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Reduced Growth Hormone Secretion in Obesity is Associated with Smaller LDL and HDL Particle Size

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Abstract

Objective—Reduced growth hormone (GH) secretion is observed in obesity and may contribute to increases in cardiovascular disease (CVD) risk. Lipoprotein characteristics including increased small dense LDL particles are known independent risk factors for CVD. We hypothesized that reduced GH secretion in obesity would be associated with a more atherogenic lipid profile including increased small dense LDL particles.

Design—To evaluate this hypothesis, we studied 102 normal weight and obese men and women using standard GH stimulation testing to assess GH secretory capacity and performed comprehensive lipoprotein analyses including determination of lipoprotein particle size and subclass concentrations using proton NMR spectroscopy.

Results—Obese subjects were stratified into reduced or sufficient GH secretion based on the median peak stimulated GH ($6.25 \mu\text{g/l}$). Obese subjects with reduced GH secretion ($n=35$) demonstrated a smaller mean LDL and HDL particle size in comparison to normal weight subjects ($n=33$) or obese subjects with sufficient GH ($n=34$) by ANOVA ($P<0.0001$). Univariate analyses demonstrated peak stimulated GH was positively associated with LDL ($r=0.50$; $P<0.0001$) and HDL ($r=0.57$; $P<0.0001$) but not VLDL ($P=0.06$) particle size. Multivariate regression analysis controlling for age, gender, race, ethnicity, tobacco, use of lipid lowering medication, BMI and HOMA demonstrated peak stimulated GH remained significantly associated with LDL particle size ($\beta=0.01$; $P=0.01$; $R^2=0.42$; $P<0.0001$ for overall model) and HDL particle size ($\beta=0.008$; $P=0.001$; $R^2=0.44$; $P<0.0001$ for overall model).

Conclusions—These results suggest reduced peak stimulated GH in obesity is independently associated with a more atherogenic lipoprotein profile defined in terms of particle size.

Keywords

relative growth hormone deficiency; lipoprotein particle size; cardiovascular risk

Introduction

Reduced GH secretion observed in obesity is associated with increased cardio-metabolic complications including dyslipidemia, insulin resistance, inflammation and increased carotid intima-media thickness^{1,2}. In addition to traditional lipid profiles, specific lipoprotein

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characteristics such as increased concentrations of small dense low-density lipoprotein (LDL) particles have been associated with increased risk of cardiovascular disease (CVD), including incident CVD events³. In subjects with GH deficiency due to hypothalamic/pituitary disease, despite similar total LDL concentrations, an increase in the concentration of small dense LDL particles was noted compared to age and BMI matched controls⁴. However, a detailed analysis of lipoprotein particle profile, including size and concentration of the lipoprotein sub-species, has not been performed to our knowledge in obese subjects with reduced GH secretion. We hypothesized that obese subjects with reduced GH secretion would have higher concentrations of small LDL particles which may contribute to the increased cardio-metabolic risk associated with reduced GH secretion in obesity. We therefore performed standard GH stimulation tests and determined the particle size and concentrations of lipoprotein sub-species by proton nuclear magnetic resonance (NMR) spectroscopy in a group of well phenotyped normal weight and obese subjects.

Methods and Procedures

Study subjects

Obese (n=69, BMI >30 kg/m²) and normal weight (n=33, BMI <25 kg/m²) men and women from the Boston area were enrolled. Data from these subjects were previously published in a study evaluating the relationship between reduced GH secretion and increased carotid intima-media thickness in obesity², however, detailed lipoprotein profiles of these subjects have yet to be reported. These subjects were relatively healthy adults, age 18 to 55 years, without known pituitary disease. Exclusion criteria included a history of diabetes mellitus, thyroid disorders, chronic medical condition such as HIV infection, or any medical condition known to affect the GH axis. Subjects taking medications known to affect GH secretion were excluded. Subjects with serum creatinine >1.5 mg/dl, hemoglobin <11g/dl, or aspartate aminotransferase >2.5 times the upper limit of the normal range were also excluded. Subjects underwent written informed consent in compliance with the guidelines of the Subcommittee on Human Studies at the Massachusetts General Hospital prior to the administration of any study procedures.

