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Fat Distribution and Metabolic Health: The Effects of Macronutrient Manipulation on Fat Distribution, Weight Loss, and Glucose Metabolism

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FAT DISTRIBUTION AND METABOLIC HEALTH: THE EFFECTS OF MACRONUTRIENT MANIPULATION ON FAT DISTRIBUTION, WEIGHT LOSS, AND GLUCOSE METABOLISM

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ABSTRACT

Fat distribution pattern may contribute to risk of development of metabolic diseases such and type 2 diabetes and cardiovascular disease. However, the precise nature of the relationships between adipose tissue depots and metabolic health remains controversial. Additionally, further research is needed to identify optimal dietary approaches to reduce disease risk and visceral adiposity among overweight and obese individuals, who may already be on a trajectory for development of metabolic disease. Therefore, the goal of this project was to examine relationships of adipose tissue depots with insulin sensitivity, and then to determine if diets differing in CHO and fat content can modulate fat distribution, thereby improving indices of metabolic health. Thus, the first aim of this project was to identify independent associations of adipose tissue depots with insulin sensitivity among postmenopausal women. Additional aims of this project were to determine whether consumption of a low vs high glycemic load (GL) diet would reduce total and visceral adipose tissue under both eucaloric and hypocaloric conditions, and further to determine if under eucaloric conditions, the low GL/higher fat diet influenced sex hormone-binding globulin (SHBG) and parameters of glucose metabolism.

To address the first aim, 97 healthy, early postmenopausal women underwent testing of: body composition by dual-energy X-ray absorptiometry (DXA), fat

distribution by computed tomography (CT), and insulin sensitivity by frequently sampled intravenous glucose tolerance test. For the next two aims, 69 healthy overweight participants were assigned to either a low GL diet (43%CHO, 18%PRO, 39%FAT) or high GL diet (55%CHO, 18%PRO, 27%FAT). Body composition was assessed by DXA and fat distribution by CT at baseline and after 8 weeks of a eucaloric diet intervention and 8 weeks of a hypocaloric diet intervention. Additionally, for the third aim, serum fasting SHBG, fasting glucose, fasting insulin, and glucose area under the curve following a standard liquid mixed macronutrient meal were assessed at baseline and following the eucaloric phase.

Results from these studies indicate that maintaining greater SAT and lesser IAAT or thigh IMAT promotes or reflects greater insulin sensitivity. Also, consumption of a low vs high GL diet resulted in preferential loss of IAAT during weight maintenance and greater total fat loss during caloric restriction. However, during eucaloric conditions this diet also resulted in reduced SHBG, which was associated with change in fasting and post-challenge glucose.

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INTRODUCTION

Overview of Fat Distribution and Metabolic Health

It is well known that obesity is a risk factor for development of cardiovascular and metabolic disease. However, research over the past two decades has clearly demonstrated that use of body mass index (BMI) alone may not accurately predict disease development. Elevated disease risk may be less dependent on the amount of triglyceride accumulation, but rather on the location of triglyceride storage (1). Specifically, abdominal adiposity has emerged as a stronger predictor of metabolic disease than BMI. Accumulation of adipose tissue in the intra-abdominal cavity has been associated with poor cardiovascular and metabolic outcomes independent of total adiposity (2-4). Similar associations have also been observed when lipid accumulates in the liver and skeletal muscle (5;6), whereas accumulation of lipid in subcutaneous depots has been associated favorably with indices of metabolic health (7;8). Thus, proportionally greater adipose tissue distributed to visceral and ectopic depots compared to the subcutaneous depots describes a high risk fat distribution phenotype. However, it remains equivocal whether distribution of lipid to non-subcutaneous spaces is causal in regards to poor metabolic health, or rather is a marker for underlying metabolic abnormalities.

Intra-abdominal adipose tissue (IAAT) correlates directly with intrahepatic fat content and intermuscular adipose tissue (IMAT) and it has been suggested that these are coordinated depots (9-12). Lipid deposition to the intra-abdominal cavity and ectopic

depots is likely related to dysfunction of subcutaneous adipose tissue. Under normal conditions, subcutaneous adipose tissue sequesters non-esterified fatty acids (NEFA) released from adipose tissue, and fatty acids from dietary sources (13). It has been suggested that under certain pathological conditions, such as obesity, there is a downregulation of lipid storage to subcutaneous depots resulting from a maladaptive response to postprandial increases in fatty acids (14). A reduction in the uptake of triglyceride to subcutaneous adipose tissue depots may lead to greater lipid storage to the visceral and ectopic depots (13;14). Factors contributing to reduced lipid uptake to subcutaneous adipose tissue and increased lipid storage to ectopic depots remain unclear; however it is likely a complex, multifactorial process involving the interaction of genetic and environmental factors including sex, physical activity and diet.

Lipid storage to the visceral and ectopic depots may not only serve as a biomarker for the downregulation of lipid storage to subcutaneous depots, but it may also directly contribute to metabolic disease progression. Adipose tissue acts as energy storage organ, but it has also been identified as an endocrine organ secreting adipokines, such as tumor necrosis factor-α, interleukin-6, leptin, resistin, visfatin, and adiponectin (15). Evidence suggests visceral and ectopic adipose tissue depots are associated with the dysregulation of production and secretions of these bioactive substances which may contribute to hepatic and skeletal muscle insulin resistance (15). It is also possible that free fatty acids (FFA) originating from these depots directly influence processes related to glucose metabolism and insulin sensitivity (16-18). Studies have reported evidence suggesting that FFAs released from IAAT directly into hepatic portal vein may be responsible for deleterious effects on insulin signaling and gluconeogenesis (19). Likewise, IMAT may

have similar effects on skeletal muscle insulin sensitivity due to the close proximity of this adipose tissue depot to the muscle (20); however, results from studies examining this depot are discrepant (21-23). Further research is warranted to identify the precise mechanisms linking visceral and ectopic adipose tissue to metabolic disease, and to determine if modifiable lifestyle factors, such as diet, can modulate fat distribution and disease risk.

Effects of Dietary Macronutrient Manipulation on Weight Loss, Fat Distribution, and Glucose Homeostasis

Excess caloric intake leads to excess adiposity and altered metabolic outcomes; however, diet quality may have metabolic affects unique from caloric content. In particular, glycemic index (GI), the extent to which a food increases serum glucose concentrations, has been proposed as potentially affecting weight change, body composition, or even fat distribution. By increasing insulin to a greater extent or for a longer time, foods with a relatively high GI may affect specific metabolic processes, such as lipolysis, lipogenesis, or substrate oxidation (24-26). These processes in turn may affect hunger, satiety, food intake, or energy expenditure, factors that could impact energy balance, body composition, and location of triglyceride storage. A diet comprised of low glycemic index foods may elicit a reduced insulin response and thus, have significant effects on weight loss and fat distribution.

Weight Loss

Numerous studies have been conducted in a variety of populations, ranging from young children to adults, examining the efficacy of low glycemic load (GL) or GI diets on weight and fat mass loss (27). The majority of the controlled trials used a parallel design in order to compare a low vs high GL/GI diet (27). However, these studies have

shown inconsistent findings in regards to the effectiveness of low GL/GI diets yielding greater weight loss and total fat loss compared to other dietary approaches. These inconsistencies may be due to differences in methodology, underlying physiological differences in study populations, and other confounding factors affecting diet adherence and efficacy.

Fat distribution

In addition to affecting total body weight and fat mass, diet quality may also affect the specific location of the fat that is deposited or mobilized. Results of some studies have indicated that relatively greater consumption of lower GI foods is associated with a smaller waist circumference (28;29). Among the studies that have reported associations between diet and fat distribution independent of weight loss, many have also reported sex differences in these associations (29). Halkjaer et al found that carbohydrate (CHO) energy intake from fruits and vegetables was inversely associated with change in waist circumference over a 5 year period, and conversely, CHO energy intake from all other food groups was positively associated with change in waist circumference (29). Further, these associations were significantly stronger in women than in men. Similarly, high intake of refined grains was associated with gain in waist circumference adjusted for BMI over 6 years in women but not men (28). A dietary counseling study among men and women with type 2 diabetes reported consumption of a moderately reduced CHO diet resulted in preferential visceral adipose tissue loss among women but not men (30). Males and females are generally thought to distribute fat differently, such that males exhibit the android or centripetal fat distribution pattern, whereas females have greater

lipid storage to the gluteofemoral region (31); therefore, it is possible diet quality influences lipid storage differently in regards to sex.

A low GL/GI diet may influence fat distribution independent of weight loss by lowering postprandial glucose and insulin response. However, whether these effects mediate improvement in metabolic outcomes is not clear. In a canine model, a eucaloric higher fat/reduced CHO diet induced hepatic insulin resistance and elevated gluconeogenic gene expression (19). This effect appeared to be mediated by an upregulation in fatty acid turnover in the visceral cavity, leading to elevated exposure of the liver to FFAs, via the hepatic portal vein. Studies have also reported that adipose tissue in the visceral cavity is resistant to insulin and suppression of lipolysis from this cavity requires 2-4 fold more insulin than subcutaneous depots (32;33). Thus, it seems plausible to speculate that a reduced insulin secretory response to a low GL/GI diet may lead to a reduction in adipose tissue in the intra-abdominal cavity given the propensity of this depot to mobilize FFA. However, to date, no human studies have been conducted to confirm this mechanism. Further studies are needed in order to identify the effects of diet on fat distribution, and more importantly whether these effects are accompanied by improvement in hepatic metabolism and glucose homeostasis.

SHBG and Glucose Homeostasis

Sex-hormone binding globulin (SHBG), a protein predominantly secreted by the liver, has been gaining momentum in the literature as a potential predictor of development of type 2 diabetes (34;35). It has been proposed that low circulating SHBG may be used as a clinical indicator of elevated risk of development of metabolic

abnormalities (35). The primary function of SHBG is classically known as the regulation of circulating sex hormone bioavailability by inhibiting receptor binding (36). However, studies have linked circulating SHBG with metabolic disease independent of the action of sex hormones. Therefore, SHBG may have a direct role in disease development or may be a biomarker for metabolic health. Numerous physiological factors associated with obesity have been linked to low circulating SHBG such as elevated fasting insulin and glucose, intrahepatic lipid content, and IAAT accumulation (37-40); however, whether SHBG plays a causal role or is simply a biomarker for underlying metabolic aberrations is unknown.

Studies have reported a link between fat distribution and SHBG production (37). IAAT is inversely correlated to circulating SHBG (37), an association which may be, in part, due to the proximity of this adipose tissue depot to the liver (41). The SHBG promoter region is regulated by transcription factor hepatic nuclear factor-4α that has a ligand binding domain for endogenous FFAs (10;42;43). The binding of FFAs to this ligand binding domain can alter the transcriptional activity of SHBG gene expression (44). Therefore, it is possible that the draining of FFAs from the intra-abdominal cavity may directly alter SHBG gene expression, or may indirectly influence SHBG production by modulating hepatic gluconeogenesis (19). Evidence suggests there is an independent relationship between glucose and hepatic SHBG production (34;40). The increased flux in NEFAs from IAAT may alter liver metabolism by increasing hepatic insulin resistance and hepatic glucose production, and through this mechanism suppress SHBG production

Recent data indicate that diet-induced changes to glucose homeostasis may be an important mediating factor in altering hepatic SHBG production (40;44). Glucose

homeostasis is determined in part by hepatic glucose production, which is increased as a result of hepatic insulin resistance and triglyceride accumulation (45). Low blood concentrations of SHBG have been observed among obese individuals (46) and studies consistently report that weight loss through caloric restriction results in increased blood concentrations (40;46), an effect that may be mediated by reduction in intrahepatic fat content and improvements in fasting insulin and glucose. However, whether dietary fat or CHO can influence hepatic SHBG production independent of weight loss is not clear, but evidence suggests a diet relatively high in fat increases fasting glucose concentration (47) and therefore, may alter SHBG production.

Objectives

In light of the evidence presented in this review, the need for further understanding of the relationships among the adipose tissue and metabolic health becomes apparent. Further, there is a lack of well-controlled dietary intervention studies in the literature elucidating how diet quality may influence fat distribution, propensity for weight loss, and parameters of glucose metabolism. Therefore, the objectives of this project were to: 1) examine the independent relationships of various fat depots with insulin sensitivity, 2) determine the effects of dietary macronutrient manipulation on changes in fat distribution under eucaloric conditions and weight loss during hypocaloric conditions , and 3) determine the effects of macronutrient manipulation on glucose metabolism and SHBG, and identify the interrelationships among changes in glucose metabolism, SHBG, and fat distribution.

Experimental Aims

Experimental Aim 1

The first aim was to examine the cross-sectional relationships of various fat depots with insulin sensitivity. To investigate this aim, 97 early, healthy postmenopausal women underwent insulin sensitivity testing, assessed by using a frequently sampled intravenous glucose tolerance test with minimal model analysis. Fat distribution was determined using computed tomography (CT) scanning of the abdomen and midthigh. Body composition data were also collected using dual energy x-ray absorptiometry (DXA).

Experimental Aim 2

The second experimental aim was to determine whether consumption of an 8-wk low GL diet vs high GL diet would facilitate changes in fat distribution under eucaloric conditions and, additionally, to determine if consumption of an 8-wk low GL diet vs high GL diet would produce greater weight loss under hypocaloric conditions among 69 healthy overweight or obese men and women. At week 0 and week 8 of the eucaloric and hypocaloric phase, fat distribution was assessed by using CT scanning and body composition was assessed by using DXA. Thus, changes in fat distribution and body composition were compared between diet groups.

Experimental Aim 3

The third experimental aim was to investigate changes in variables related to glucose metabolism (fasting glucose, fasting insulin, glucose area under the curve (AUC), and insulin AUC and SHBG in response to an 8-week eucaloric diet higher in fat vs. an 8-

week diet lower in fat. This aim included the examination of the baseline relationships between circulating SHBG, glucose metabolism, and fat distribution and the relationships of change in these variables following the diet intervention period. At week 0 and week 8 of the eucaloric diet intervention, a fasting blood draw was conducted to assess glucose, insulin, and SHBG. Additionally, glucose and insulin AUC were calculated following consumption of a standard liquid mixed meal challenge. Fat distribution was assessed by using CT scanning and body composition was assessed by using DXA.

The investigations performed to address each of the experimental aims are described in detail in the following three manuscripts.

INSULIN SENSITIVITY IS ASSOCIATED WITH THIGH ADIPOSE TISSUE DISTRIBUTION IN HEALTHY POSTMENOPAUSAL WOMEN

by

AMY M. GOSS AND BARBARA A. GOWER

Metabolism; In Press

ABSTRACT

Background

Evidence suggests intermuscular adipose tissue (IMAT) may be linked to insulin resistance, whereas thigh subcutaneous adipose tissue (SAT) may be related favorably with indices of metabolic health. However, whether adipose tissue depots of the thigh are differentially related to insulin sensitivity independent of total adiposity and other adipose tissue depots has not been determined. The objective of this study was to identify independent associations of the subcompartments of adipose tissue of the thigh with insulin sensitivity among 97 healthy early postmenopausal women.

Methods

Computed tomography (CT) scans of the mid-thigh were used to assess Thigh-SAT, Thigh perimuscular adipose tissue (PMAT), and Thigh-IMAT. CT scans at the L4-L5 intervertebral space were used to assess intra-abdominal adipose tissue (IAAT) and Abdominal-SAT. Total body fat was measured by dual-energy X-ray absorptiometry (DXA). The insulin sensitivity index (S_I) was assessed by using a frequently sampled intravenous glucose tolerance test with minimal model analysis.

Results

Results indicated S_I was positively associated with Thigh-SAT independent of total fat mass and other adipose tissue compartments. Among all women combined, S_I was inversely associated with Thigh-IMAT independent of total fat mass. However, the

relationship between S_I and Thigh-IMAT was independent of IAAT only among women with high levels of Thigh-IMAT and IAAT.

Conclusions

This is the first study to demonstrate independent, opposing relationships of Thigh-SAT and Thigh-IMAT with insulin sensitivity in healthy postmenopausal women. Further research is needed to determine if these associations are causal in nature.

INTRODUCTION

Adipose tissue depots are differentially associated with risk of diseases such as type 2 diabetes and cardiovascular disease. Whereas intra-abdominal adipose tissue (IAAT) is associated with glucose intolerance and insulin resistance, thigh adipose tissue is associated favorably with measures of glucose and lipid metabolism[1;2]. However, it is unknown whether differences in fat distribution have causal associations with metabolic health or primarily reflect underlying metabolic processes that affect both glucose/lipid metabolism and the location of triglyceride storage.

