1584.

52. Kawano T, Saito T, Yasu T, Nakamura T, Namai K, Tamemoto H, Kawakami M, Saito M, Ishikawa SE. (2005) Close associations of hypoadiponectinemia with atherosclerosis obliterans and ischemic heart disease. *Metabolism* 54: 653-657.

53. Martin LJ, Cianflone K, Zakarian R, Nagrani R, Almasy L, Rainville DL, Cole S, Hixson JE, MacCluer JW, Blangero J & Comuzzie AG. (2004) Bivariate linkage between acylation-stimulating protein and BMI and high-density lipoproteins. *Obesity Research* 12: 669-678.

54. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A & Richelsen B. (2003) Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *American Journal of Physiology Endocrinology and Metabolism* 285: E527-E533.

55. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY & Chuang LM. (2001) Weight reduction increases plasma levels of adipose-derived anti-inflammatory protein, adiponectin. *Journal of Clinical Endocrinology and Metabolism* 86: 3815-3819.

56. Ryan AS, Nicklas BJ, Berman DM & Elahi D. (2003) Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. *International Journal of Obesity Related Metabolic Disorders* 27: 1066-1071.

57. Yatagai T, Nishida Y, Nagasaka S, Nakamura T, Tokuyama K, Shin M, Tanaka H & Ishibashi S. (2003) Relationship between exercise training-induced increase in insulin sensitivity and adiponectinemia in healthy men. *Endocrinology Journal* 50: 233-238.

58. Hulver MW, Zheng D, Tanner CJ, Houmard JA, Kraus WE, Slentz CA, Sinha MK, Pories WJ, Macdonald KJ & Dohm GL. (2002) Adiponectin is not altered with exercise training despite enhanced insulin action. *American Journal of Physiology Endocrinology and Metabolism* 283: E861-E865.

59. Kriketos AD, Gan SK, Poynten AM, Furler SM, Chrisholm DJ & Campbell LV. (2004) Exercise increases adiponectin levels and insulin sensitivity in humans. *Diabetes Care* 27: 629-630.

60. Yokoyama H, Emoto M, Araki T, Fujiwara S, Motoyama K, Morioka T, Koyama H, Shoji T, Okuno Y & Nishizawa Y. (2004) Effect of aerobic exercise on plasma adiponectin levels and insulin resistance in Type 2 Diabetes. *Diabetes Care* 27: 1756-1760.

61. Hara T, Fujiwara H, Nakao H, Mimura T, Yoshikiwa T & Fujimoto S. (2005) Body composition is related to increase in plasma adiponectin levels rather than training in young obese men. *European Journal of Applied Physiology* 94: 520-528.

62. Boudou P, Sobngwi E, Mauvais-Jarvis F, Vexiau P & Gauthier JF. (2003) Absence of exercise-induced variations in adiponectin levels despite decreased abdominal adiposity and improved insulin sensitivity in type 2 diabetic men. *Europeen Journal of Endocrinology* 149: 421-424.

63. Ridker PM, Rifai N, Rose L, Buring JE & Cook JR. (2002) Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *New England Journal of Medicine* 347: 1557-1565.

64. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M, Chrousos GP & Sidossis LS. (2005) Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. *Metabolism* 54: 1472-1479.

65. Fatouros IG, Kambas A, Katabasas I, Nikolaidis K, Chatzinikolaou A, Leontsini D & Taxildaris K. (2005) Strength training and detraining effects on muscular strength, anaerobic power, and mobility of inactive older men are intensity dependent. *British Journal of Sports Medicine* 39: 776-780.

66. Jamurtas AZ, Theocharis V, Koukoulis G, Stakias N, Fatouros IG, Kouretas D & Koutedakis Y. (2006) The effects of acute exercise on serum adiponectin and resistin levels and their relation to insulin sensitivity in overweight males. *European Journal of Applied Physiology* 97: 122-126.

67. Undurti N. (2004) Anti-inflammatory nature of exercise. *Nutrition* 20: 323-326.

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

69. Ouchi N, Kihara S, Funahashi T, Matsuzawa W & Walsh K. (2003) Obesity, adiponectin and vascular inflammatory disease. *Current Opinions in Lipidology* 14: 561-566.

70. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida K, Matsuzawa Y, et al. (2002) Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. *Journal of Biology and Chemistry* 277: 37487-37491.

71. Fernandez-Real JM, Lopez-Bermejo A, Casamitjina R & Ricart W. (2004) Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *Journal of Endocrinology and Metabolism* 88: 2714-2718.

72. Okamoto Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T & Matsuzawa Y. (2002) Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 106: 2767–2770.

73. Chen H, Montagnani M, Funahashi T, Shimomura I & Quon MJ. (2003) Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *Journal of Biological Chemistry* 278: 45021-45026.

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

75. Punyadeera C, Zorenc AH, Koopman R, McAinch AJ, Smit E, MAnders R, Keizer HA, Cameron-Smith D & van Loon LJ. (2005) The effects of exercise and adipose tissue lipolysis on plasma adiponectin receptor expression in human skeletal muscle. *European Journal of Clinical Endocrinology* 152: 427-432.

76. Casanueva FF & Dieguez C. (2004) Ghrelin a new hormone implicated in the regulation of growth hormone secretion and body energy homeostasis. *Current Growth Genetics and Hormones* 20: 1-8.

77. Meiera U & Gressner AM. (2004) Endocrinology regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clinical Chemistry* 50: 1511-1525.

78. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GE, Murphy KG, Dhillo WS, Ghatei MA & Bloom SR. (2001) Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology Metabolism* 86: 5992-5995.

79. Fagerberg B, Hulten LM & Hulthe J. (2003) Plasma ghrelin, body fat, insulin resistance, and smoking in clinically healthy men: the atherosclerosis and insulin resistance study. *Metabolism* 52: 1460-1463.