Biochemical assessment

Subjects underwent standard GH stimulation testing with GH releasing hormone (GHRH) and arginine as previously reported^{2,5}. Briefly, subjects were administered GHRH 1-29 [1 µg/kg] (Sermorelin acetate, Geref, Serono Laboratories, Inc., Norwell, Ma) intravenously followed by arginine hydrochloride [30 g/300 ml (max 30 g)]. GH levels were measured at 0, 30, 45, 60, 90 and 120 minutes. GH was determined by a paramagnetic particle, chemiluminescent immunoassay (Beckman Coulter, Chaska, MN) with an analytical sensitivity of 0.002 µg/l. The intra-assay CV is 1.90-2.78 % and the inter-assay CV is 1.77-2.65 %. NMR spectroscopy was performed as previously described⁶ on fasting plasma samples by a commercial vendor (Liposcience Inc., Raleigh, NC). The NMR technique has previously been validated against lipoprotein subspecies distribution determined by gel electrophoresis with good results⁶. Oxidized LDL was measured in fasting plasma samples using commercially available ELISA kit (ALPCO Diagnostics, Salem, NH) with an intra-assay CV of 4-7.6 % and inter-assay CV of 6.2-10.7 %. Subjects also completed a 75 gram oral glucose tolerance test (OGTT) and determination of fasting cholesterol profile using standard methodologies.

Anthropometric Measurements

Height and body weight were obtained after an overnight fast. Total body fat percentage was determined by dual X-ray absorptiometry (DXA) testing using a Hologic, Inc-4500 densitometer (Hologic, Inc., Waltham, MA). In addition, 1 cm cross-sectional abdominal CT

scans were performed at the level of L4 to assess the distribution of abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) as previously described ⁷.

Statistical analysis

Lipoprotein particle size and sub-species concentrations were compared between normal weight and obese subjects using Student's t-test. Normal weight subjects were included for comparison with obese subjects as an internal control. Obese subjects were then stratified into GH sufficient (GHS) or relative GH deficient (GHD) based on the median peak stimulated GH value amongst the obese subjects ($6.25\mu\text{g/l}$). Lipoprotein particle size and sub-species concentrations were compared between normal weight, obese GHS and obese GHD using ANOVA, and adjustments for age, gender, race and BMI were also made. Subsequently, Tukey-Kramer post-hoc test to determine significant difference between groups was performed for comparisons with significant ANOVAs. Mean LDL and mean HDL particle size was related to various measures of body adiposity and to various metabolic parameters using univariate regression analyses with Pearson correlations. In addition, the association between peak stimulated GH and lipoprotein particle size and sub-species concentration was also determined using univariate regression analyses with Pearson correlations. Adjustments for possible covariates including age, gender, race, ethnicity, tobacco use, use of lipid lowering medication, anthropometric measurements, HOMA and their respective cholesterol concentrations was performed using multivariate regression analyses with standard least squares modeling. Variables were selected for entry into the multivariate models based on known associations with lipoprotein particle size from the literature (such as age, gender, race and ethnicity, and insulin sensitivity ⁸⁻¹³) and based on significant associations with mean LDL or HDL particle size on univariate analyses (measures of adiposity, cholesterol and insulin sensitivity). In addition, sensitivity analyses using the median peak GH responses among normal weight subjects as cutoffs were performed. Statistical analysis was performed using JMP 8.0.2 (SAS Institute, Cary, North Carolina, USA). Statistical significance was defined as $P<0.05$.