Further, the nature of the relationship between leg fat and insulin sensitivity is not entirely clear. While some studies demonstrate relationships linking proportionally greater leg fat to favorable fasting insulin and glucose concentrations and blood lipid profile[3], other studies have demonstrated an inverse relationship between leg fat and insulin sensitivity[4;5]. Discrepancies among studies may be explained by differences in adjustment for confounders in statistical models and differences in the specific adipose tissue compartments examined. The thigh region is comprised of multiple adipose tissue compartments including subcutaneous adipose tissue (SAT), perimuscular adipose tissue

(PMAT), and intermuscular adipose tissue (IMAT). Lipid is also found within muscle cells as intramyocellular lipid (IMCL). Further, divergent relationships may exist as to the way in which these adipose tissue compartments contribute to metabolic health. Whole body-IMAT is proposed to be an adipose tissue depot similar in size to IAAT[6;7]. Evidence suggests adipose tissue infiltration in skeletal amuscle, like IAAT, is associated with greater circulating inflammatory markers and may contribute to insulin resistance and other cardio-metabolic disease risk factors[8;9]. Conversely, Thigh-SAT may be positively related to insulin sensitivity when examined independently of the other adipose tissue depots of the thigh[10;11]. Thus, use of total thigh fat as an independent variable does not allow for visualization of the opposing effects of the individual compartments, and may lead to discrepant results depending upon the extent to which each of the compartments contributes to the total measure. No study has simultaneously shown independent, opposing relationships among these thigh adipose tissue depots and a robust measure of insulin sensitivity.

Among the studies that have aimed to characterize the multiple adipose tissue compartments of the thigh and their contributions to metabolic health, differences in the definition of Thigh-IMAT, study populations, and scan location and modality may have lead to discrepant results. Although Thigh-IMAT is often considered as all adipose tissue deposited beneath the fascia lata within and adjacent to skeletal muscle, sub-compartments within Thigh-IMAT have been identified[5]. Goodpaster et al described the adipose tissue compartments of the thigh as Thigh SAT, Thigh-PMAT (also described as subfascial adipose tissue), and Thigh-IMAT[5]. With this characterization, Thigh-IMAT was inversely associated, Thigh-PMAT tended to be inversely associated, and Thigh-

SAT was not associated with insulin sensitivity in subjects with obesity and type 2 diabetes[5]. These relationships were not independent of total body fat. Other study populations within which Thigh-IMAT and insulin resistance are associated include elderly men or those with at least one risk factor for diabetes[9]. No study has examined the relationship of insulin sensitivity with thigh adipose tissue distribution in a healthy, relatively homogeneous study population, specifically early postmenopausal women. These associations may be important considering that changes occur in both disease risk[12;13] and body fat distribution[14;15] following menopause.

The primary purpose of this study was to investigate independent associations of Thigh-SAT, Thigh-PMAT, and Thigh-IMAT with insulin sensitivity in healthy early postmenopausal women. A secondary aim was to identify associations between Thigh-IMAT and other adipose tissue compartments such as IAAT, Thigh-PMAT, and Thigh-SAT. We hypothesized that Thigh-SAT would be positively associated with, and Thigh-IMAT and Thigh-PMAT would be inversely associated with, insulin sensitivity, and furthermore, that Thigh-IMAT would be positively associated with IAAT.

METHODS AND PROCEDURES

Participants and protocol

Subjects were 97 healthy postmenopausal women aged 45-60 years who participated in one of two studies at the University of Alabama at Birmingham (UAB). Metabolic testing and body composition assessment took place under controlled conditions during an in-patient visit to the Department of Nutrition Sciences and the General Clinical Research Center (GCRC) at UAB. Women who experienced a natural

menopause, with the time of cessation of menstruation of at least 6 months, or hysterectomy, and FSH level >30 IU/mL (FSH ranged 44–138 IU/mL) were included in the study. Fifty-two percent of the women recruited for the study were using hormone replacement therapy (HRT) and had been using HRT for an of average 2.8 yrs. Among the women recruited, 9% were African American (n=9) and 91% European American (n=78). Five of the African American women were HRT users. None of the women smoked. The protocol was approved by the Institutional Review Board for Human Use at UAB, and all subjects signed an informed consent prior to testing.

Body composition and fat distribution

Mid-thigh and abdominal cross-sectional tissue areas were analyzed by computed tomography (CT) scanning using a HiLight/Advantage scanner (General Electric, Milwaukee, WI). Thigh muscle and thigh adipose tissue areas were determined using a one slice mid-thigh (between the superior border of the patella and the inferior anterior iliac crest) CT scan. A scout scan was conducted to identify the L4-L5 intervertebral spaces and was followed by a 5-mm scan of the abdomen at the identified site. Scans were later analyzed for cross-sectional area (cm^2) of adipose tissue and muscle tissue using SliceOmatic image analysis software (version 4.2: Tomovision, Montreal, Canada). The abdomen scan was used to analyze IAAT and Abdominal-SAT. Thigh-IMAT and Thigh-PMAT were separated from Thigh-SAT by manually drawing a line along the fascia lata surrounding the thigh muscle. Subsequently, Thigh-IMAT was partitioned from Thigh-PMAT by manually drawing a line around the muscle itself to capture adipose tissue located directly between and within muscle groups.

Total and regional body composition were measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L densitometer (LUNAR Radiation Corp., Madison, WI). Subjects were scanned in light clothing while lying flat on their backs with arms at their sides. DXA scans were performed and analyzed with adult software version 1.5g. The software provided measures of total fat (kg), total lean (kg), and leg fat (kg).

Insulin sensitivity testing

Glucose tolerance and insulin sensitivity were measured by frequently sampled intravenous glucose tolerance (FSIGT) test[16] and details were described elsewhere[17;18]. In brief, the FSIGT test involves intravenous glucose administration (11.4 g/m^2)at time "0", and subsequent tolbutamide injection(125 mg/m^2) or insulin infusion (0.02 units/kg over 5 min) 20 minutes later. For both tests, three samples were collected prior to glucose administration, and glucose and insulin values averaged to determine mean fasting values. For the tolbutamide-modified test, 29 additional blood samples (2 ml each) were collected at time points +2 to +180 minutes relative to the initiation of glucose administration. For the insulin-modified test, 31 additional blood samples (2 ml each) were collected at time points +2 to +240 minutes. All samples were analyzed for insulin and glucose concentrations. The insulin sensitivity index (S_I) was calculated using MINMOD computer software (version 3.0)[19;20]. In this study, the tolbutamide-modified method was used for 81 women and the insulin-modified method was used for 16 women. S_I values from tolbutamide-modified tests are reported to be approximately 16% higher than those from insulin-modified tests[21]. Between-study differences in FSIGT

were accounted for statistically by including a variable for FSIGT method ("test type" variable) in analyses as previously described[22].

Assay of glucose and insulin

Glucose was measured in 10 µl sera using an Ektachem DT II System (Johnson and Johnson Clinical Diagnostics, Rochester, NY). For the majority of the postmenopausal women (those who had the tolbutamide-modified FSIGT), insulin was assayed in duplicate 200 µl aliquots with "Coat-A-Count" kits (Diagnostic Products Corporation, Los Angeles, CA). In our laboratory, this assay has a sensitivity of 1.9 µIU/ml, a mean intra-assay coefficient of variation of 5%, and a mean interassay coefficient of variation of 6%. For the remainder of the women (those who had the insulin-modified FSIGT), insulin was assayed in duplicate 100 µl aliquots with reagents from Linco Research Products Inc. (St. Charles. MO). In our laboratory, this assay has a sensitivity of 3.35 µIU/ml, a mean intra-assay coefficient of variation of 3.49%, and a mean interassay coefficient of variation of 5.57%. Commercial quality control sera of low, medium, and high insulin concentration are included in every assay to monitor variation over time. Difference in methodology was accounted for by inclusion of a "test type" variable in analyses.

Statistical methods

Descriptive statistics were computed for all study variables of interest. Variables known to deviate from a normal distribution, such as S_I, were log 10 transformed prior to statistical analysis. All statistical tests were two-sided and were performed using a type I error rate of 0.05. Statistical analyses were performed using SAS (version 9.1; SAS

Institute, Inc., Cary, NC). Pearson correlation coefficients were calculated to determine associations of S_I with Thigh-SAT, Thigh-PMAT, Thigh-IMAT, IAAT, Abdominal - SAT, and total fat mass. Partial correlation coefficients were calculated to determine associations of S_I with Thigh-SAT, Thigh-PMAT, Thigh-IMAT, IAAT, and Abdominal-SAT adjusted for total fat mass. Thigh-IMAT was further adjusted for thigh muscle area. Multiple linear regression analysis was conducted to determine independent associations of S_I with Thigh-SAT, Thigh-IMAT, total fat, and IAAT. This model was also adjusted for thigh muscle area. All final models were adjusted for "test type." HRT use was also tested as in all models as a confounding variable; however, HRT use was not significant and subsequently was excluded from models.

To further explore the interrelationships among IAAT, Thigh-SAT, and S_I, subjects were characterized into four fat distribution phenotype groups based on IAAT and Thigh-SAT. The median IAAT (103.2 cm^2) and median Thigh-SAT (238.5 cm^2) were used to classify subjects into 1 of 4 groups including 1) high IAAT/high Thigh-SAT, 2) high IAAT/low Thigh-SAT, 3) low IAAT/high Thigh-SAT, and 4) low IAAT/low Thigh-SAT. Analysis of covariance (ANCOVA) was used to compare group differences in S_I adjusted for total fat. Additionally, because IAAT has a profound effect on hepatic S_I that may mask other contributions to whole-body insulin sensitivity, subjects were dichotomized into 2 groups, low (<103.2 cm^2) or high (>103.2 cm^2) IAAT using the median IAAT value of the entire study population. Multiple linear regression analysis was conducted to determine independent associations of S_I and Thigh-IMAT, % fat, and IAAT within each group.

Pearson correlation coefficients were calculated to determine associations among fat distribution variables: Thigh-SAT, Thigh-PMAT, Thigh-IMAT, IAAT, and Abdominal-SAT. Partial correlation coefficients were also calculated to determine associations among Thigh-SAT, Thigh-PMAT, Thigh-IMAT, IAAT, and Abdominal-SAT independent of total fat mass.

RESULTS

Subject characteristics are shown in **Table 1**. Pearson simple correlation analysis indicated all fat distribution variables were significantly inversely associated with S_I (**Table2**). Partial correlation analysis indicated that Thigh-IMAT and IAAT were inversely associated with S_I independent of total fat mass. Partial correlation analysis also indicated that Thigh-SAT was positively associated with S_I independent of total fat (**Table 2**). Multiple linear regression analysis indicated that S_I was positively associated with Thigh-SAT and inversely associated with IAAT independent of total fat mass and other leg fat variables (**Table 3**).

Subgroup analysis by fat distribution phenotype (**Figure 1**) indicated subjects in the high IAAT/low Thigh-SAT group had significantly lower adjusted S_I when compared with all other subgroups (high IAAT/high Thigh-SAT; $p=0.01$, low IAAT/high Thigh-SAT; $p<0.001$, low IAAT/low Thigh-SAT; $p=0.005$). There were no other significant between-group differences in adjusted S_I. Multiple linear regression analysis by subgroup ("high" or "low" IAAT) indicated that S_I was inversely associated Thigh-IMAT independent of %fat and IAAT amongst subjects with >130 cm^2 IAAT, and S_I was

inversely associated with IAAT and %fat amongst subjects with <130 cm^2 IAAT (**Table 4**).

Simple correlation coefficients among all fat distribution variables are shown in **Table 5a.** All variables were significantly positively associated. The highest correlation coefficients calculated were between Abdominal-SAT and thigh SAT (r=0.73), IAAT and Thigh-IMAT (r=0.69), and IAAT and Abdominal-SAT (0.69). Partial correlation coefficients for adipose tissue compartments of the thigh and IAAT adjusted for total fat mass are shown in **Table 5b**. Thigh-IMAT was significantly positively associated with IAAT and Thigh-PMAT. Thigh-SAT was significantly inversely associated with IAAT independent of total fat.

DISCUSSION

The major finding of this study is that Thigh-SAT was positively and independently associated with S_I, whereas Thigh-IMAT was inversely associated with S_I. This is the first study to demonstrate opposing relationships between thigh adipose tissue compartments of the thigh and S_I among postmenopausal women. Our findings suggest maintenance of greater Thigh-SAT and lesser Thigh-IMAT may promote or reflect favorable metabolic health among early postmenopausal women.

In this study, Thigh-SAT, after adjustment for total adiposity, was positively and independently associated with insulin sensitivity in all women combined. When women were divided based on their fat distribution pattern, those with low Thigh-SAT/high IAAT had lower S_I. These findings agree with other studies linking greater Thigh-SAT, relative to other fat depots, to favorable metabolic indices[10;11;23]. However, few other

studies have used well-accepted measures of insulin sensitivity. Among obese HIV-positive women, Thigh-SAT was positively associated with S_I from FSIGT[10]. However, to our knowledge, this is the first study to observe an independent association of Thigh-SAT with S_I among healthy postmenopausal women.

The physiological basis for the positive association between Thigh-SAT and S_I is not clear. Subcutaneous adipose tissue under normal conditions sequesters nonesterified fatty acids released from adipose tissue, and fatty acids from dietary sources[24]. It has been suggested that under certain pathological conditions, such as obesity, there is a down regulation of lipid storage to subcutaneous depots resulting from a maladaptive response to postprandial increases in fatty acids[25]. A reduction in the uptake of triglyceride to subcutaneous adipose tissue depots may lead to greater lipid storage to ectopic depots known to be adversely associated with both hepatic and peripheral insulin sensitivity[24;25]. It is possible that other factors involved in regulating insulin sensitivity, such as genotype, dietary intake, physical activity, and inflammation, are also responsible for the degree of triglyceride storage to subcutaneous depots, thus mediating the association between insulin sensitivity and subcutaneous adipose tissue. Although in the present study we cannot draw cause–and-effect conclusions, we believe our findings support the hypothesis that Thigh-SAT either exhibits protective effects on insulin sensitivity, or reflects a metabolic state compatible with maintenance of relatively high insulin sensitivity among postmenopausal women.

In our study, greater Thigh-IMAT was associated with lower insulin sensitivity, independent of total body fat. Few studies have examined the relationship between Thigh-IMAT and insulin sensitivity using appropriate measures of insulin sensitivity,

such as clamp or FSIGT, and appropriately adjusting for other confounding variables. In a study involving middle-aged men and women, calf IMAT was significantly inversely associated with glucose infusion rate from the hyperinsulinemic euglycemic clamp[4]. Among subjects with obesity and type 2 diabetes, an inverse association was observed between insulin sensitivity assessed by hyperinsulinemic euglycemic clamp and Thigh-IMAT[5]. However, neither study considered whether the relationship was independent of total fat mass. Similarly in a study of premenopausal women, whole-body IMAT was inversely associated with insulin sensitivity in both African Americans and Caucasians; however this relationship was not adjusted for total adiposity[26]. Thus, our study is the first to demonstrate that Thigh-IMAT is associated with a robust measure of insulin sensitivity after accounting for total body adiposity.

Although our observed association between Thigh-IMAT and S_I was independent of total fat, it was not independent of IAAT. In all women combined, IAAT but not Thigh-IMAT was independently associated with S_I. However, when women were divided based on their degree of intra-abdominal adiposity, Thigh-IMAT was independently associated with S_I among those women with high, but not low, IAAT. In this group, IAAT was not independently associated with S_I, possibly because all women had levels of IAAT above the threshold for metabolic dysfunction[26;27]. It has been suggested that IAAT specifically impairs hepatic insulin sensitivity by increasing exposure of the liver to fatty acids. In contrast, due to its location, Thigh-IMAT is likely to affect skeletal muscle insulin sensitivity. Because the S_I index we used captures both hepatic and peripheral insulin sensitivity, it may be difficult to isolate relationships that are specific to skeletal muscle in subjects with high volumes of IAAT. Thus, when

variance attributable to IAAT was minimized, the association of Thigh-IMAT with insulin sensitivity was apparent. However, it is also possible that the greater Thigh-IMAT in the women with greater IAAT played a role in the stronger association of Thigh-IMAT with SI in this group.

A secondary aim of this study was to characterize the associations of Thigh-IMAT and Thigh-PMAT with other adipose tissue depots. Our results suggested a positive, independent, relationship between Thigh-IMAT and IAAT, in agreement with other studies[6;7]. These results support the concept of coordinated accumulation of ectopic adipose tissue. While the mechanisms leading to this coordinate deposition are unclear, lipid deposition to both IAAT and Thigh-IMAT may result from the downregulation of lipid uptake by the subcutaneous adipose tissue depots. An estrogenic hormone profile is also thought to direct lipid deposition to the subcutaneous depots[28]. Therefore, among postmenopausal women, decline in circulating estrogen may further contribute to a shift in lipid deposition from subcutaneous depots to ectopic depots. Our results also indicated that Thigh-IMAT and Thigh-PMAT are highly correlated. However, only Thigh-IMAT was independently associated with S_I. Therefore we recommend that these adipose tissue compartments be separated when conducting analyses concerning thigh fat distribution and metabolic health.