80. Cummings DE, Weigle DS & Frayo RS. (2003) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine* 346: 1623-1630.

81. Nakasoto M, Murakami N, Date Y, et al. (2001) A role for ghrelin in the central regulation of feeding. *Nature* 409: 194-198.

82. Foster-Schubert KE, McTiernan A, Frayo RS, Schwartz RS, Rajan KB, Yasui Y, Tworoger SS & Cummings DE. (2005) Human plasma ghrelin levels increase during a one-year exercise program. *The Journal of Clinical Endocrinology and Metabolism* 90: 820-825.

83. Bagnasco M, Kalra PS & Kalra SP. (2002) Ghrelin and leptin pulse discharge in fed and fasted rats. *Endocrinology* 143: 726-729.

84. Leidy HJ, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL & Williams NI. (2004) Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. *Journal of Clinical Endocrinology and Metabolism* 89: 2659-2664.

85. Lanfranco F, Gianotti L, Giordano R, Pellegrino M, Maccario M & Arvat E. (2003) Ageing, growth hormone and physical performance. *Journal of Endocrinology Investigation* 26: 861-872.

86. Vahl N, Klausen I, Christiansen JS & Jergensen JOL. (1999) Growth hormone status is an independent determinant of serum levels of cholesterol and triglycerides in healthy adults. *Clinical Endocrinology* 51: 309-316.

87. Weltman A, Despres JP, Clasey JL, Weltman JY, Wideman L, Kanaley J, Patrie J, Bergeron J, Thorner MO, Bouchard C & Hartman ML. (2003) Impact of abdominal visceral fat, growth hormone, fitness, and insulin on lipids and lipoproteins in older adults. *Metabolism* 52: 73-80.

88. Jorgensen JO, Krag M, Kanaley J, Hansen TK, Moller N, Christiansen JS & Orskov H. (2003) Exercise, hormones, and body temperature. Regulation and action of GH during exercise. *Journal of Endocrinology Investigation* 26: 838-842.

89. DeVries WR, Abdesselam SA, Schers TJ, Maas HC, Osman-Dualeh M, Maitimu I & Koppeschaar HP. (2002) Complete inhibition of hypothalamic somatostatin activity is only partially responsible for the growth hormone response to strenuous exercise. *Metabolism* 51: 1093-1096.

90. De Vries WR, Schers TJ, Ait Abdesselam S, Osman-Dualch M, Maitimu I & Koppeschaar HP. (2003) Involvement of endogenous growth hormone-releasing hormone in the exercise-related response of growth hormone. *International Journal of Sports Medicine* 24: 208-211.

91. Schmidt A, Maier C, Schaller G, Nowotny P, Bayerle-Eder M, Buranyi B, Luger A & Wolzt M. (2004) Acute exercise has no effect on ghrelin plasma concentrations. *Hormone Metabolic Research* 36: 174-177.

92. Kraemer RR, Durand RJ, Acevedo EO, Johnson LG, Kraemer GR, Hebert EP & Castracane VD. (2004) Rigorous running increases growth hormone and insulin-like growth factor-1 without altering ghrelin. *Experimental Biological Medicine (Maywood)* 229: 240-246.

93. Dall R, Kanaley J, Hansen TK, Moller N, Christiansen JS, Hosoda H, Kangawa K & Jorgensen JO. (2002) Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. *European Journal of Endocrinology* 47: 65-70.

94. Fletcher GF. (1997) How to implement physical activity in primary and secondary prevention: a statement for healthcare professionals from the Task Force on Risk Reduction, American Heart Association. *Circulation* 96: 355-357.

95. Varady KA, Ebine N, Vanstone CA, Parsons WE & Jones P. (2004) Plant sterols and endurance training combine to favorably alter plasma lipid profiles in previously sedentary hypercholesterolemic adults after 8 wk. *American Journal of Clinical Nutrition* 80: 1159-1166.

96. Varady KA, St-Pierre AC, Lamarche B & Jones PJ. (2005) Effect of plant sterols and endurance training on LDL particle size and distribution in previously sedentary hypercholesterolemic adults. *European Journal of Clinical Nutrition* 59: 518-25.

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CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

LAWRENCE HUTCHISON, MD
MICHAL ABRAHAMOWICZ, PHD
ARTHUR CANDIB, MED
CATHERINE GARDNER, BSC
NEIL MACDONALD, MD
ROBERT L. MUNRO, BCL
ROBERTA PALMOUR, PHD
LUCILLE PANET-RAYMOND, BA
MARGARET SWAINE, BA

has examined the research project **AØ6-M50-02A** entitled **"Effects of Plant Sterol Supplementation and Exercise Training on Plasma Lipid Levels and Lipid Metabolism in the Elderly"**

as proposed by: Peter J.H. Jones (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

June 18, 2002
Date

Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458

June 5, 2006

To Whom it May Concern:

The purpose of the present letter is to confirm that the co-authors (Peter Jones and Krista Varady) agree that the candidate (Melissa Collins) includes the manuscript entitled *The effects of plant sterols and endurance training on apoA, apoB, adiponectin, growth hormone and ghrelin* in her thesis.

The candidate's roles in this study included participating in the design and protocol for the clinical trial, analyzing stored plasma samples for adiponectin, growth hormone and ghrelin, compiling the data and conducting the analyses. The candidate wrote the manuscript under the guidance of the co-authors and made modifications to it in response to their comments.

Melissa Collins

I, the co-author, agree that the candidate, Melissa Collins, include the manuscript entitled *The effects of plant sterols and endurance training on apoA, apoB, adiponectin, growth hormone and ghrelin* in her thesis.

Peter Jones

Krista Varady