Results

Clinical characteristics of study subjects

Thirty-three normal weight and 69 obese subjects were studied. The two groups were similar in age (40.4 ± 1.9 vs. 42.1 ± 1.1 years; $P=0.42$), gender (64 vs. 57 % male; $P=0.49$), race (64 vs. 51 % Caucasian; $P=0.22$) and tobacco use (5.2 ± 1.7 vs. 7.4 ± 1.6 pack years; $P=0.39$). Obese subjects had higher BMI (22.5 ± 0.3 vs. 38.1 ± 0.8 kg/m^2 ; $P<0.0001$) and waist circumference (80 ± 1 vs. 119 ± 2 cm; $P<0.0001$) as expected. The two groups had similar levels of total cholesterol (174 ± 6 vs. 182 ± 4 mg/dl; $P=0.26$) but obese subjects had higher triglycerides (75 ± 7 vs. 127 ± 10 mg/dl; $P=0.0009$) and LDL cholesterol (101 ± 5 vs. 116 ± 4 mg/dl; $P=0.02$) and lower HDL cholesterol (58 ± 3 vs. 45 ± 1 mg/dl; $P<0.0001$). Obese subjects had lower fasting GH (0.8 ± 0.2 vs. 0.1 ± 0.03 $\mu\text{g/l}$; $P<0.0001$), peak stimulated GH (44.7 ± 4.9 vs. 8.8 ± 0.9 $\mu\text{g/l}$; $P<0.0001$) as well as a lower IGF-1 (93.1 ± 7.6 vs. 73.5 ± 4.1 $\mu\text{g/l}$; $P=0.01$) (all comparisons normal weight vs. obese).

Lipoprotein particle size in obesity

Obese subjects had smaller mean LDL (21.6 ± 0.1 vs. 20.8 ± 0.1 nm; $P<0.0001$) and HDL (9.3 ± 0.08 vs. 8.8 ± 0.05 nm; $P<0.0001$) and tended to have a larger VLDL (49.0 ± 1.5 vs. 52.4 ± 1.2 nm; $P=0.09$) particle size compared to normal weight subjects. Obese subjects also had greater concentrations of small (376.9 ± 44.4 vs. 808.4 ± 51.6 nmol/l; $P<0.0001$), medium small (79.1 ± 9.0 vs. 168.1 ± 10.5 nmol/l; $P<0.0001$) and very small (297.7 ± 35.6 vs. 640.3 ± 41.5 nmol/l; $P<0.0001$) LDL particles with lower concentrations of large LDL

particles (441.9 ± 24.2 vs. 325.4 ± 21.0 nmol/l; $P=0.001$) compared to normal weight subjects. Similarly, obese subjects had greater concentrations of small HDL (18.2 ± 0.9 vs. 21.0 ± 0.6 nmol/l; $P=0.01$) but lower concentrations of large HDL (9.2 ± 0.6 vs. 5.5 ± 0.4 nmol/l; $P<0.0001$) particles. The two groups had similar concentrations of medium HDL particles (3.5 ± 0.5 vs. 3.5 ± 0.4 nmol/l; $P=0.98$). Obese subjects had higher concentrations of IDL particles compared to normal weight subjects (29.8 ± 7.1 vs. 56.1 ± 6.1 nmol/l; $P=0.01$). There were no differences in the concentration of oxidized LDL (2394 ± 607 vs. 3001 ± 1019 mU/l; $P=0.69$) (all comparisons normal weight vs. obese, respectively).

Growth hormone in obesity

The obese subjects had a mean GH value of 8.8 ± 0.9 $\mu\text{g/l}$ and a median GH value of 6.25 $\mu\text{g/l}$ (25-75% range: 4.4-9.5 $\mu\text{g/l}$). The median GH value of 6.25 $\mu\text{g/l}$ was utilized as the cut-off criteria to define relative GH deficiency in obesity so as to obtain equal number of subjects in the GH sufficient and the relative GH deficient group.

The obese GHD subjects (peak GH 6.25 $\mu\text{g/l}$; $n=35$) tended to be older ($P=0.07$ by ANOVA) and more likely to be Caucasian ($P=0.06$ by ANOVA) and had higher percentage of male subjects ($P=0.03$ by ANOVA) and higher BMI ($P<0.0001$ by ANOVA) compared to normal weight and obese GHS subjects (peak GH >6.25 $\mu\text{g/l}$; $n=34$) (Table 1). Therefore, all results comparing normal weight, obese GHS and obese GHD subjects for lipoprotein particle size and sub-species concentration were adjusted for age, gender, race and BMI.