This study has several strengths. Measures of fat distribution and body composition were obtained using DXA and CT scanning. To our knowledge, this is the only study identifying independent relationships of the adipose tissue compartments of the thigh with S_I among healthy early postmenopausal women. Limitations to this study include the cross-sectional study design and relatively small number of subjects. We did

not have statistical power to examine potential race/ethnicity differences in the relationships of interest. We did not assess hepatic fat content or liver function, therefore, we are unable to determine independent relationships of hepatic fat with other ectopic lipid depots and insulin sensitivity. Further, we did not assess IMCL which may also adversely affect insulin sensitivity.

In conclusion, results suggested that among healthy early postmenopausal women, maintenance of greater Thigh-SAT may exhibit protective effects on insulin sensitivity, or may reflect a fat distribution pattern synonymous with good metabolic health. Thigh-IMAT was inversely and independently associated with insulin sensitivity among women with high but not low levels of IAAT. These results identify independent, opposing relationships of adipose tissue depots of the thigh with metabolic health among a relatively homogenous population of healthy postmenopausal women, thus emphasizing the importance of considering fat distribution phenotype in identifying risk of development of metabolic disease among otherwise healthy, aging women. Further studies are needed to determine whether a cause-and-effect association exists between Thigh-SAT and insulin sensitivity, and to also determine whether adipose tissue infiltration in skeletal muscle independently influences peripheral insulin sensitivity.

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REFERENCE LIST

1. Van Pelt RE, Jankowski CM, Gozansky WS, et al: Lower-body adiposity and metabolic protection in postmenopausal women. J.Clin.Endocrinol.Metab 90:4573-4578, 2005

2. Williams MJ, Hunter GR, Kekes-Szabo T, et al: Regional fat distribution in women and risk of cardiovascular disease. Am.J.Clin.Nutr. 65:855-860, 1997

3. Hunter GR, Chandler-Laney PC, Brock DW, et al: Fat distribution, aerobic fitness, blood lipids, and insulin sensitivity in African-American and European-American women. Obesity.(Silver.Spring) 18:274-281, 2010

4. Boettcher M, Machann J, Stefan N, et al: Intermuscular adipose tissue (IMAT): association with other adipose tissue compartments and insulin sensitivity. J.Magn Reson.Imaging 29:1340-1345, 2009

5. Goodpaster BH, Thaete FL, Kelley DE: Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am.J.Clin.Nutr. 71:885-892, 2000

6. Gallagher D, Kuznia P, Heshka S, et al: Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. Am.J.Clin.Nutr. 81:903-910, 2005

7. Song MY, Ruts E, Kim J, et al: Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. Am.J.Clin.Nutr. 79:874-880, 2004

8. Yim JE, Heshka S, Albu JB, et al: Femoral-gluteal subcutaneous and intermuscular adipose tissues have independent and opposing relationships with CVD risk. J.Appl.Physiol 104:700-707, 2008

9. Zoico E, Rossi A, Di F, V, et al: Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. J.Gerontol.A Biol.Sci.Med.Sci. 65:295-299, 2010

10. Albu JB, Kenya S, He Q, et al: Independent associations of insulin resistance with high whole-body intermuscular and low leg subcutaneous adipose tissue distribution in obese HIV-infected women. Am.J.Clin.Nutr. 86:100-106, 2007

11. Pigeon E, Couillard E, Tremblay A, et al: Mid-thigh subcutaneous adipose tissue and glucose tolerance in the Quebec family study. Obes.Facts. 1:310-318, 2008

12. Janssen I, Powell LH, Crawford S, et al: Menopause and the metabolic syndrome: the Study of Women's Health Across the Nation. Arch.Intern.Med. 168:1568-1575, 2008

13. Park YW, Zhu S, Palaniappan L, et al: The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch.Intern.Med. 163:427-436, 2003

14. Gower BA, Munoz J, Desmond R, et al: Changes in intra-abdominal fat in early postmenopausal women: effects of hormone use. Obesity.(Silver.Spring) 14:1046-1055, 2006

15. Sowers M, Zheng H, Tomey K, et al: Changes in body composition in women over six years at midlife: ovarian and chronological aging. J.Clin.Endocrinol.Metab 92:895-901, 2007

16. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. Endocr.Rev. 6:45-86, 1985

17. Munoz J, Derstine A, Gower BA: Fat distribution and insulin sensitivity in postmenopausal women: influence of hormone replacement. Obes.Res. 10:424-431, 2002

18. Pacini G, Tonolo G, Sambataro M, et al: Insulin sensitivity and glucose effectiveness: minimal model analysis of regular and insulin-modified FSIGT. Am.J.Physiol 274:E592-E599, 1998

19. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J.Clin.Invest 68:1456-1467, 1981

20. Pacini G, Bergman RN: MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput.Methods Programs Biomed. 23:113-122, 1986

21. Welch S, Gebhart SS, Bergman RN, et al: Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. J.Clin.Endocrinol.Metab 71:1508-1518, 1990

22. Gower BA, Ard JD, Hunter GR, et al: Elements of the metabolic syndrome: association with insulin sensitivity and effects of ethnicity. Metab Syndr.Relat Disord. 5:77-86, 2007

23. Snijder MB, Visser M, Dekker JM, et al: Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. Diabetologia 48:301-308, 2005

24. Despres JP, Lemieux I: Abdominal obesity and metabolic syndrome. Nature 444:881-887, 2006

25. McQuaid SE, Hodson L, Neville MJ, et al: Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes 60:47-55, 2011

26. Albu JB, Kovera AJ, Allen L, et al: Independent association of insulin resistance with larger amounts of intermuscular adipose tissue and a greater acute insulin response to glucose in African American than in white nondiabetic women. Am.J.Clin.Nutr. 82:1210-1217, 2005

27. Hunter GR, Snyder SW, Kekes-Szabo T, et al: Intra-abdominal adipose tissue values associated with risk of possessing elevated blood lipids and blood pressure. Obes.Res. 2:563-568, 1994

28. Elbers JM, Asscheman H, Seidell JC, et al: Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am.J.Physiol 276:E317-E325, 1999

Table 1. Subject characteristics (n=97)

Variable	Mean±SD
Age (yrs)	50.8 ± 2.8
BMI (kg/m2)	26.2 ± 4.7
Fasting Insulin (µIU/ml)	9.9 ± 5.1
Fasting Glucose (mg/dL)	94.4 ± 8.7
$\mathbf{S_I}$ [$x10^{-4}min^{-1}/(\mu IU/ml)$]	5.7 ± 3.7
Total Fat (kg)	27.3 ± 9.1
Total Lean (kg)	38.9 ± 4.4
Thigh Muscle (cm^2)	213.5 ± 31.1
Thigh-SAT (cm^2)	256.4 ± 91.1
Thigh-PMAT (cm^2)	15.9 ± 6.2
Thigh-IMAT (cm^2)	10.9 ± 5.3
IAAT (cm^2)	114.1 ± 51.4
Abdominal-SAT (cm^2)	316.8 ± 122.4

BMI, body mass index; S_I, insulin sensitivity index; SAT, subcutaneous adipose tissue; PMAT, perimuscular adipose tissue; IMAT, intermuscular adipose tissue; IAAT, intra-abdominal adipose tissue.

Table 2. Pearson simple and partial correlation analysis with S_I as the dependent variables

	S_I	
	Simple r	**Partial r**
Thigh-SAT	-0.36***	0.34***
Thigh-PMAT	-0.37***	0.07
Thigh-IMAT	-0.54***	-0.24*
IAAT	-0.40***	-0.36***
Abdominal-SAT	-0.53***	0.03
Total fat	-0.61***	---

All partial correlations adjusted for total fat mass. Thigh-IMAT is also adjusted for thigh muscle area; S_I, insulin sensitivity index; SAT, subcutaneous adipose tissue; PMAT, perimuscular adipose tissue; IMAT, intermuscular adipose tissue; IAAT, intra-abdominal adipose tissue; ***p<0.001; **p<0.01; *p<0.05

Table 3. Multiple linear regression models with S_I as the dependent variables

	Variable estimate ± SEE	Std β	*P*
Thigh-SAT	0.96 ±0.30	0.48	<0.01
Thigh-IMAT	0.00 ± 0.01	0.05	0.72
Total fat	-1.42 ± 0.41	-0.71	<0.001
IAAT	-0.00 ± 0.00	-0.40	<0.01

Model adjusted for thigh muscle area and test type; data reported as standardized β; S_I, insulin sensitivity index; SAT, subcutaneous adipose tissue; IMAT, intermuscular adipose tissue; IAAT, intra-abdominal adipose tissue.

Table 4. Multiple linear regression models with S_I as the dependent variable

	High IAAT (>103.2 cm^2)		Low IAAT (<103.2 cm^2)	
	Std β	***P***	**Std β**	***P***
Thigh-IMAT	-0.81	**0.01**	0.12	0.48
IAAT	-0.05	0.80	-0.33	**0.05**
% Fat	0.28	0.24	-0.37	**0.03**

Models also adjusted for test type. Data reported as standardized β; S_I, insulin sensitivity index; IMAT, intermuscular adipose tissue; IAAT, intra-abdominal adipose tissue

Table 5. Pearson simple (a) and partial (b) correlation analysis among fat distribution variables

a. Simple (r)	Thigh IMAT	Thigh PMAT	Thigh SAT	SAAT
IAAT	0.69***	0.52***	0.54***	0.69***
SAAT	0.63***	0.55***	0.73***	
Thigh SAT	0.49***	0.52***		
Thigh PMAT	0.62***			

b. Partial (r)	Thigh IMAT	Thigh PMAT	Thigh SAT	SAAT
IAAT	0.25**	<0.01	-0.29***	0.18
SAAT	0.20	-0.01	0.09	-----
Thigh SAT	-0.09	0.02	-----	-----
Thigh PMAT	0.38***			

Data for partial analysis are adjusted for total fat mass; SAT, subcutaneous adipose tissue; PMAT, perimuscular adipose tissue; IMAT, intermuscular adipose tissue; IAAT, intra-abdominal adipose tissue; SAAT, subcutaneous abdominal adipose tissue; ***p <0.001; **p<0.01; *p<0.05

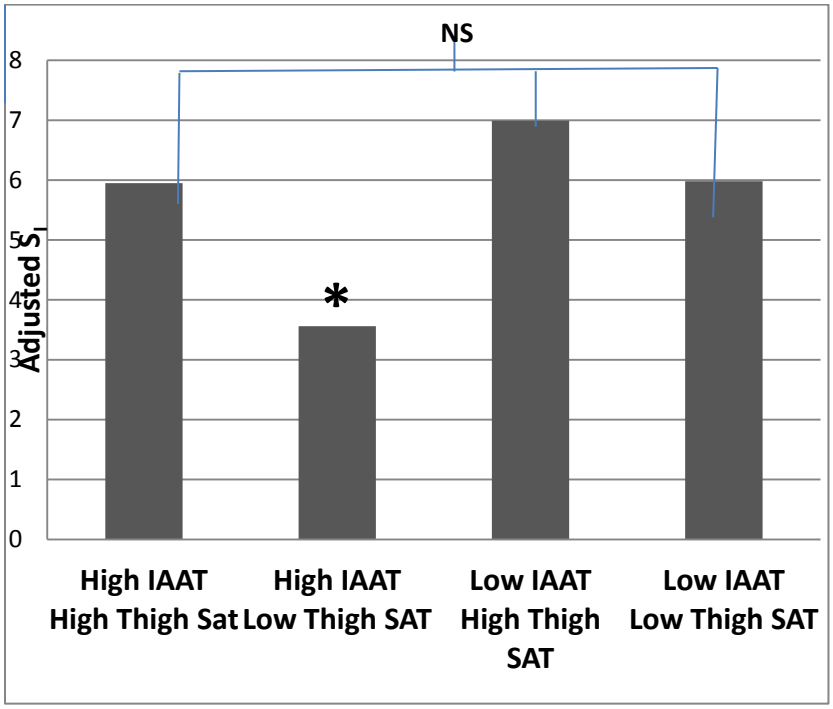

Figure 1. S_I adjusted means by fat distribution phenotype group (S_I adjusted for total fat and FSIGT method). Subjects with high IAAT/low Thigh-SAT had significantly lower adjusted S_I than all other groups. There were no other significant between-group differences. NS; not significant, *P<0.01

EFFECTS OF DIET MACRONUTRIENT COMPOSITION ON BODY COMPOSITION AND FAT DISTRIBUTION DURING WEIGHT MAINTENANCE AND WEIGHT LOSS

by

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ABSTRACT

Background

Qualitative aspects of diet may affect body composition and propensity for weight gain or loss. We tested the hypothesis that consumption of a relatively low glycemic load (GL) diet would reduce total and visceral adipose tissue under both eucaloric and hypocaloric conditions.

Methods

Participants were 69 healthy overweight men and women. Body composition was assessed by DXA and fat distribution by CT scan at baseline, after 8 weeks of a eucaloric diet intervention, and after 8 weeks of a hypocaloric (1000 kcal/d deficit) diet intervention. Participants were provided all food for both phases, and randomized to either a low GL diet ($\leq$45 points per 1000 kcal; n=40) or high GL diet (>75 points per 1000 kcal, n=29).

Results

After the eucaloric phase, participants who consumed the low GL diet had 11% less intra-abdominal fat (IAAT) than those who consumed the high GL diet ($P<0.05$, adjusted for total fat mass and baseline IAAT). Participants lost an average of 5.8 kg during the hypocaloric phase, with no differences in the amount of weight loss with diet assignment ($P=0.39$). Following weight loss, participants who consumed the low GL diet had 4.4% less total fat mass than those who consumed the high GL diet ($P<0.05$, adjusted for lean mass and baseline fat mass).

Conclusion

Consumption of a relatively low GL diet may affect energy partitioning, both inducing reduction in IAAT independent of weight change, and enhancing loss of fat relative to lean mass during weight loss.

INTRODUCTION

The concept that diet quality may have metabolic effects unique from caloric content has been gaining momentum. In particular, glycemic index (GI), the extent to which a food increases serum glucose concentrations, has been proposed as potentially affecting weight change or body composition. By increasing insulin to a greater extent or for a longer time, foods with a relatively high GI may affect specific metabolic processes, such as lipolysis, lipogenesis, or substrate oxidation[1-3]. These processes in turn may affect hunger, satiety, food intake, or energy expenditure, factors that could impact energy balance and body composition.

In addition to affecting body weight per se, diet quality also may affect energy partitioning; i.e., the amount of fat mass relative to fat-free mass that is deposited or lost. Consumption of high GL diets, and subsequent elevated insulin response, may selectively preserve fat mass due to the lipogenic actions of insulin. Further, diet quality also may affect the specific location of the fat that is deposited or mobilized. Results of some studies have indicated that relatively greater consumption of lower GI foods is associated with a smaller waist circumference[4]; although a sex-effect has been reported[4-6]. Males and females are generally thought to distribute weight differently, such that males have an android or centripetal fat distribution pattern, whereas females have greater lipid

storage to the gluteo-femoral region. Whether sex affects the impact of diet quality on body composition and fat distribution under weight maintenance conditions has not been extensively examined.

The objective of this study was to test the hypothesis that consumption of a relatively low GL diet would reduce total and regional adipose tissue during both weight maintenance and weight loss conditions. A secondary aim was to determine if there is a sexual dimorphism in outcomes of interest.

METHODS

Participants

Participants were 69 healthy overweight or obese (BMI>25) African American and European Americans (52% European American; 45% male), aged 21-50 years. Females were all premenopausal. Race was self reported during a telephone screen. Inclusion and exclusion criteria have been described elsewhere[7]. In brief, participants were relatively sedentary (<2 hr/wk activity), non-diabetic, non-smokers, and weight stable for 6 months prior to enrolling in the study (i.e. no weight change greater than 2.29 kg). The protocol was approved by the Institutional Review Board for Human Use at UAB, and all subjects signed an informed consent prior to testing.

Procedures

Participants completed a 4 day food record (3 week days, 1 weekend day) for assessment of typical, free-living, nutrient intake prior to beginning the 16-wk dietary intervention. After completing the food record, all subjects consumed the same diet for habituation (3 days). The dietary intervention included 2 phases: 8-wks under eucaloric conditions followed by 8-wks under hypocaloric conditions. Dual-energy X-ray

absorptiometry (DXA) and computed tomography (CT) scans were acquired for all participants at baseline, following the 8-wk eucaloric phase, and following the subsequent 8-wk hypocaloric phase. For the duration of the intervention, participants reported to the General Clinical Research Center each weekday morning to be weighed, eat breakfast, and collect food for their remaining meals. On Fridays, participants picked up food for Saturday and Sunday to consume at home. All food was provided by the General Clinical Research Unit (GCRC) Metabolic Kitchen. Body weight was recorded five times weekly to monitor weight maintenance and weight loss.