The obese GHS and GHD subjects had similar percent adipose tissue by DXA and similar abdominal SAT. However, the obese GHD subjects had a greater waist circumference and higher VAT compared to the obese GHS subjects (Table 1).

Lipoprotein particle size in normal weight, obese GHS and obese GHD subjects

The obese GHD group demonstrated a smaller mean LDL particle size in comparison with normal weight and obese GHS group by overall ANOVA ($P<0.0001$). Using Tukey-Kramer post-hoc analyses, the obese GHD group had smaller mean LDL particle size compared to normal weight subjects ($P<0.05$). The obese GHS group also had smaller mean LDL particle size compared to normal weight subjects ($P<0.05$). The obese GHD group had higher mean concentrations of small, medium small, and very small LDL particles in comparison to the normal weight group. The obese GHD subjects also had lower mean concentrations of large LDL particles in comparison to normal weight subjects but similar levels compared to obese GHS subjects. The obese GHS subjects similarly had greater levels of small, medium small and very small LDL sub-species in comparison with normal weight subjects (Table 1).

The obese GHD group had smaller mean HDL particle size compared to normal weight and the obese GHS group by ANOVA ($P<0.0001$) and Tukey-Kramer post-hoc test ($P<0.05$). The obese GHD group had higher mean concentrations of small HDL particles and lower mean concentrations of large HDL particles compared to normal weight subjects. The obese GHS group had lower mean concentrations of large HDL particles compared to normal weight subjects as well but had similar levels of medium and small HDL particles (Table 1).

There was no difference in the mean VLDL particle size. The obese GHD group had higher mean concentrations of medium and large VLDL and chylomicron particles compared to normal weight and obese GHS subjects, however, there were no differences in the small VLDL particle concentrations. The obese GHS subjects had similar level of VLDL and chylomicrons in comparison with the normal weight subjects (Table 1).

There were no differences in oxidized LDL among the three groups (2394 ± 607 vs. 2196 ± 451 vs. 3783 ± 1966 ; adjusted $P=0.93$ comparing normal weight vs. obese GHS vs. obese GHD; Table 1).

Relationship of LDL and HDL particle size to metabolic parameters

Mean LDL particle size was inversely associated with measures of adiposity including BMI ($r=-0.48$; $P<0.0001$), waist circumference ($r=-0.47$; $P<0.0001$), percent body fat by DXA ($r=-0.36$; $P=0.0002$), VAT ($r=-0.51$; $P<0.0001$) and SAT ($r=-0.36$; $P=0.0004$) (Table 2). While LDL particle size was not associated with total cholesterol ($P=0.23$), it was inversely associated with serum concentrations of LDL cholesterol ($r=-0.26$; $P=0.007$) and triglycerides ($r=-0.60$; $P<0.0001$) and positively associated with serum concentrations of HDL cholesterol ($r=0.68$; $P<0.0001$) (Table 2). In addition, mean LDL particle size was inversely associated with measures of insulin sensitivity including HbA1c ($r=-0.31$; $P=0.001$), fasting glucose ($r=-0.27$; $P=0.006$), 2-hour glucose ($r=-0.43$; $P<0.0001$), fasting insulin ($r=-0.37$; $P=0.0001$) and HOMA ($r=-0.37$; $P=0.0002$) (Table 2).

Mean HDL particle size was also inversely associated with measures of adiposity including BMI ($r=-0.44$; $P<0.0001$), waist circumference ($r=-0.51$; $P<0.0001$), percent body fat ($r=-0.33$; $P=0.0007$), VAT ($r=-0.58$; $P<0.0001$) and SAT ($r=-0.39$; $P<0.0001$) (Table 3). Mean HDL particle size was not associated with serum total cholesterol concentrations ($P=0.11$) but it was associated with serum HDL concentrations ($r=0.81$; $P<0.0001$) and was inversely associated with serum LDL concentrations ($r=-0.41$; $P<0.0001$) and triglyceride concentrations ($r=-0.49$; $P<0.0001$) (Table 3). HDL particle size was inversely associated with HbA1c ($r=-0.31$; $P=0.002$), fasting glucose ($r=-0.24$; $P=0.02$), 2-hour glucose ($r=-0.38$; $P<0.0001$), fasting insulin ($r=-0.38$; $P<0.0001$) and HOMA ($r=-0.35$; $P=0.0003$) (Table 3).