Diets

Participants were blinded to an assigned diet which was either the low GL diet ($\leq$45 points per 1000 kcal; 43% CHO, 18% protein, 39% fat, n=40) or the high GL diet ($\geq$75 points per 1000 kcal, 59% CHO, 18% protein, 27% fat, n=29). Macronutrient composition and GL points for the intervention diets were identical for both the eucaloric and hypocaloric phases. Intervention diet menus were designed using Nutrition Data System for Research (NDSR) software versions 2006 and 2007 (Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN). Diet composition and sample menus were previously reported elsewhere[7;8]. In brief, both the low GL diet and the high GL diet included foods typical to an American diet. Breakfast menus on the low GL diet included (but were not limited to) items such as: oatmeal or rye bread, bacon, eggs, and fruit. Breakfast menus on the high GL diet included (but were not limited to) items such as: pancakes, waffles, or cereal with milk or yogurt, and fruit juice. Lunch and dinner menus on both diets generally consisted of a main entrée supplemented with items such as a roll with margarine and vegetables (e.g. green beans, broccoli, or salad).

Main entrée items for lunch and dinner were either frozen packaged meals such as roasted turkey, lasagna, or chicken with pasta by Lean Cuisine (Stouffer's Nestle, Solon, OH) or Healthy Choice (ConAgra Foods, Omaha, NE) or entrées prepared by the metabolic kitchen staff (e.g. sandwich or grilled chicken breast). Glucose was used as the reference for determining GL points. Energy requirements were determined by the Harris Benedict equation with an activity factor of 1.35 for females and 1.5 for males during the eucaloric phase. Energy intake was adjusted if necessary to maintain body weight within 2 kg of baseline weight. The addition or reduction in calories to the assigned diets did not affect the macronutrient compositions or GL. Estimated energy requirements established during the eucaloric phase were reduced by 1000 kcal to achieve a 1-2 lb weight reduction per wk during the hypocaloric phase of the study. Subjects were asked to maintain their baseline physical activity level throughout the intervention time period.

Body composition and fat distribution

Total body fat mass and lean mass were measured by DXA using a Lunar Prodigy densitometer (GE-Lunar Corporation, Madison, WI, software version 12.3). Participants were required to wear light clothing, remove all metal objects from their body, and lie supine with arms at their sides while undergoing a total body scan. Intra-abdominal adipose tissue (IAAT), subcutaneous abdominal adipose tissue (SAAT), thigh muscle, thigh subcutaneous adipose tissue (SAT), thigh perimuscular adipose tissue (PMAT), and thigh intermuscular adipose tissue (IMAT) were determined by computed tomography (CT) scanning. A five millimeter axial scan at the level of the umbilicus (approximately the L4-L5 intervertebral space) and another at mid-thigh were taken. Scans were later

analyzed for cross-sectional area (cm^2) of adipose tissue and muscle tissue using SliceOmatic image analysis software (version 4.3: Tomovision, Montreal, Canada). The abdomen scan was used to analyze IAAT and SAAT. Thigh IMAT and PMAT were separated from thigh SAT by manually drawing a line along the fascia lata surrounding the thigh muscle. Subsequently, IMAT was partitioned from PMAT by manually drawing a line around the muscle itself to capture adipose tissue located directly between and within muscle groups[9;10]. All scans were analyzed by the same image analyst (AG).

Statistical methods

Descriptive statistics were computed for all variables of interest. Variables known to deviate from a normal distribution were log 10 transformed prior to statistical analysis. All statistical tests were two-sided and were performed using a type I error rate of 0.05. Statistical analyses were performed using SAS (version 9.1; SAS Institute, Inc., Cary, NC). Two-way repeated measures analysis of variance was used to examine the effects of time (baseline to follow-up), diet group (high GL vs low GL diets) and time x diet group interaction for measures of body composition and fat distribution from both eucaloric and hypocaloric diet phases. Analysis of covariance (ANCOVA) was used to determine the effect of diet on changes in individual adipose tissue depots after adjusting for change in total fat mass during the eucaloric phase. ANCOVA was also used to determine the effect of diet on change in total fat mass independent of total lean mass during the hypocaloric phase. Dependent variables were 8-wk (post-intervention) outcomes, and baseline outcome measures were used as covariates. For subgroup analyses by sex, paired t-tests were used to examine changes in body composition and fat distribution from baseline to follow-up during the eucaloric phase. Subgroup analysis by

sex was not conducted following the hypocaloric phase due to subgroup sample size limitations during this phase of the study.

RESULTS

Descriptive information on the subject population is shown in **Table 1**. By study design, subjects were overweight or obese at baseline of the eucaloric phase (BMI 25-46.9 kg/m^2). BMI was significantly higher in the high GL diet group at baseline of both phases. At baseline of the eucaloric phase, average weight and age did not statistically differ by diet group, and the low GL group had significantly greater total fat mass ($P=0.02$) and greater thigh SAT ($p \leq 0.05$). There were no other significant differences in regional adiposity by diet group at baseline of the eucaloric or hypocaloric phase.

Eucaloric phase

Although each subject's daily energy intake was calculated on an individual basis to maintain body mass during the eucaloric phase, fluctuations in body mass occurred over the 8 week intervention period. On average, a change of - 1.03% (-1.0 kg) in body mass (range = -2.10% to +4.05%; -2.07 kg to +4.00 kg) was observed, which did not statistically differ with diet assignment.

Changes in body composition and fat distribution variables over the 8-week eucaloric dietary intervention period are reported in **Table 2**. Significant time effects were observed for SAAT such that it decreased in both diet groups over the 8-wk eucaloric period. A significant time by group effect was observed for IAAT, such that a greater loss over the eucaloric intervention period was observed in the low GL group relative to the high GL group. IAAT remained significantly lower (11%) after the 8-wk

eucaloric intervention in the low GL diet group compared to the high GL diet group after adjustment for baseline IAAT and 8-wk total fat mass (P<0.05).

Subgroup analysis by sex indicated all groups lost total fat mass from baseline to 8 wks of the eucaloric phase, such that men lost 4.2% on the high GL diet (P<0.001) and 6.3% on the low GL diet (P<0.001) and women lost 4.9% on the high GL diet (P<0.01) and 3.1% on the low GL diet (P<0.05). Only women in the low GL group lost IAAT, such that on average women in this group lost 15.1% (P=0.001), while women in the high GL diet group lost 1% (P=0.82), men in the high GL diet group gained 4.2% (P=0.55), and men in the low GL diet group lost 4.3% (P=0.44) (**Figure 1**). All subgroups, except the women in the low GL diet group, lost SAAT, such that men on the high GL diet lost 5.7%(P<0.01), men on the low GL diet lost 8%(P<0.001), women on the high GL diet lost 6.9%(P<0.01), and women on the low GL diet lost 1% (P<0.12). Women on both the low GL diet and high GL diet lost thigh SAT (5.8%, 9.8%, respectively, P<0.01) and thigh IMAT (9.4%, 11.9%, respectively, P<0.05). Only men in the low GL diet group lost thigh SAT (10.4%) and thigh PMAT (15.5%) (P< 0.01 for both; data not shown). Race did not have an effect on outcomes of interest and was equally distributed across subgroups by sex.

Hypocaloric phase

Changes in body composition and fat distribution resulting from the 8-wk hypocaloric diet intervention phase are reported in **Table 3.** Significant time effects were observed for weight, total lean, and IAAT reflecting that these outcomes decreased in both groups over the 8-wk intervention. A significant time by group effect was observed

for total fat, such that the Low GL diet group lost more fat mass over the hypocaloric intervention period. No group effects were observed for the hypocaloric diet phase. Total fat mass remained significantly lower after the 8-wk hypocaloric intervention in the low GL diet group compared to the high GL diet group after adjustment for baseline total fat mass and follow up total lean mass ($P<0.05$) (**Figure 2**).

DISCUSSION

The goal of the present study was to test the hypothesis that consumption of a relatively low GL diet compared to a high GL diet would result in preferential visceral fat loss and greater total fat loss following both weight maintenance and weight loss conditions. We also aimed to examine if there were sex-specific differences in outcomes of interest. Following the eucaloric phase, we found participants who consumed the low GL diet had 11% less IAAT after adjustment for total fat mass than those who consumed the high GL diet. However, in subgroup analysis, loss of IAAT in the low GL group was specific to women, who lost an average of 15.1% IAAT. Following the hypocaloric phase, we found participants who consumed the low GL diet had 4.4 % less total fat mass than those who consumed the high GL diet. Our findings suggest that consuming a low GL diet may promote loss of abdominal fat, even with little or no change in weight, and may also promote greater loss of total body fat during weight loss when compared to a high GL diet.

Our results indicate that consuming a low GL diet may promote loss of IAAT, even under weight maintenance conditions. Several cross-sectional studies have linked greater intake of low GI foods to smaller waist circumference, a proxy measure of visceral adiposity[4-6]. However, to our knowledge this is the first tightly controlled

dietary intervention including a robust, direct measure of body fat distribution to report a significant reduction in IAAT as the result of a low GL diet in healthy overweight and obese subjects. The precise mechanisms leading to preferential IAAT loss during weight maintenance conditions following the consumption of a low GL diet are not clear, however may be related to insulin secretion. We previously reported, in this same population, that consumption of a low GL diet for 8 weeks relative to a high GL diet resulted in a lower insulin secretory response to a fixed meal challenge[7]. The reduced postprandial insulin response following consumption of a low GL diet may be permissive to increased fatty acid mobilization from adipose tissue within the abdominal cavity, as has been observed with total body fat[11].

We found the reduction in IAAT during eucaloric conditions to be specific to women on the low GL diet, such that this was the only subgroup to significantly lose IAAT. Our findings are in congruence with other studies linking the consumption of lower GI foods to a smaller waist circumference specifically in women[4-6]. Halkjaer et al found that CHO energy intake from fruits and vegetables was inversely associated with change in waist circumference over a 5 year period, and conversely, CHO energy intake from all other food groups was positively associated with change in waist circumference[5]. Further, these associations were significantly stronger in women than in men. Similarly, high intake of refined grains was associated with gain in waist circumference adjusted for BMI over 6 years in women but not men[4]. A dietary counseling study among men and women with type 2 diabetes reported consumption of a moderately reduced CHO diet resulted in preferential visceral adipose tissue loss among women but not men[12]. Taken together, findings from these studies suggest macronutrient composition of the diet and

CHO quality may have an effect on fat distribution that is specific to women. However, the mechanisms regulating specific loss of IAAT among women following a low GL diet are unknown. Further investigation is warranted to explore whether interactions between the changes in postprandial insulin dynamics and the sex hormone environment may underlie sex differences in adipose distribution.

The reason for sexual dimorphic results in response to the diets is not clear; however it is possible that a repartitioning of lipid played a role. Women on the low GL diet tended to have more thigh SAT ($P=0.09$) and SAAT ($P<0.05$) than women on the high GL diet at the end of the eucaloric phase (data not shown; adjusted for baseline value, and changes in total fat and IAAT), which may suggest that triglyceride was preferentially stored in subcutaneous adipose tissue in women consuming the low GL diet. Greater circulating estrogen in women may promote deposition of lipid in the hip/thigh area[13], an effect that may have been facilitated by the low GL diet.

Following the 8-wk hypocaloric diet phase, participants who consumed the low GL diet had significantly greater total body fat loss (4.4 %) than those who consumed the high GL diet. Other studies have shown inconsistent findings in regards to the effectiveness of low GL diets yielding greater weight loss and total fat loss compared to other dietary approaches[14]. These inconsistencies may be due to differences in methodology, underlying physiological differences in study populations, and other confounding factors affecting diet adherence and efficacy. To our knowledge this is the first tightly controlled dietary intervention study to report a significant difference in total body fat loss after 8 wks of consuming a low GL diet with only a modest reduction in % CHO when compared to a high GL.

Greater fat loss resulting from consumption of the hypocaloric low GL diet may be related to effects of glycemic load on fat oxidation and energy expenditure. Animal and human studies have demonstrated impaired metabolic flexibility and reduced fat oxidation resulting from consumption of a high CHO diet[15;16]. The observed changes in fat oxidation in these studies may be related to a greater postprandial insulin response following consumption of a diet with high GI or CHO content. Evidence also suggests a low GL vs. high GL diet may increase postprandial energy expenditure, also known as diet-induced thermogenesis (DIT), by reducing the rate of CHO absorption and disposal[17-19]. However, in the present study, it is also possible the higher fat content on the low GL diet influenced energy metabolism during weight loss. Both the percentage fat from omega-3 and oleic acid and the absolute amount of omega-3 and oleic acid were higher in the low GL diet[7], Data from animal and human studies indicate long chain omega-3 fatty acids may induce body fat loss by influencing fat oxidation and energy expenditure[20]. Therefore, it seems possible the difference in fat and/or CHO content between the low and high GL diet may have affected the outcomes. The effects of elevated postprandial energy expenditure in conjunction with an increased propensity to oxidize fat may have contributed to greater fat mass loss among those consuming the low GL diet in our study. Further research is needed to determine if the here-observed greater total body fat loss under weight loss conditions on a low GL diet vs. high GL diet is attributable to greater fatty acid oxidation or postprandial energy expenditure, and whether these effects were induced by CHO or fat content in a low GL diet.

Strengths of this study included control of subject intake by supplying all food over the study period; use of a eucaloric diet arm, which avoided confounding by large

changes in energy balance; use of robust measures to determine body composition and fat distribution; diets comprised of foods that may be practically consumed and a macronutrient profile with only a modest reduction in CHO. Limitations to this study included a relatively small sample size in subgroups by gender and inability to determine independent effects of dietary CHO vs. fat. Also, observed changes in total fat and fat distribution during the eucaloric phase may have hindered observation of further changes during the hypocaloric phase of the intervention.

In conclusion, consumption of a relatively low GL diet may induce loss of IAAT during weight maintenance conditions, especially in women. During weight loss, consumption of a low GL diet may affect energy partitioning, enhancing loss of fat relative to lean mass compared to a high GL, low-fat diet. Further studies are needed to identify mechanisms linking low GL diet to loss of visceral and total fat, and for gender-specific effects.

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REFERENCE LIST

1. Ludwig DS: Dietary glycemic index and the regulation of body weight. Lipids 38:117-121, 2003

2. Mozaffarian D, Hao T, Rimm EB, et al: Changes in diet and lifestyle and long-term weight gain in women and men. N.Engl.J.Med. 364:2392-2404, 2011

3. Armendariz-Anguiano AL, Jimenez-Cruz A, Bacardi-Gascon M, et al: Effect of a low glycemic load on body composition and Homeostasis Model Assessment (HOMA) in overweight and obese subjects. Nutr.Hosp. 26:170-175, 2011

4. Halkjaer J, Sorensen TI, Tjonneland A, et al: Food and drinking patterns as predictors of 6-year BMI-adjusted changes in waist circumference. Br.J.Nutr. 92:735-748, 2004

5. Halkjaer J, Tjonneland A, Thomsen BL, et al: Intake of macronutrients as predictors of 5-y changes in waist circumference. Am.J.Clin.Nutr. 84:789-797, 2006

6. Romaguera D, Angquist L, Du H, et al: Dietary determinants of changes in waist circumference adjusted for body mass index - a proxy measure of visceral adiposity. PLoS.One. 5:e11588, 2010

7. Goree LL, Chandler-Laney P, Ellis AC, et al: Dietary macronutrient composition affects beta cell responsiveness but not insulin sensitivity. Am.J.Clin.Nutr. 94:120-127, 2011

8. Gower BA, Goree LL, Chandler-Laney PC, et al: A higher-carbohydrate, lower-fat diet reduces fasting glucose concentration and improves beta-cell function in individuals with impaired fasting glucose. Metabolism , 2011

9. Song MY, Ruts E, Kim J, et al: Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. Am.J.Clin.Nutr. 79:874-880, 2004

10. Goodpaster BH, Thaete FL, Kelley DE: Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am.J.Clin.Nutr. 71:885-892, 2000

11. Ebbeling CB, Leidig MM, Feldman HA, et al: Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. JAMA 297:2092-2102, 2007

12. Sasakabe T, Haimoto H, Umegaki H, et al: Effects of a moderate low-carbohydrate diet on preferential abdominal fat loss and cardiovascular risk factors in patients with type 2 diabetes. Diabetes Metab Syndr.Obes. 4:167-174, 2011

13. Elbers JM, Asscheman H, Seidell JC, et al: Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am.J.Physiol 276:E317-E325, 1999

14. Esfahani A, Wong JM, Mirrahimi A, et al: The application of the glycemic index and glycemic load in weight loss: A review of the clinical evidence. IUBMB.Life 63:7-13, 2011

15. Bray MS, Tsai JY, Villegas-Montoya C, et al: Time-of-day-dependent dietary fat consumption influences multiple cardiometabolic syndrome parameters in mice. Int.J.Obes.(Lond) 34:1589-1598, 2010

16. Isken F, Klaus S, Petzke KJ, et al: Impairment of fat oxidation under high- vs. low-glycemic index diet occurs before the development of an obese phenotype. Am.J.Physiol Endocrinol.Metab 298:E287-E295, 2010

17. Agus MS, Swain JF, Larson CL, et al: Dietary composition and physiologic adaptations to energy restriction. Am.J.Clin.Nutr. 71:901-907, 2000

18. Pereira MA, Swain J, Goldfine AB, et al: Effects of a low-glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. JAMA 292:2482-2490, 2004

19. Scazzina F, Del RD, Benini L, et al: The effect of breakfasts varying in glycemic index and glycemic load on dietary induced thermogenesis and respiratory quotient. Nutr.Metab Cardiovasc.Dis. 21:121-125, 2011

20. Baillie RA, Takada R, Nakamura M, et al: Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. Prostaglandins Leukot.Essent.Fatty Acids 60:351-356, 1999

Table 1. Baseline characteristics of study population by diet group during eucaloric and hypocaloric phases

Variable	Diet	Eucaloric Phase	Hypocaloric Phase
n	High GL	29	28
	Low GL	40	31
Sex (% male)	High GL	48% (n=14)	46% (n=13)
	Low GL	43% (n=17)	45% (n=14)
Race (% EA)	High GL	48% (n=14)	50% (n=14)
	Low GL	55% (n=22)	58% (n=18)
Age (yr)	High GL	34.6 ± 8.1[1]	34.7 ± 8.1[1]
	Low GL	35.6 ± 8.5[1]	35.9 ± 8.4[1]
BMI (kg/m^2)[2]	High GL	31.4 ± 4.4[1]	30.9 ± 4.5[1]
	Low GL	33.5 ± 4.3[1]	32.4 ± 4.1[1]

[1]Data reported as mean ± SD; EA, European American; BMI, body mass index

[2] $P \leq 0.05$ (2-sample t test for significant differences between diet groups at baseline of both phases).

Table 2. Fat distribution and body composition outcomes for <u>eucaloric</u> phase by diet

Variable	Diet	Baseline	Follow-up	Change (%)	Time †	Group †	Group *time†
Weight (kg)	High GL	96.1±20.3	95.2±20.7	-1.6±0.4	0.47	0.36	0.39
	Low GL	99.8±18.1	98.6±17.9	-1.9±2.2			
Total Lean (kg)	High GL	56.4±1.6	56.2±1.5	-0.4±0.4	0.60	0.99	0.73
	Low GL	55.7±1.3	55.3±1.3	-0.5±0.5			
Total Fat (kg)	High GL	36.8±7.9	35.1±8.1	-4.7±0.8	0.07	**0.02**	0.69
	Low GL	41.3±8.7	39.4±9.7	-5.0±1.0			
IAAT (cm^2)	High GL	80.6±48.3	82.4±57.9	-1.3±3.5	0.12	0.19	**0.03**
	Low GL	89.5±46.3	81.5±49.4	-10.9±3.0			
SAAT (cm^2)	High GL	409.9±125.3	384.5±118.6	-6.1±1.5	**0.02**	0.33	0.66
	Low GL	426.0±112.0	404.2±121.6	-6.1±1.2			
Thigh SAT (cm^2)	High GL	241.7± 89.1	229.5±87.9	-3.9±3.2	0.82	**0.02**	0.26
	Low GL	298.2±110.6	276.5±109.8	-8.3±8.3			
Thigh IMAT (cm^2)	High GL	14.1±6.9	13.0±6.7	-5.6±4.7	0.40	0.36	0.98
	Low GL	15.0±6.4	13.8±6.4	-10.1±3.2			
Thigh PMAT (cm^2)	High GL	18.2±5.5	18.0±6.0	-0.9±3.3	0.45	0.20	0.15
	Low GL	20.7±7.9	19.4±8.2	-5.2±3.6			
Thigh muscle (cm^2)	High GL	317.1± 93.5	322.9±75.4	-0.5±1.0	0.17	0.70	0.12
	Low GL	333.4± 72.6	325.7±12.5	-2.2±1.1			

Baseline and follow-up data reported as mean ± SD. The percent change from baseline is reported as mean±SEM. *P*, p-value; IAAT, intra-abdominal adipose tissue; SAAT, subcutaneous abdominal adipose tissue; SAT, subcutaneous adipose tissue; IMAT, intermuscular adipose tissue; PMAT, perimuscular adipose tissue.

†, P-value for 2-way ANOVA for unadjusted data

Table 3. Fat distribution and body composition outcomes for <u>hypocaloric</u> phase by diet

Variable	Diet	Baseline	Follow-up	Change (%)	Time†	Group†	Group *time†
Weight (kg)	High GL	94.3±20.4	89.4±20.9	-4.3±0.8	**<0.001**	0.52	0.41
	Low GL	98.4±17.9	92.9±18.1	-6.1±3.9			
Total Lean (kg)	High GL	55.5±1.5	53.7±1.5	-3.3±0.7	**0.01**	0.87	0.36
	Low GL	55.3±1.3	54.1±1.3	-2.2±0.5			
Total Fat (kg)	High GL	34.9±8.2	32.4±9.4	-8.3±1.4	0.16	**0.03**	**0.02**
	Low GL	39.1±9.7	35.3±9.8	-10.5±1.2			
IAAT (cm^2)	High GL	86.6±59.3	71.1±50.0	-13.6±3.3	**0.05**	0.92	0.45
	Low GL	84.5±50.4	73.6±45.5	-13.8±3.3			
SAAT (cm^2)	High GL	378.4±116.6	342.7±131.3	-10.8±1.6	0.07	0.60	0.37
	Low GL	404.8±107.7	358.0±99.3	-11.7±8.0			
Thigh SAT (cm^2)	High GL	225.4±89.1	207.2±81.6	-7.9±2.3	0.34	0.06	0.31
	Low GL	285.6±104.9	253.7±102.4	-9.2±1.4			
Thigh IMAT (cm^2)	High GL	12.9±6.8	10.5±5.5	-17.4±3.6	0.39	0.36	0.99
	Low GL	14.3±6.2	11.8±5.3	-17.5±1.7			
Thigh PMAT (cm^2)	High GL	17.8±5.8	16.6±6.6	-0.08±3.3	0.21	0.37	0.55
	Low GL	19.1±7.8	18.4±7.3	-2.6±2.6			
Thigh muscle (cm^2)	High GL	320.2±71.9	312.4±70.3	-2.3±0.7	0.11	0.95	0.90
	Low GL	324.8±35.7	317.4±73.2	-2.1±0.6			

Baseline and follow-up data reported as mean ± SD. The percent change from baseline is reported as mean±SEM. *P*, p-value; IAAT, intra-abdominal adipose tissue; SAAT, subcutaneous abdominal adipose tissue; SAT, subcutaneous adipose tissue; IMAT, intermuscular adipose tissue; PMAT, perimuscular adipose tissue.

†, P-value for 2-way ANOVA for unadjusted data

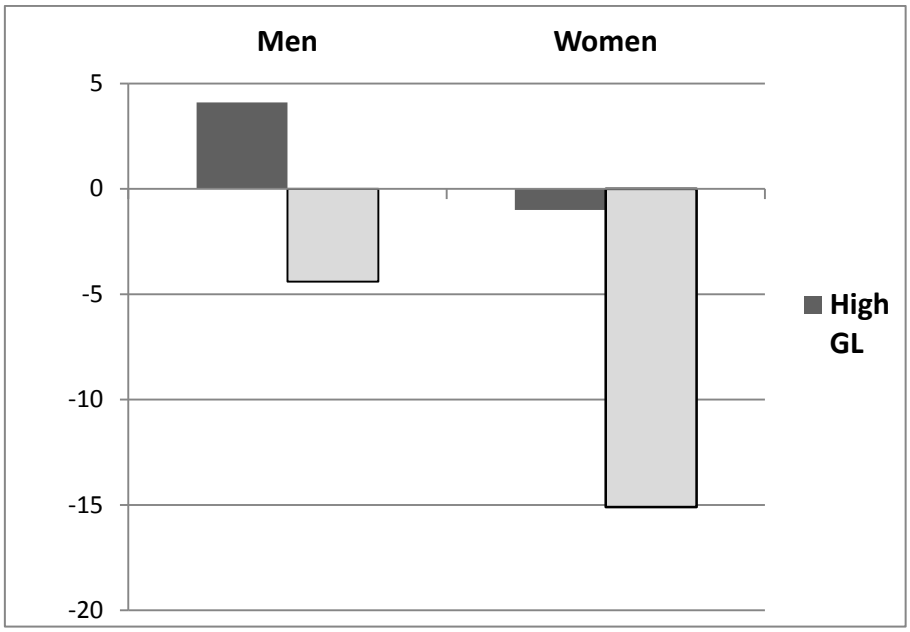

Figure 1. Mean % change in IAAT (cm^2) following consumption of the eucaloric high GL and low GL diets by gender. Men consuming the high GL diet gained 4.1% (NS) and men consuming the low GL diet lost 4.4% (NS) IAAT. Women consuming the high GL diet lost 1% (NS) and women consuming the low GL diet lost 15.1% ($P<0.01$) IAAT.

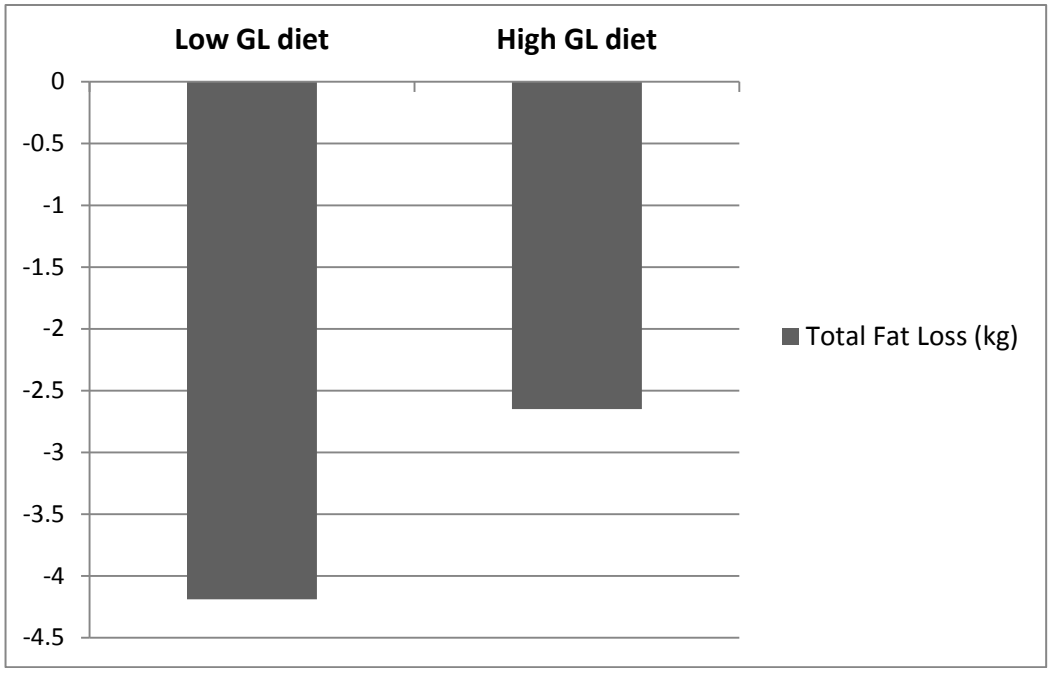

Figure 2. Change in total fat mass (kg) adjusted for total lean mass (kg) following the 8-wk hypocaloric phase by diet. Low GL diet group had significantly less total fat mass relative to lean mass following the hypocaloric phase ($P<0.05$)

REDUCED SEX HORMONE-BINDING GLOBULIN FOLLOWING A HIGH FAT WEIGHT MAINTENANCE DIET IS ASSOCIATED WITH CHANGE IN FASTING AND POST-CHALLENGE GLYCEMIA IN OVERWEIGHT MEN AND WOMEN.

by

GOSS AM, GOREE LL, AZRAD M, ARD J, GOWER BA

Journal of Clinical Endocrinology and Metabolism; Under Review
Format adapted for dissertation

ABSTRACT

Background

Low **c**irculating sex hormone binding globulin (SHBG) has been linked to increased risk of type 2 diabetes. Previous studies have reported an association between fasting glycemia and circulating SHBG. Macronutrient composition of the diet may regulate SHBG production mediated by dietary effects on glucose homeostasis. The objective of this study was to examine the effects of an 8-wk high fat weight maintenance diet on SHBG concentrations and determine independent associations of fasting and post-challenge glucose with SHBG in healthy overweight and obese men and women.

Methods

69 healthy overweight men and women were assigned to either a 8-wk high fat (39% energy from fat) or control (27% energy from fat) weight maintenance diet Serum SHBG, fasting glucose, fasting insulin, and glucose area under the curve (AUC) were assessed. Body composition was determined by dual-energy X-ray absorptiometry and fat distribution was determined by computed tomography scanning.

Results

In the group consuming the high fat diet, there was a significant reduction in SHBG and a significant increase in fasting glucose. There was a significant association of fasting glucose with change in SHBG ($P=0.03$). Glucose AUC following a fixed meal challenge was also significantly associated with change in SHBG ($P<0.01$).

Conclusions

During weight maintenance conditions, consumption of a high fat diet was associated with suppression of SHBG. Associations between changes in fasting or post-challenge glucose and changes in SHBG suggested that these variables may be linked in a cause-and-effect manner.

INTRODUCTION

Sex-hormone binding globulin (SHBG) is a protein predominantly secreted by the liver. The primary function of SHBG is classically known as the regulation of the bound fraction of circulating sex hormones (1). However, studies have linked circulating SHBG with insulin sensitivity and type 2 diabetes independent of the action of sex hormones(2-4). Therefore, SHBG may have a direct role in disease development or may be a biomarker for metabolic health.

Type 2 diabetes and metabolic syndrome are associated with low circulating SHBG (5-7). Obesity also results in low circulating SHBG (8). Numerous physiological factors associated with obesity have been linked to low circulating SHBG such as elevated fasting insulin and glucose, hepatic lipid accumulation, and intra-abdominal adipose tissue (IAAT) accumulation (9-12). While data from some studies indicate that insulin may decrease the release of SHBG from the hepatocyte (10;11), more recent studies provide convincing evidence that fasting glucose and hepatic lipid, not insulin, are important determinants of hepatic SHBG secretion (12). The precise nature of the relationships among these factors and hepatic SHBG secretion has yet to be fully elucidated.

Recent data indicate that diet-induced changes to glucose homeostasis may be an important mediating factor in altering hepatic SHBG production (12;13). Glucose homeostasis is determined in part by hepatic glucose production, which is increased as a result of hepatic insulin resistance and triglyceride accumulation (14). Whether dietary fat can increase hepatic glucose production through this mechanism is not clear, but we have shown that a diet relatively high in fat increases fasting glucose concentrations (15). Based on these findings, it seems plausible that consumption of a higher fat diet may induce suppression of hepatic SHBG production mediated by changes in glucose homeostasis. Currently, no studies have examined the effects of macronutrient composition on circulating SHBG concentrations under weight maintenance conditions and to what extent diet-effects may be mediated by glucose metabolism.

The primary purpose of this investigation was to examine the effects of an 8-wk high fat weight maintenance diet on SHBG concentrations in healthy overweight and obese men and women. Further, we aimed to investigate the independent associations of fasting and post-challenge glucose with SHBG. We hypothesized that SHBG would be reduced following an 8-wk higher fat weight maintenance diet (39% energy from fat) relative to a control diet (27% energy from fat), and that change in SHBG would be associated with change in fasting glucose and glucose area-under-the-curve (AUC).

METHODS

Participants

Participants were 69 healthy overweight or obese (BMI>25) African American and European Americans (52% European American; 45% male), aged 21-50 years. Females were all premenopausal. Race was self reported during a telephone screen. Inclusion and exclusion criteria have been described elsewhere (16). In brief, participants were relatively sedentary (<2 hr/wk activity), non-diabetic, non-smokers, and weight stable for 6 months prior to enrolling in the study (i.e. no weight change greater than 2.29 kg). The protocol was approved by the Institutional Review Board for Human Use at UAB, and all subjects signed an informed consent prior to testing.