Relationship of peak stimulated GH to lipoprotein particle size

Among all subjects, fasting GH ($r=0.27$; $P=0.006$), peak stimulated GH ($r=0.50$; $P<0.0001$) (Figure 1A) and IGF-1 ($r=0.25$; $P=0.02$) were all associated with mean LDL particle size (Table 2). Similarly, fasting GH ($r=0.23$; $P=0.02$), peak stimulated GH ($r=0.57$; $P<0.0001$) (Figure 1B) and IGF-1 ($r=0.24$; $P=0.02$) were all associated with mean HDL particle size (Table 3). While mean VLDL particle size trended to an association with peak stimulated GH ($r=-0.19$; $P=0.06$) (Figure 1C), it was not significantly associated with fasting GH ($P=0.15$) or IGF-1 levels ($P=0.10$).

In regards to lipoprotein sub-species, peak stimulated GH was inversely associated with mean concentrations of small ($r=-0.48$; $P<0.0001$), medium small ($r=-0.50$; $P<0.0001$) and very small LDL ($r=-0.47$; $P<0.0001$) particles while it was positively associated with large LDL particle concentrations ($r=0.27$; $P=0.007$). Similarly, peak stimulated GH was inversely associated with small HDL ($r=-0.31$; $P=0.002$) and positively associated with large HDL ($r=0.49$; $P<0.0001$) particle concentrations. Peak GH tended to an inverse association with medium HDL particle concentration ($r=-0.18$; $P=0.07$). Peak stimulated GH was not associated with oxidized LDL ($P=0.86$) (Table 4).

In multivariate regression modeling controlling for the effects of age, gender, race, ethnicity, tobacco use, lipid lowering medication use, BMI, and HOMA, peak stimulated GH remained significantly associated with LDL particle size ($\beta=0.01$; $P=0.01$; $R^2=0.42$; $P<0.0001$ for overall model) and HDL particle size ($\beta=0.008$; $P=0.001$; $R^2=0.44$; $P<0.0001$ for overall model) in separate models. Peak stimulated GH remained significantly associated with LDL or HDL particle size when the model was further controlled for LDL or HDL concentrations respectively in separate models (Model for LDL: Peak GH: $\beta=0.01$; $P=0.02$;

$R^2=0.43$; $P<0.0001$ for overall model; Model for HDL: Peak GH: $\beta=0.006$; $P=0.0009$; $R^2=0.73$; $P<0.0001$ for overall model) (Table 5A and B). The addition of waist-circumference to the overall model also did not change the significant relationship between GH and LDL or HDL particle size (Model for LDL: Peak GH: $\beta=0.01$; $P=0.01$; $R^2=0.43$; $P<0.0001$ for overall model; Model for HDL: Peak GH: $\beta=0.006$; $P=0.003$; $R^2=0.73$; $P<0.0001$ for overall model). Furthermore, the use of direct measurements of adiposity such as body fat percentage by DXA or measures of central adiposity such as VAT by CT scan did not change the significant relationship between peak stimulated GH and mean LDL or HDL particle size. The use of other measures of insulin sensitivity such as HbA1c, fasting glucose, 2-hour glucose, or fasting insulin also did not change the significant relationship between peak GH and mean LDL or HDL particle size.

However, when multivariate modeling was performed for VLDL particle size, HOMA, and not peak stimulated GH, was the significant variable associated with VLDL particle size (HOMA: $\beta=1.2$; $P=0.01$; Peak GH: $\beta=-0.01$; $P=0.82$; overall model: $R^2=0.18$; $P=0.03$). The use of percent body fat by DEXA or VAT by CT scan did not affect the significant relationship between HOMA and VLDL particle size.