Dietary Interventions

Participants completed a 4-day food record (3 week days, 1 weekend day) for assessment of typical, free-living, nutrient intake prior to beginning the 8-wk dietary intervention. After completing the food record, all subjects consumed the same diet for habituation (3 days). The dietary intervention included 8-wks of weight maintenance conditions. For the duration of the intervention, participants reported to the General Clinical Research Center (GCRC) each weekday morning to be weighed, eat breakfast, and collect food for their remaining meals. On Fridays, participants picked up food for Saturday and Sunday to consume at home. All food was provided by the GCRC Metabolic Kitchen. Body weight was recorded five times weekly to monitor adherence and weight maintenance.

Participants were assigned to a diet which was either the high fat diet (43% CHO, 18% protein, 39% fat, with <10% saturated fat, n=40) or the control diet (55% CHO,

18% protein, 27% fat, with <10% saturated fat, n=29). The high fat diet was higher in both percentage and absolute amount of fat from omega-3 and oleic acid. Intervention diet menus were designed using Nutrition Data System for Research (NDSR) software versions 2006 and 2007 (Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN). Specific diet composition and sample menus were previously reported elsewhere (15;16). Energy requirements were determined by the Harris Benedict equation with an activity factor of 1.35 for females and 1.5 for males for weight maintenance. Energy intake was adjusted if necessary to maintain body weight within 2 kg of baseline weight.

Analysis of glucose and hormones

Concentrations of glucose, insulin, and SHBG were analyzed in the Core Laboratory of the GCRC, Nutrition Obesity Research Center, and Diabetes Research and Training Center. SHBG was measured in 10 µl aliquots with IRMA (immunoradiometric assay) (Siemens Corporation [previously DPC, Los Angeles, CA]). In our laboratory, this assay had an intra-assay coefficient of variation (CV) of 6.2%, and an interassay coefficient of variation of 9.2 % with a sensitivity of 0.68 nmol/L. Glucose was measured in 3 µl sera using the glucose oxidase method (Stanbio Laboratory, Boerne, TX). In our laboratory, this assay had an intra-assay coefficient of variation (CV) of 1.2%, and an interassay coefficient of variation of 3.1%. Insulin was assayed in 50 µl aliquots with immunofluorescence technology on a TOSOH AIA-II analyzer (TOSOH, South San Francisco, CA).). In our laboratory, this assay had an intra-assay coefficient of variation (CV) of 1.5%, and an interassay coefficient of variation of 4.4 %.

Liquid meal tolerance test

Glucose AUC was determined from data derived from a liquid meal tolerance test. The details of this test have been described elsewhere(16). In brief, participants fasted overnight prior to the test. Following the placement of a flexible intravenous catheter, participants consumed a liquid meal (7 kcal/kg of body weight as 24% fat, 58.6% as CHO, and 17.4% protein) over 5 minutes, starting at time point "zero". Blood samples were collected at time point -15 and -5 minutes prior to liquid meal consumption (time zero). Samples were collected every five minutes from zero to 30 minutes, every 10 minutes from 30 to 180 minutes, and at 210 and 240 minutes. Sera were stored at -85°C. The glucose and insulin AUC was calculated by using the trapezoidal method (17;18).

Body composition and fat distribution

Total body fat mass and lean mass were measured by DXA using a Lunar Prodigy densitometer (GE-Lunar Corporation, Madison, WI, software version 12.3). Participants were required to wear light clothing, remove all metal objects from their body, and lie supine with arms at their sides while undergoing a total body scan. Intra-abdominal adipose tissue (IAAT) was determined by computed tomography (CT) scanning. A five millimeter axial scan at the level of the umbilicus (approximately the L4-L5 intervertebral space) was taken. Scans were later analyzed for cross-sectional area (cm^2) of adipose tissue using SliceOmatic image analysis software (version 4.3: Tomovision, Montreal, Canada). All scans were analyzed by the same image analyst (AG).

Statistical analyses

Descriptive statistics were computed for all study variables of interest. Variables known to deviate from a normal distribution were log 10 transformed prior to statistical analysis. All statistical tests were two-sided and were performed using a type I error rate of 0.05. Statistical analyses were performed using SAS (version 9.1; SAS Institute, Inc., Cary, NC). Paired t-test was used to determine the difference in baseline and 8-wk post intervention serum analytes, AUC data, body composition, and fat distribution by diet group. Simple correlation coefficients were determined for baseline SHBG, total fat mass, total lean mass, IAAT, fasting insulin, insulin AUC, fasting glucose, and glucose AUC. Multiple linear regression modeling was used to determine independent associations of SHBG with IAAT, fasting insulin, fasting glucose, insulin AUC, and glucose AUC at baseline. In order to determine associations among changes in these variables over time, change scores (baseline minus follow-up) were calculated, and simple correlation coefficients were determined for changes in SHBG, IAAT, fasting insulin, fasting glucose, insulin AUC, and glucose AUC. Repeated-measures mixed-model analyses, a robust method for determining longitudinal associations among variables, were used to examine the associations among dependent variable SHBG with independent variables IAAT, fasting insulin, fasting glucose, insulin AUC, and glucose AUC after adjusting for time, diet group, and weight change. Fasting glucose adjusted for fasting insulin, and glucose AUC adjusted for insulin AUC, were examined as independent variables in separate mixed-models to avoid collinearity among the variables.

RESULTS

Descriptive information on the subject population is shown in **Table 1**. By study design, subjects were overweight or obese at baseline (BMI 25-46.9 kg/m^2). Average weight and age at baseline did not statistically differ by diet group. Although each subject's daily energy intake was calculated on an individual basis to maintain body mass, fluctuations in body mass occurred over the 8 week intervention period. On average, a change of - 1.03% (-1.0 kg) in body mass (range = -2.10% to +4.05%; -2.07 kg to +4.00 kg) was observed, which did not statistically differ with diet assignment.

Changes in body composition, fat distribution, and serum analyte variables over the 8-week weight maintenance dietary intervention period are reported in **Table 2**. In both groups, there were significant reductions in weight and total fat mass. Serum triglycerides were significantly reduced in the control group. Only the high fat diet group had significant reductions in SHBG and IAAT, and a significant increase in fasting glucose.

In univariate analyses of baseline data, there were significant correlations between SHBG and fasting glucose ((r=-0.51, P<0.001), fasting insulin (r=-0.36, <0.01), IAAT (r=-0.43, P<0.001), and glucose AUC (r=-0.60, P<0.001). **Table 3** shows the results from multiple linear regression models for dependent variable baseline SHBG. As shown in model 1, fasting glucose was significantly associated with SHBG independent of IAAT and fasting insulin. In model 2, glucose AUC was significantly associated with SHBG independent of IAAT and insulin AUC.

Table 4 shows results from repeated-measures mixed models for dependent variable change in SHBG. As shown in this model, there was a significant association of fasting glucose with change in SHBG (P=0.03), independent of diet group and changes in IAAT, fasting insulin, and weight. When glucose AUC was substituted for fasting glucose (**Table 5**), glucose AUC was significantly associated with change in SHBG (P<0.01) independent of diet group and changes in IAAT, insulin AUC, and weight.

DISCUSSION

The goal of the present study was to determine the effects of consumption of a high fat weight maintenance diet on circulating SHBG in a healthy overweight/obese population. We also aimed to identify the independent determinants of SHBG, and if diet-induced changes in these determinants were related to changes in SHBG over the 8 week intervention. We found that participants who consumed the high fat diet showed a decrease in SHBG and an increase in fasting glucose. At baseline, and over the course of the intervention, SHBG was significantly associated with both fasting and post-challenge glucose, independent of IAAT and insulin. Our findings suggest that consumption of a high fat diet may inhibit hepatic SHBG production, which may in part be mediated by alterations in fasting glucose and glucose response to a meal.

As a result of consumption of the high fat weight maintenance diet, there was a significant reduction in SHBG. This finding is consistent with one previous study observing reduced SHBG among 6 normal weight men following consumption of a high fat diet for 4 weeks(19). A number of studies have suggested that a reduction in CHO intake or glycemic load may increase SHBG by limiting hepatic de novo lipogenesis and

triglyceride production. However, based on our data, it seems possible that limiting CHO and consuming relatively more fat instead may, in fact, suppress SHBG production. Studies have shown that elevated hepatic fat content is strongly correlated with circulating SHBG in humans (12). Although triglycerides decreased in high fat diet group suggesting no diet-effect on hepatic lipogenesis, it is possible that increased dietary fat results in increased exposure of the liver to fatty acids, which in turn may directly inhibit SHBG gene expression by binding to hepatic nuclear factor-4α (20) or may have increased hepatic gluconeogenesis (14).

The observed reduction in circulating SHBG with the high fat diet may have been, in part, mediated by diet-induced changes in glucose metabolism. In this study, we observed independent, inverse associations of SHBG with fasting and post-challenge glucose, both at baseline and following the intervention. Results from several previous studies support the concept that glucose may affect SHBG production (12;20). In mouse and in vitro models, Selva et al found that treatment with glucose and fructose suppressed hepatic SHBG gene expression, an effect that was associated with induction of lipogenesis(20). Peters et al observed a cross-sectional relationship between fasting glucose and SHBG, independent of hepatic lipid and other confounding factors (12). Taken together, we believe these observations provide convincing evidence that glucose may be involved in the regulation of SHBG production. However, it is also possible that SHBG influences hepatic gluconeogenesis, thus regulating fasting glycemia (21). Further studies are needed to clarify the cause and effect nature of associations between glucose and SHBG.

Although several studies have examined associations between fasting glucose and SHBG, we are aware of one other study reporting data concerning post-challenge glucose (22). In the present study, we observed that glucose AUC was strongly associated with SHBG at baseline, and that the change in glucose AUC over the course of the intervention was a strong predictor of the change in SHBG. Glucose AUC appeared more strongly related to SHBG than fasting glucose, and when both were simultaneously placed in multiple regression models, only glucose AUC was significant (data not shown). Glucose AUC integrates and reflects a number of processes, including insulin secretion, insulin sensitivity, glucose production, and glucose disposal. We previously reported that, in this study, the high fat weight maintenance diet was associated with reductions in both beta-cell responsiveness to glucose and insulin sensitivity (15;16). Thus it is possible that changes in these processes could lead to a depression in postprandial glucose disappearance and thereby to increased exposure of the liver to glucose. Further studies are needed to identify if and how postprandial glucose regulates SHBG production.

Consistently, studies have shown that weight loss results in an increase in circulating SHBG. In particular, loss of IAAT has been associated with an increase in SHBG, presumably by decreasing exposure of the liver to free fatty acids. Although the current study was designed for weight maintenance, both diet groups experienced weight and fat loss within ~2 kg. In addition, the high fat diet group experienced a significant reduction in IAAT. Thus, it is possible that changes in IAAT over the course of the intervention affected SHBG production. However, we did not observe an association between the change in IAAT and the change in SHBG. In fact, given that the higher fat

diet was associated with decreases in both IAAT and SHBG, our data do not support the hypothesis that greater IAAT is causally linked to lower SHBG.

Altogether, when taking into account data from previous studies and data from the present study, it seems likely that SHBG production is regulated by a number of processes related to glucose metabolism. Thus, we propose a model whereby hepatic fat content, insulin sensitivity, and beta-cell responsiveness contribute to the regulation of hepatocyte SHBG production (**Fig. 1**). Hepatic fat content may directly influence SHBG production by the hepatocyte, but also indirectly by influencing hepatic gluconeogenesis (12;14). Beta-cell responsiveness, skeletal muscle insulin-stimulated glucose uptake, and hepatic insulin-mediated suppression of glucose production are also major determinants of glucose homeostasis that may be indirectly related to SHBG production.

Strengths of this study included control of subject intake by supplying all food over the study period; weight maintenance study design; use of robust measures to determine body composition and fat distribution; and diets comprised of foods that may be practically consumed and a macronutrient profile with only a modest reduction in carbohydrate. Limitations to this study included a relatively small sample size and inability to determine independent effects of dietary CHO vs. fat. Also, measurement of hepatic fat would have provided greater insight into mechanistic involvement of this variable in the regulation of SHBG production.

In conclusion, during weight maintenance conditions, consumption of a high fat diet was associated with suppression of SHBG. Associations between changes in fasting or post-challenge glucose and changes in SHBG suggested that these variables may be linked in a cause-and-effect manner. Further study is needed to clarify whether dietary

fat depresses SHBG by elevating glucose, and if so, whether the target for this effect is the liver, pancreas, skeletal muscle, or a combination of these tissues.

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REFERENCE LIST

1. Mendel CM. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. J Androl 1992; 13(2):107-116.

2. Kalyani RR, Franco M, Dobs AS et al. The association of endogenous sex hormones, adiposity, and insulin resistance with incident diabetes in postmenopausal women. J Clin Endocrinol Metab 2009; 94(11):4127-4135.

3. Lakshman KM, Bhasin S, Araujo AB. Sex hormone-binding globulin as an independent predictor of incident type 2 diabetes mellitus in men. J Gerontol A Biol Sci Med Sci 2010; 65(5):503-509.

4. Vikan T, Schirmer H, Njolstad I, Svartberg J. Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. Eur J Endocrinol 2010; 162(4):747-754.

5. Agirbasli M, Agaoglu NB, Orak N et al. Sex hormones and metabolic syndrome in children and adolescents. Metabolism 2009; 58(9):1256-1262.

6. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2006; 295(11):1288-1299.

7. Pugeat M, Nader N, Hogeveen K, Raverot G, Dechaud H, Grenot C. Sex hormone-binding globulin gene expression in the liver: drugs and the metabolic syndrome. Mol Cell Endocrinol 2010; 316(1):53-59.

8. Morisset AS, Blouin K, Tchernof A. Impact of diet and adiposity on circulating levels of sex hormone-binding globulin and androgens. Nutr Rev 2008; 66(9):506-516.

9. Azrad M, Gower BA, Hunter GR, Nagy TR. Intra-Abdominal Adipose Tissue Is Independently Associated With Sex-Hormone Binding Globulin in Premenopausal Women. Obesity (Silver Spring) 2012.

10. Maggio M, Lauretani F, Basaria S et al. Sex hormone binding globulin levels across the adult lifespan in women--the role of body mass index and fasting insulin. J Endocrinol Invest 2008; 31(7):597-601.

11. Nayeem F, Nagamani M, Anderson KE, Huang Y, Grady JJ, Lu LJ. Dietary beta-tocopherol and linoleic acid, serum insulin, and waist circumference predict circulating sex hormone-binding globulin in premenopausal women. J Nutr 2009; 139(6):1135-1142.

12. Peter A, Kantartzis K, Machann J et al. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. Diabetes 2010; 59(12):3167-3173.

13. Bonnet F, Balkau B, Malecot JM et al. Sex hormone-binding globulin predicts the incidence of hyperglycemia in women: interactions with adiponectin levels. Eur J Endocrinol 2009; 161(1):81-85.

14. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 2011; 14(6):804-810.

15. Gower BA, Goree LL, Chandler-Laney PC, Ellis AC, Casazza K, Granger WM. A higher-carbohydrate, lower-fat diet reduces fasting glucose concentration and improves beta-cell function in individuals with impaired fasting glucose. Metabolism 2012; 61(3):358-365.

16. Goree LL, Chandler-Laney P, Ellis AC, Casazza K, Granger WM, Gower BA. Dietary macronutrient composition affects beta cell responsiveness but not insulin sensitivity. Am J Clin Nutr 2011; 94(1):120-127.

17. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L. Blood glucose area under the curve. Methodological aspects. Diabetes Care 1990; 13(2):172-175.

18. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ 1990; 300(6719):230-235.

19. Reed MJ, Cheng RW, Simmonds M, Richmond W, James VH. Dietary lipids: an additional regulator of plasma levels of sex hormone binding globulin. J Clin Endocrinol Metab 1987; 64(5):1083-1085.

20. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. J Clin Invest 2007; 117(12):3979-3987.

21. Meyer C, Pimenta W, Woerle HJ et al. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. Diabetes Care 2006; 29(8):1909-1914.

22. Kim C, Kong S, Laughlin GA et al. Endogenous Sex Hormone Changes in Postmenopausal Women in the Diabetes Prevention Program. J Clin Endocrinol Metab 2012.

Table 1. Baseline characteristics of study population by diet group

	Diet		
	High Fat (n=40)	**Control (n=29)**	***P* for difference**
Gender, % male	43%	48%	0.64
Race, % EA	55%	48%	0.58
Age, yr	35.6 ± 8.5[1]	34.6 ± 8.1[1]	0.63
BMI, kg/m^2	33.5 ± 4.3[1]	31.4 ± 4.4[1]	**0.05**

[1]Data reported as mean ± SD; EA, European American; BMI, body mass index

Table 2. Baseline and follow-up measurements of body composition and serum analytes by diet group

Variable	Diet	Baseline	Follow-up	*P* for time
Body Composition				
Weight, kg	High fat	100.5±3.1	98.6±3.1	<0.001
	Control	96.6±3.8	95.1±3.8	<0.001
Total fat mass, kg	High fat	41.3±1.5	39.4±1.7	<0.001
	Control	36.8±1.5	35.1±1.5	<0.001
Total lean mass, kg	High fat	55.7±2.3	55.3±2.3	0.12
	Control	56.4±2.9	56.2±2.8	0.26
IAAT, cm^2	High fat	89.5±7.93	81.5±8.5	0.01
	Control	80.6±9.1	82.4±10.9	0.61
Serum Analytes				
SHBG, nmol/L	High fat	45.7±7.6	39.0±6.2	<0.01
	Control	41.7±6.9	41.1±6.1	0.91
Fasting Glucose, mg/dL	High fat	100.1±1.9	103.0±1.7	0.03
	Control	98.1±1.7	97.6±8.8	0.81
Fasting Insulin, µIU/mL	High fat	12.3±1.3	11.5±7.9	0.26
	Control	11.8±1.2	11.4±6.3	0.55
Glucose AUC	High fat	26270.6±3403.1	26911.7±3465.3	0.19
	Control	26829.7±3614.6	26633.4±3304.7	0.69
Insulin AUC	High fat	874.2±675.2	974.8±7480.5	0.07
	Control	1339.8±368.3	1301.7±1003.9	0.64
Triglycerides, mg/dL	High fat	125.0±77.1	112.8±72.9	0.07
	Control	134.8±77.4	120.5±71.2	0.02

LDL, mg/dL	High fat	121.8±24.6	121.1±27.3	0.87
	Control	117.4±32.1	112.5±33.6	0.07

Baseline and follow-up data reported as mean ± SD; IAAT, intra-abdominal adipose tissue; SHBG, sex hormone-binding globulin; AUC, area under the curve; LDL, low-density lipoprotein.

Table 3. Multiple linear regression models with baseline SHBG as the dependent variable

Model 1	**Variable estimate ± SEE**	**Std β**	***P***
IAAT	-0.00 ± 0.00	-0.11	0.39
Fasting insulin	-0.79 ± 0.40	-0.25	0.06
Fasting glucose	-6.60 ± 1.92	-0.39	**<0.01**
Model 2	**Variable estimate ± SEE**	**Std β**	***P***
IAAT	-0.00 ± 0.00	-0.11	0.42
Insulin AUC	-0.41 ± 0.22	-0.19	0.07
Glucose AUC	-3.41 ± 0.67	-0.62	**<0.001**

Models also adjusted for total body fat. Bold values indicate significant effects. Data reported as standardized β; IAAT, intra-abdominal adipose tissue; AUC, area under the curve.

Table 4. Repeated measures mixed models for dependent variable SHBG

Term	Estimate	s.e	*P*
Intercept	3.841	0.295	<0.001
Time	-0.072	0.132	0.586
Diet	-0.179	0.139	0.199
IAAT	0.004	0.003	0.290
Fasting insulin	-0.003	0.016	0.806
Fasting glucose	-0.019	0.009	**0.034**

Model also adjusted for weight change. Bold values indicate significant effects. IAAT, fasting insulin, and fasting glucose reported as change variables (baseline – follow-up values). IAAT, intra-abdominal adipose tissue

Table 5. Repeated measures mixed models for dependent variable SHBG

Term	Estimate	s.e	*P*
Intercept	3.841	0.295	<0.001
Time	-0.066	0.134	0.619
Diet	-0.217	0.150	0.152
IAAT	0.005	0.004	0.199
Insulin AUC	-0.000	0.000	0.473
Glucose AUC	-0.000	0.000	**0.006**

Model also adjusted for weight change. Bold values indicate significant effects. IAAT, insulin AUC, and glucose AUC reported as change variables (baseline – follow-up values). IAAT, intra-abdominal adipose tissue; AUC, area under the curve.

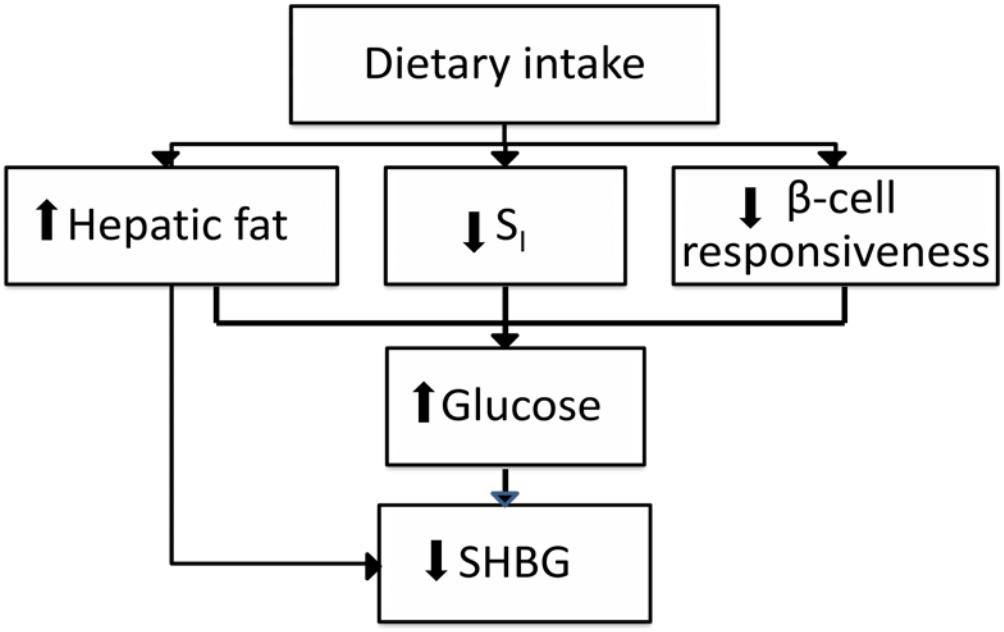

Figure 1. Proposed model incorporating data from previous studies and the current study regarding the major determining factors of hepatic SHBG production. Dietary intake may indirectly regulate the production of SHBG by influencing processes related to glucose metabolism. Data from the current study suggests a high fat diet suppresses SHBG, an effect potentially mediated by reduced whole body insulin sensitivity and a decline in β-cell responsiveness. Hepatic fat content may directly regulate SHBG production or by influencing hepatic gluconeogenesis.

GENERAL DISCUSSION

The primary aims of these studies were to 1) examine the relationships of various fat depots with insulin sensitivity, 2) compare the effects of an 8-week low vs high GL diet on changes in fat distribution during eucaloric conditions and on weight loss during hypocaloric conditions, and 3) examine the interrelationships among changes in glucose metabolism, SHBG, and fat distribution in response to a eucaloric 8-week high vs low fat diet. In a cross-sectional study design, we observed significant differential associations among fat depots and insulin sensitivity among healthy, early postmenopausal women. These findings indicate that maintenance of relatively greater fat distribution to thigh subcutaneous adipose tissue and lesser accumulation of adipose tissue to the intra-abdominal cavity and thigh muscle may either contribute to or reflect greater insulin sensitivity. We then conducted a dietary intervention in order to examine diet as a potential modifiable lifestyle factor that may reduce the risk of metabolic disease. We found that a low GL diet resulted in preferential loss of IAAT and greater weight loss when compared to a high GL diet. However, despite a significant reduction in IAAT in response to consumption of the low GL diet during eucaloric conditions, this did not result in improved metabolic outcomes. We observed that consumption of the low GL diet resulted in higher fasting glycemia and reduced circulating SHBG, effects that may have been mediated by changes in beta cell responsiveness and insulin sensitivity (47;48). Further investigation is warranted to identify causal relationships among fat

distribution and insulin sensitivity, and also therapeutic dietary approaches to improve metabolic processes among overweight and obese individuals independent of weight loss.

Fat Distribution and Insulin Sensitivity

Among our population of healthy postmenopausal women, we observed opposing relationships of the thigh adipose tissue depots and S_I, a robust measure of insulin sensitivity. Few studies have employed such a well accepted measure of insulin sensitivity in observing such relationships (20), and even those studies did not report simultaneous differential relationships among thigh adipose tissue depots and insulin sensitivity. Thigh SAT was positively and independently associated with insulin sensitivity following adjustment for total adiposity. Likewise, results also indicated that a fat distribution phenotype characterized by high IAAT and low thigh SAT had significantly lower insulin sensitivity than a phenotype characterized be high IAAT and high thigh SAT. These findings suggest that maintenance of greater thigh SAT may either promote or reflect greater insulin sensitivity.

Data from the present study suggest IAAT and thigh IMAT may be coordinated depots. IAAT and thigh IMAT were inversely associated with insulin sensitivity, independent of total adiposity. However, thigh IMAT was not associated with S_I independent of IAAT. Subjects were then dichotomized into groups based on high vs low IAAT. Among those with high IAAT, thigh IMAT was significantly, inversely associated with insulin sensitivity. We believe this data suggests that those with high levels of IAAT may also have high levels of thigh IMAT, perhaps above a threshold for impairment of skeletal muscle insulin sensitivity. It is also possible that by

dichotomizing the group based on high vs low IAAT, variance in insulin sensitivity attributable to IAAT was minimized, therefore the relationship between thigh IMAT and insulin sensitivity became apparent. Further studies assessing insulin sensitivity by the hyperinsulinemic euglycemic clamp are warranted to differentiate the contributions of adipose tissue depots to hepatic vs skeletal muscle insulin sensitivity. Given the cross-sectional design of the current study, we cannot draw cause and effect conclusions; however, we believe this data supports the need for interventions designed to reduce IAAT and thigh IMAT, in light of the observed relationships with insulin sensitivity even in a relatively normal weight group of postmenopausal women. This study also emphasizes the need to determine if reduction in visceral and ectopic adiposity is linked to improvement in insulin sensitivity and glucose metabolism.

Macronutrient Manipulation: Fat Distribution and Weight Loss

Numerous studies have reported associations between the intake of low GI foods and smaller waist circumference (28-30); however few studies have reported preferential loss of IAAT following a dietary intervention under weight maintenance conditions. In the present study, consumption of a low vs high GL diet resulted in significant reduction in IAAT in the absence of significant weight loss. Sub-group analysis by diet group and sex indicated that the significant loss in IAAT was specific to women consuming the low GL diet. These findings are congruent with previous studies reporting sex differences in associations of diet with waist circumference, such that a smaller waist circumference has been associated intake in low GI foods in women but not men (28-30). It seems possible that an interaction between changes in postprandial insulin dynamics and the sex

hormone environment in response to the low GL diet may underlie the observed sex-dimorphic results.

The reason for this difference between subgroups is not clear, however these differences may be related to suppression of SHBG. Peter et al reported an inverse association of circulating SHBG with fasting glucose (40). In our data, it was previously reported that consumption of the low vs high GL diet resulted in reduced beta-cell responsiveness and insulin sensitivity (47;48). Changes in these regulatory mechanisms involved in glucose homeostasis may have acted to suppress SHBG. Lower SHBG would increase bioavailability of estradiol, which promotes deposition of fat in the gluteo-femoral region (31). Thus, it seems possible that the interaction of gender and diet resulted in a unique repartitioning of fat from visceral to subcutaneous depots in women who consumed the lower GL diet.

The low GL diet resulted in greater weight and fat loss than the high GL diet under hypocaloric conditions in a healthy overweight and obese population. Those consuming the low GL diet had 4.4kg less total fat mass compared to those consuming the high GL diet following the 8-week intervention. The mechanisms contributing to the observed greater loss in fat mass in response to the low GL diet are unclear. However, animal and human studies suggest a high GI diet may suppress fat oxidation (49;50), an effect that is likely mediated by insulin. Evidence also suggests a low GL vs. high GL diet may increase postprandial energy expenditure, also known as dietary induced thermogenesis (DIT) (51). Scazzina et al reported that a low GL, low GI breakfast consistently resulted in increased postprandial energy expenditure when compared to

breakfasts containing high GI foods (51). Taken together, the findings suggest that the low GL diet may have affected a number of processes that would have led to greater fat mass loss when compared to a high GL diet.

The literature currently lacks tightly controlled dietary intervention studies regarding the efficacy of low GL/GI diets to induce greater weight or fat loss than other dietary approaches. Therefore, findings from the current study mark an important contribution to the literature, indicating that a low GL diet may be a useful strategy for weight loss in the management of obesity. However, in regards to lowering disease risk, a low GL diet may not be an optimal approach for overweight or obese individuals who are maintaining their weight. Significant loss of IAAT among those consuming the low GL diet during the eucaloric phase was not accompanied by improved metabolic outcomes. Our population included overweight and obese individuals with either normal glucose-tolerance or impaired fasting glucose. The low GL diet appeared to reduce insulin sensitivity among the normal glucose tolerant subjects and reduce beta cell responsiveness among all subjects (47;48). While these effects may be perceived as detrimental to the progression of overt disease development, we cannot rule out the possibility that these attributes of the eucaloric low GL diet arm influenced degree of weight loss in the subsequent hypocaloric arm of the study. It is also possible that a low GL diet may be ideal for populations without underlying metabolic abnormalities; thus, it may be important to identify the metabolic effects of diets differing in GL in lean, healthy populations. Conversely, a low GL diet may further perturb processes related to glucose metabolism among those with a predisposition to disease development.

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Macronutrient Manipulation: SHBG and Glucose Metabolism

The low GL diet was 39% calories from fat, an aspect of the diet that may have altered metabolic outcomes among overweight and obese individuals under eucaloric conditions. Consumption of the 8-week low GL diet resulted in significantly elevated fasting glycemia and reduced SHBG. These findings suggest that any positive contribution of loss of IAAT to metabolic health was masked by the adverse effects of the diet on aspects of glucose metabolism. It is also possible that the loss of IAAT contributed to changes in glucose metabolism. Elevated exposure of the liver to FFA draining into the hepatic portal vein from the intra-abdominal cavity may have altered gluconeogenesis and hepatic insulin sensitivity (41). In a canine model, Kabir et al reported an increase in both hepatic insulin resistance and gluconeogenic gene expression following feeding of a high fat eucaloric diet for 12 weeks (19). These changes in hepatic metabolism appeared to be related to elevated FFA mobilization in the visceral cavity. Thus, it is possible loss of IAAT affected hepatic glucose production. Although a relationship between IAAT and SHBG has been previously reported (37), in the current study change in IAAT was not directly associated with SHBG.

Following the 8-week eucaloric diet phase, change in SHBG was significantly associated with change in fasting and postchallenge glucose, independent of diet, insulin, IAAT, and total fat mass. Epidemiological data reports low circulating SHBG among populations with type 2 diabetes (35); thus it has been hypothesized that SHBG is directly involved in the development of type 2 diabetes by influencing hepatic glucose production. One previous human study has reported a cross-sectional relationship between fasting glucose and SHBG, independent of possible confounding factors such as

insulin, IAAT, and liver fat (40). Therefore, the current study adds to the literature by demonstrating an independent relationship between change in SHBG and fasting glucose following a dietary intervention. We also observed a significant relationship between change in SHBG and glucose AUC. While other factors are likely involved in the regulation of SHBG production such as total fat mass, hepatic fat content, and IAAT, the here-observed associations suggest that under the eucaloric conditions of this dietary intervention, changes to glucose metabolism were likely involved in suppressing SHBG production. Given that glucose AUC reflects a number of processes related to glucose metabolism, rather than just hepatic glucose production, we believe this provides further evidence supporting the hypothesis that SHBG is a biomarker for underlying metabolic aberrations.

Strengths and Limitations

The strengths of these studies included robust methods used to assess body composition, fat distribution, and insulin sensitivity. For the dietary intervention studies, strengths included strict control of food intake by providing all foods to be consumed over the course of both the eucaloric intervention period and the hypocaloric diet period. Also, registered dietitians planned menus for both dietary approaches, and provided oversight for food distribution and compliance. The diet menus included foods that could be practically consumed on a typical American diet. By design, the eucaloric arm allowed for elimination of confounding of results by significant weight loss. Limitations to these studies include the relatively small number of subjects and the cross-sectional design of the first aim. We did not assess hepatic fat content or liver function; therefore, we are unable to determine independent relationships of hepatic fat with other ectopic

lipid depots and insulin sensitivity. Also, measurement of hepatic fat would have provided greater insight into mechanistic involvement of this variable in the regulation of SHBG production. We did not have statistical power to examine potential ethnic differences in the relationships of interest or to examine data in subgroups by ethnicity. The dietary intervention was designed to examine diets differing in percent fat and CHO, therefore it is possible that differences in the types of CHO and fats included in each of the diets affected outcomes of interest.