Sensitivity analyses among normal weight subjects

We performed additional analyses limited to normal weight subjects. Normal weight subjects had a mean peak stimulated GH of $44.7 \pm 4.9 \mu\text{g/l}$ with a median peak stimulated GH of $43.8 \mu\text{g/l}$ (25-75% range: 18.9 to $66.7 \mu\text{g/l}$). There were no differences in mean LDL, HDL or VLDL particle size comparing normal weight subjects with peak stimulated GH above or below the median value of $43.8 \mu\text{g/l}$. Among normal weight subjects only ($n=33$), peak stimulated GH was significantly related to mean HDL particle size ($r=0.34$; $P=0.05$) but was not associated with LDL particle size ($r=0.27$; $P=0.13$) or VLDL particle size ($r=-0.03$; $P=0.86$).

Discussion

In this study, we provide the first comprehensive report of lipoprotein profile, including particle size and sub-species concentrations, among obese patients in whom GH secretory capacity was characterized. Our data demonstrate a significant association between peak stimulated GH on standard stimulation testing and LDL and HDL particle size. The results suggest that reduced peak stimulated GH is associated with a more atherogenic lipoprotein profile with decreased LDL and HDL particle size, which may contribute to the increased CVD risk associated with reduced GH secretion in obesity. In our data, peak stimulated GH was not associated with oxidized LDL.

For the purposes of this study, we used the median peak stimulated GH value among obese subjects to define relative GH sufficiency or deficiency of obesity. Using this cut-off value of $6.25 \mu\text{g/l}$, we obtained equal numbers of obese subjects with relative GH sufficiency or deficiency and demonstrated a strong relationship of GH status on LDL and HDL particle size. More importantly, we demonstrate a continuum of abnormality in lipoprotein particle size and subspecies concentrations associated with reduced GH secretory capacity as demonstrated in the univariate analyses and confirmed in the multivariate analyses.

Previous studies have demonstrated an association between small dense LDL cholesterol particles and increased CVD³ and small HDL particles have also been associated with the presence of coronary artery disease¹⁴. Our results demonstrate a significant association between reduced GH secretion in obesity and reduced LDL and HDL particle size with a shift to a greater concentration of smaller LDL and HDL particles. The shift in the specific

lipoprotein sub-species and size may contribute to the recently demonstrated association between reduced GH secretion and increased CVD risk in obesity ^{1, 2}.

As age, gender, race, ethnicity and insulin resistance have been shown to play a role in lipoprotein distribution ⁸⁻¹³, we controlled for these covariates, as well as the use of lipid lowering medications and their respective serum cholesterol levels, in our multivariate regression modeling. Our results suggest the association of peak stimulated GH with LDL and HDL particle size may be independent from the effects of these covariates.

The current study was not designed to specifically address the mechanism through which reduced GH secretion in obesity may be associated with circulating lipoprotein concentrations or particle size. Previous studies have demonstrated GH reduces total cholesterol in hypercholesterolemic and normocholesterolemic men ¹⁵ either through induction of hepatic LDL receptor to potentiate clearing of LDL ¹⁶ or through activation of cholesterol 7 α -hydroxylase to metabolize cholesterol to bile acids ¹⁷ or even through decreased de novo lipogenesis as seen in subjects with HIV infection ¹⁸. In regards to particle size, LDL particle size is inversely related to hepatic lipase activity ¹⁹. As hepatic lipase activity is low in conditions of GH excess such as acromegaly ^{20, 21}, reduced GH secretion associated with obesity may be associated with elevated hepatic lipase activity contributing to reduced LDL particle size. The size of the lipoprotein particles, HDL and VLDL/chylomicrons in particular, may also be affected by the activity of cholesterol ester transfer protein (CETP) which is expressed in the GH target organs of adipose tissue and liver ²². While GH excess, as seen in acromegaly, was associated with higher plasma CETP activity in one study ²⁰, another study demonstrated reduced CETP activity in acromegaly and an inverse association between plasma IGF-1 levels and CETP activity ²³. GH treatment has also been shown to decrease CETP ^{24, 25}, further supporting this negative association. Increased CETP activity in the setting of reduced GH secretion in obesity may result in increased transfer of cholesterol esters from HDL to VLDL/chylomicrons and IDL resulting in smaller, more dense HDL particles and to larger VLDL/chylomicrons and IDL particles, as seen in our study. The combination of increased CETP activity and hepatic lipase activity also may contribute to the formation of more dense, lipid poor, LDL particles.