Future Directions

The studies included in this project contribute to the current understanding of the relationship between fat distribution and metabolic health, and the impact of dietary macronutrient composition on fat distribution, weight loss, and glucose metabolism. This was the first study to report differential relationships of adipose tissue depots of the thigh with insulin sensitivity among healthy, relatively normal weight postmenopausal women. The causal nature of these associations remains equivocal and in order to address this issue, it seems imperative to first understand the factors determining the degree of triglyceride storage to subcutaneous adipose tissue, assuming the downregulation of this process is the primary defect leading to accrual of lipid elsewhere. Further investigations are warranted to identify whether visceral and ectopic adipose tissue directly influences insulin sensitivity, or whether other mediating factors explain these observed associations.

The low GL diet resulted in greater weight loss than the high GL load diet over 8 weeks, but whether this particular dietary approach is optimal in regards to maintaining long-term weight loss is an important issue that needs to be addressed. Further, the effects of the low GL dietary approach on metabolic outcomes during weight maintenance may have been unique to an overweight/obese phenotype and cannot be extrapolated to lean or weight-reduced populations. In addition, future studies are needed to determine the effects of a higher fat/reduced CHO diet on SHBG production in populations with hyperandrogenemia, polycystic ovary syndrome, or other disease states that may benefit from greater circulating SHBG.

GENERAL LIST OF REFERENCES

1. Despres JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis 1990; 10(4):497-511.

2. Alexander CM, Landsman PB, Teutsch SM, Haffner SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes 2003; 52(5):1210-1214.

3. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care 1994; 17(9):961-969.

4. Despres JP. Intra-abdominal obesity: an untreated risk factor for Type 2 diabetes and cardiovascular disease. J Endocrinol Invest 2006; 29(3 Suppl):77-82.

5. Yim JE, Heshka S, Albu JB, Heymsfield S, Gallagher D. Femoral-gluteal subcutaneous and intermuscular adipose tissues have independent and opposing relationships with CVD risk. J Appl Physiol 2008; 104(3):700-707.

6. Zoico E, Rossi A, Di F, V et al. Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. J Gerontol A Biol Sci Med Sci 2010; 65(3):295-299.

7. Van Pelt RE, Jankowski CM, Gozansky WS, Schwartz RS, Kohrt WM. Lower-body adiposity and metabolic protection in postmenopausal women. J Clin Endocrinol Metab 2005; 90(8):4573-4578.

8. Williams MJ, Hunter GR, Kekes-Szabo T, Snyder S, Treuth MS. Regional fat distribution in women and risk of cardiovascular disease. Am J Clin Nutr 1997; 65(3):855-860.

9. Gallagher D, Kuznia P, Heshka S et al. Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. Am J Clin Nutr 2005; 81(4):903-910.

10. Hwang JH, Stein DT, Barzilai N et al. Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. Am J Physiol Endocrinol Metab 2007; 293(6):E1663-E1669.

11. Jakobsen MU, Berentzen T, Sorensen TI, Overvad K. Abdominal obesity and fatty liver. Epidemiol Rev 2007; 29:77-87.

12. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 2008; 134(5):1369-1375.

13. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006; 444(7121):881-887.

14. McQuaid SE, Hodson L, Neville MJ et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes 2011; 60(1):47-55.

15. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89(6):2548-2556.

16. Arner P. Not all fat is alike. Lancet 1998; 351(9112):1301-1302.

17. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 1990; 10(4):493-496.

18. Bjorntorp P. Metabolic implications of body fat distribution. Diabetes Care 1991; 14(12):1132-1143.

19. Kabir M, Catalano KJ, Ananthnarayan S et al. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. Am J Physiol Endocrinol Metab 2005; 288(2):E454-E461.

20. Albu JB, Kenya S, He Q et al. Independent associations of insulin resistance with high whole-body intermuscular and low leg subcutaneous adipose tissue distribution in obese HIV-infected women. Am J Clin Nutr 2007; 86(1):100-106.

21. Albu JB, Kovera AJ, Allen L et al. Independent association of insulin resistance with larger amounts of intermuscular adipose tissue and a greater acute insulin response to glucose in African American than in white nondiabetic women. Am J Clin Nutr 2005; 82(6):1210-1217.

22. Boettcher M, Machann J, Stefan N et al. Intermuscular adipose tissue (IMAT): association with other adipose tissue compartments and insulin sensitivity. J Magn Reson Imaging 2009; 29(6):1340-1345.

23. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am J Clin Nutr 2000; 71(4):885-892.

24. Ludwig DS. Dietary glycemic index and the regulation of body weight. Lipids 2003; 38(2):117-121.

25. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med 2011; 364(25):2392-2404.

26. Armendariz-Anguiano AL, Jimenez-Cruz A, Bacardi-Gascon M, Hurtado-Ayala L. Effect of a low glycemic load on body composition and Homeostasis Model Assessment (HOMA) in overweight and obese subjects. Nutr Hosp 2011; 26(1):170-175.

27. Esfahani A, Wong JM, Mirrahimi A, Villa CR, Kendall CW. The application of the glycemic index and glycemic load in weight loss: A review of the clinical evidence. IUBMB Life 2011; 63(1):7-13.

28. Halkjaer J, Sorensen TI, Tjonneland A, Togo P, Holst C, Heitmann BL. Food and drinking patterns as predictors of 6-year BMI-adjusted changes in waist circumference. Br J Nutr 2004; 92(4):735-748.

29. Halkjaer J, Tjonneland A, Thomsen BL, Overvad K, Sorensen TI. Intake of macronutrients as predictors of 5-y changes in waist circumference. Am J Clin Nutr 2006; 84(4):789-797.

30. Sasakabe T, Haimoto H, Umegaki H, Wakai K. Effects of a moderate low-carbohydrate diet on preferential abdominal fat loss and cardiovascular risk factors in patients with type 2 diabetes. Diabetes Metab Syndr Obes 2011; 4:167-174.

31. Elbers JM, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am J Physiol 1999; 276(2 Pt 1):E317-E325.

32. Mittelman SD, Van Citters GW, Kirkman EL, Bergman RN. Extreme insulin resistance of the central adipose depot in vivo. Diabetes 2002; 51(3):755-761.

33. Ostman J, Arner P, Engfeldt P, Kager L. Regional differences in the control of lipolysis in human adipose tissue. Metabolism 1979; 28(12):1198-1205.

34. Kalyani RR, Franco M, Dobs AS et al. The association of endogenous sex hormones, adiposity, and insulin resistance with incident diabetes in postmenopausal women. J Clin Endocrinol Metab 2009; 94(11):4127-4135.

35. Lakshman KM, Bhasin S, Araujo AB. Sex hormone-binding globulin as an independent predictor of incident type 2 diabetes mellitus in men. J Gerontol A Biol Sci Med Sci 2010; 65(5):503-509.

36. Mendel CM. The free hormone hypothesis. Distinction from the free hormone transport hypothesis. J Androl 1992; 13(2):107-116.

37. Azrad M, Gower BA, Hunter GR, Nagy TR. Intra-Abdominal Adipose Tissue Is Independently Associated With Sex-Hormone Binding Globulin in Premenopausal Women. Obesity (Silver Spring) 2012.

38. Maggio M, Lauretani F, Basaria S et al. Sex hormone binding globulin levels across the adult lifespan in women--the role of body mass index and fasting insulin. J Endocrinol Invest 2008; 31(7):597-601.

39. Nayeem F, Nagamani M, Anderson KE, Huang Y, Grady JJ, Lu LJ. Dietary beta-tocopherol and linoleic acid, serum insulin, and waist circumference predict circulating sex hormone-binding globulin in premenopausal women. J Nutr 2009; 139(6):1135-1142.

40. Peter A, Kantartzis K, Machann J et al. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. Diabetes 2010; 59(12):3167-3173.

41. Jensen MD. Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model. Obesity (Silver Spring) 2006; 14 Suppl 1:20S-24S.

42. Hertz R, Magenheim J, Berman I, Bar-Tana J. Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. Nature 1998; 392(6675):512-516.

43. Janne M, Hammond GL. Hepatocyte nuclear factor-4 controls transcription from a TATA-less human sex hormone-binding globulin gene promoter. J Biol Chem 1998; 273(51):34105-34114.

44. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. J Clin Invest 2007; 117(12):3979-3987.

45. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 2011; 14(6):804-810.

46. Morisset AS, Blouin K, Tchernof A. Impact of diet and adiposity on circulating levels of sex hormone-binding globulin and androgens. Nutr Rev 2008; 66(9):506-516.

47. Gower BA, Goree LL, Chandler-Laney PC, Ellis AC, Casazza K, Granger WM. A higher-carbohydrate, lower-fat diet reduces fasting glucose concentration and improves beta-cell function in individuals with impaired fasting glucose. Metabolism 2012; 61(3):358-365.

48. Goree LL, Chandler-Laney P, Ellis AC, Casazza K, Granger WM, Gower BA. Dietary macronutrient composition affects beta cell responsiveness but not insulin sensitivity. Am J Clin Nutr 2011; 94(1):120-127.

49. Isken F, Klaus S, Petzke KJ, Loddenkemper C, Pfeiffer AF, Weickert MO. Impairment of fat oxidation under high- vs. low-glycemic index diet occurs before the development of an obese phenotype. Am J Physiol Endocrinol Metab 2010; 298(2):E287-E295.

50. Roberts R, Bickerton AS, Fielding BA et al. Reduced oxidation of dietary fat after a short term high-carbohydrate diet. Am J Clin Nutr 2008; 87(4):824-831.

51. Scazzina F, Del RD, Benini L et al. The effect of breakfasts varying in glycemic index and glycemic load on dietary induced thermogenesis and respiratory quotient. Nutr Metab Cardiovasc Dis 2011; 21(2):121-125.

APPENDIX A

INSTITUTIONAL REVIEW BOARD FORMS

Project Revision/Amendment Form

Form version: October 28, 2010

In MS Word, click in the white boxes and type your text; double-click checkboxes to check/uncheck.

- Federal regulations require IRB approval before implementing proposed changes. See Section 14 of the IRB Guidebook for Investigators for additional information.
- Change means any change, in content or form, to the protocol, consent form, or any supportive materials (such as the Investigator's Brochure, questionnaires, surveys, advertisements, etc.). See Item 4 for more examples.

1. Today's Date	06/15/2012

2. Principal Investigator (PI)

Name (with degree)	Barbara Gower, PhD	Blazer ID	bgower
Department	Nutrition Science	Division (if applicable)	
Office Address	WEBB 423, zip 3360	Office Phone	4-4087
E-mail	bgower@uab.edu	Fax Number	4-7050

Contact person who should receive copies of IRB correspondence (Optional)

Name	Amy Miskimon	E-Mail	amymiski@uab.edu
Phone	975-9980	Fax Number	
Office Address (if different from PI)	JT 1566		

3. UAB IRB Protocol Identification

3.a. Protocol Number: F070322005

3.b. Protocol Title: Intramuscular Lipid and Insulin Action: Ethnic Aspects

3.c. Current Status of Protocol—Check ONE box at left; provide numbers and dates where applicable

- ☐ Study has not yet begun — No participants, data, or specimens have been entered.
- ☐ In progress, open to accrual — Number of participants, data, or specimens entered:
- ☐ Enrollment temporarily suspended by sponsor
- ☐ Closed to accrual, but procedures continue as defined in the protocol (therapy, intervention, follow-up visits, etc.)
  - Date closed: — Number of participants receiving interventions: — Number of participants in long-term follow-up only:
- ☒ Closed to accrual, and only data analysis continues
  - Date closed: 6/30/09 — Total number of participants entered: 69 ✓

4. Types of Change

Check all types of change that apply, and describe the changes in Item 5.c. or 5.d. as applicable. To help avoid delay in IRB review, please ensure that you provide the required materials and/or information for each type of change checked.

- ☐ **Protocol revision (change in the IRB-approved protocol)**
  In Item 5.c., if applicable, provide sponsor's protocol version number, amendment number, update number, etc.
- ☐ **Protocol amendment (addition to the IRB-approved protocol)**
  In Item 5.c., if applicable, provide funding application document from sponsor, as well as sponsor's protocol version number, amendment number, update number, etc.
- ☒ **Add or remove personnel**
  In Item 5.c., include name, title/degree, department/division, institutional affiliation, and role(s) in research, and address whether new personnel have any conflict of interest. See "Change in Principal Investigator" in the IRB Guidebook if the principal investigator is being changed.
  - ☒ **Add graduate student(s) or postdoctoral fellow(s) working toward thesis, dissertation, or publication**
    In Item 5.c., (a) identify these individuals by name; (b) provide the working title of the thesis, dissertation, or publication; and (c) indicate whether or not the student's analysis differs in any way from the purpose of the research described in the IRB-approved HSP (e.g., a secondary analysis of data obtained under this HSP). ✓
- ☐ **Change in source of funding; change or add funding**
  In Item 5.c., describe the change or addition in detail, include the applicable OGCA tracking number(s), and provide a copy of the application as funded (or as submitted to the sponsor if pending). Note that some changes in funding may require a new IRB application.

☐ **Add or remove performance sites**
In Item 5.c., identify the site and location, and describe the research-related procedures performed there. If adding site(s), attach notification of permission or IRB approval to perform research there. Also include copy of subcontract, if applicable. If this protocol includes acting as the Coordinating Center for a study, attach IRB approval from any non-UAB site added.

☐ **Add or change a genetic component or storage of samples and/or data component—this could include data submissions for Genome-Wide Association Studies (GWAS)**
To assist you in revising or preparing your submission, please see the IRB Guidebook for Investigators or call the IRB office at 934-3789.

☐ **Suspend, re-open, or permanently close protocol to accrual of individuals, data, or samples (IRB approval to remain active)**
In Item 5.c., indicate the action, provide applicable dates and reasons for action; attach supporting documentation.

☐ **Report being forwarded to IRB (e.g., DSMB, sponsor or other monitor)**
In Item 5.c., include date and source of report, summarize findings, and indicate any recommendations.

☐ **Revise or amend consent, assent form(s)**
Complete Item 5.d.

☐ **Addendum (new) consent form**
Complete Item 5.d.

☐ **Add or revise recruitment materials**
Complete Item 5.d.

☐ **Other (e.g., investigator brochure)**
Indicate the type of change in the space below, and provide details in Item 5.c. or 5.d. as applicable.
Include a copy of all affected documents, with revisions highlighted as applicable.

▶

5. Description and Rationale

In Item 5.a. and 5.b, check Yes or No and see instructions for Yes responses.
In Item 5.c. and 5.d, describe—and explain the reason for—the change(s) noted in Item 4.

☐ Yes ☐ No **5.a. Are any of the participants enrolled as normal, healthy controls?**
If yes, describe in detail in Item 5.c. how this change will affect those participants.

☐ Yes ☐ No **5.b. Does the change affect subject participation, such as procedures, risks, costs, location of services, etc.?**
If yes, FAP-designated units complete a FAP submission and send to fap@uab.edu. Identify the FAP-designated unit in Item 5.c.
For more details on the UAB FAP, see www.uab.edu/cto.

5.c. Protocol Changes: In the space below, briefly describe—and explain the reason for—all change(s) to the protocol.

▶ Please add graduate student, Amy Miskimon, MS, RD from the Department of Nutrition Sciences, UAB, to the HSP. She will be conducting a secondary data analysis for publication and her dissertation entitled, *Fat distribution and metabolic health: The effects of macronutrient manipulation on weight loss, fat distribution, and glucose metabolism*. This student has no conflicts of interest to disclose.

√ SIRB √ IC

5.d. Consent and Recruitment Changes: In the space below,
(a) describe all changes to IRB-approved forms or recruitment materials and the reasons for them;
(b) describe the reasons for the addition of any materials (e.g., addendum consent, recruitment); and
(c) indicate either how and when you will reconsent enrolled participants or why reconsenting is not necessary (not applicable for recruitment materials).

Also, indicate the number of forms changed or added. For new forms, provide 1 copy. For revised documents, provide 3 copies:
- **a copy of the currently approved document (showing the IRB approval stamp, if applicable)**
- **a revised copy highlighting all proposed changes with "tracked" changes**
- **a revised copy for the IRB approval stamp.**

▶

Signature of Principal Investigator [signature] Date 6/15/12

FOR IRB USE ONLY

☐ Received & Noted ☑ Approved Expedited* ☐ To Convened IRB

f Clark Williams June 18, 2012

Signature (Chair, Vice-Chair, Designee) Date

DOLA 10-26-11

Change to Expedited Category Y / N / NA

*No change to IRB's previous determination of approval criteria at 45 CFR 46.111 or 21 CFR 56.111