Interestingly, while peak stimulated GH remained significantly associated with both LDL and HDL particle size independent of measures of insulin resistance, VLDL particle size was related to HOMA IR and not peak GH in multivariate modeling. This suggests that for VLDL particle size, unlike for LDL and HDL, insulin resistance rather than peak GH is a primary determinant of particle size. This is consistent with the known role of insulin in VLDL metabolism and the association between insulin resistance and hypertriglyceridemia ²⁶. Previous studies have demonstrated the increase in VLDL particle size associated with insulin resistance occurs primarily due to an increase in the concentration of circulating large VLDL particles without significant changes in the concentrations of small or medium VLDL particles ²⁷. This pattern of VLDL particle concentration is confirmed in our subset of obese subjects with reduced GH secretion and may reflect the increased insulin resistance seen in this sub-group of obese subjects.

One potential explanation for our data is that obesity results in reduced GH, and reduced GH further contributes to abnormal lipoprotein particle size in obesity. However, this study is limited by its observational nature and we can not conclude whether the changes in lipoprotein sub-species are a consequence of the reduced GH secretion. Therefore an alternative explanation for our data could involve other covariates such as age, BMI and insulin resistance which may affect lipoprotein particle size independent of GH. Although we controlled for these factors statistically using multivariate modeling, further interventional studies would be necessary to demonstrate whether normalization of the

reduced GH secretion would yield any beneficial effects on specific lipoprotein profiles in obesity. Nonetheless, this study is the first study to perform a detailed evaluation of the association between GH secretion and various lipoprotein particle size and sub-species using proton NMR spectroscopy and suggests a potential relationship between reduced GH and a more atherogenic lipoprotein particle size in obesity. In addition, we characterized GH secretory capacity using a standardized GHRH-arginine test. We did not characterize endogenous GH secretion including various pulsatility parameters which would have been difficult in a study of this size but may relate differentially to lipoprotein particle size. Lastly, the obese GHD subjects, compared to the obese GHS subjects, were older, with more central obesity and tended to be male. This is consistent with the known effects of these covariates, particularly, central obesity, on GH secretion, and may account for some of the more unfavorable lipoprotein characteristics. However, we control for these covariates and report both an un-adjusted and an adjusted P value in the stratified analyses. Furthermore, the relationship between GH and lipoprotein particle size and sub-species concentrations was also demonstrated on univariate analyses including all subjects and in more complex multivariate modeling. Further studies are needed into the mechanism by which reduced GH secretion may contribute to abnormal lipoprotein particle size in obesity.

In summary, we demonstrate a more adverse LDL and HDL subpopulation distribution in obese subjects with reduced GH secretion. These changes were independent of obesity and insulin resistance and may contribute to the increased CVD risk previously demonstrated in obese subjects with reduced GH secretion. Further interventional studies are now needed to demonstrate whether improvement of reduced GH secretion by exogenous GH or GH releasing factors would provide beneficial effects on lipoprotein profile and cardio-metabolic risk associated with obesity.

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Figure 1.

Univariate regression analyses demonstrating association between peak stimulated GH and (A) LDL particle size, (B) HDL particle size, and a trend towards an association with (C) VLDL particle size among all subjects.

Table 1

Characteristics of study subjects stratified to normal weight and obese subjects with GH sufficiency and relative GH deficiency (n=102). GH sufficiency or relative GH deficiency was determined by the median peak stimulated GH among obese subjects of 6.25 μ g/l. Results are presented as mean $\pm$ SEM.

	Normal Weight (n=33)	Obese GHS (n=34)	Obese GHD (n=35)	P	Adjusted P*
Age	40.4 $\pm$ 1.9	39.5 $\pm$ 1.7	44.5 $\pm$ 1.2	0.07	N/A
Gender (Male [%])	21 (64%)	14 (41%)	25 (71%)	0.03	N/A
Race (Caucasian [%])	21 (64%)	13 (38%)	22 (65%)	0.06	N/A
Ethnicity (Hispanic [%])	1 (3%)	4 (12%)	2 (6%)	0.35	1.0
Tobacco use (pack years)	5.2 $\pm$ 1.7	8.2 $\pm$ 2.2	6.7 $\pm$ 2.3	0.61	0.14
HMG CoA Reductase Inhibitor use (%)	0 (0%)	3 (9%)	2 (6%)	0.12	1.0
Fish Oil use (%)	2 (6%)	7 (21%)	4 (11%)	0.19	0.99
Niacin use (%)	0 (0%)	0 (0%)	0 (0%)	N/A	N/A
EtOH use (g/day)	8.2 $\pm$ 2.3	4.0 $\pm$ 2.3	6.2 $\pm$ 4.3	0.62	0.15
BMI (kg/m ²)	22.5 $\pm$ 0.3	36.4 $\pm$ 1.0 [†]	39.8 $\pm$ 1.2 ^{†‡}	<0.0001	N/A
Waist circumference (cm)	79.9 $\pm$ 1.3	114.4 $\pm$ 2.5 [†]	122.9 $\pm$ 2.0 ^{†‡}	<0.0001	<0.0001
Body fat by DXA (%)	19.1 $\pm$ 1.3	35.3 $\pm$ 1.5 [†]	35.3 $\pm$ 1.4 [†]	<0.0001	<0.0001
VAT (cm ²)	51 $\pm$ 7	141 $\pm$ 10 [†]	227 $\pm$ 12 ^{†‡}	<0.0001	<0.0001
SAT (cm ²)	129 $\pm$ 11	450 $\pm$ 22 [†]	503 $\pm$ 28 [†]	<0.0001	<0.0001
HOMA	0.67 $\pm$ 0.1	1.60 $\pm$ 0.17	3.24 $\pm$ 0.52 ^{†‡}	<0.0001	<0.0001
VLDL & Chylomicron: (nmol/l)					
Total VLDL & Chylomicron	48.0 $\pm$ 4.2	52.2 $\pm$ 4.0	67.9 $\pm$ 4.8 ^{†‡}	0.004	0.01
Large VLDL & Chylomicron	1.2 $\pm$ 0.2	2.7 $\pm$ 0.8	5.3 $\pm$ 0.9 ^{†‡}	0.0004	0.008
Medium VLDL	15.5 $\pm$ 1.8	16.9 $\pm$ 2.0	25.5 $\pm$ 2.8 ^{†‡}	0.004	0.008
Small VLDL	31.3 $\pm$ 2.8	32.6 $\pm$ 2.7	37.1 $\pm$ 2.5	0.27	0.36
IDL Particles:(nmol/l)	29.8 $\pm$ 7.1	52.0 $\pm$ 8.7	60.1 $\pm$ 8.6 [†]	0.03	0.04
LDL Particles: (nmol/l)					
Total LDL	848.4 $\pm$ 48.5	1125.1 $\pm$ 67.1 [†]	1252.6 $\pm$ 69.8 [†]	<0.0001	0.0005
Large LDL	441.9 $\pm$ 24.2	352.9 $\pm$ 33.6	298.7 $\pm$ 25.1 [†]	0.002	0.02

	Normal Weight (n=33)	Obese GHS (n=34)	Obese GHD (n=35)	P	Adjusted P [*]
Small LDL	376.9 ± 44.4	720.3 ± 77.5 [‡]	893.9 ± 66.4 [‡]	<0.0001	<0.0001
Medium Small LDL	79.1 ± 9.0	153.8 ± 17.3 [‡]	181.9 ± 11.9 [‡]	<0.0001	<0.0001
Very Small LDL	297.7 ± 35.6	566.5 ± 60.4 [‡]	712 ± 55.3 [‡]	<0.0001	<0.0001
HDL Particles: (nmol/l)					
Total HDL	30.9 ± 0.9	29.8 ± 0.8	30.2 ± 0.9	0.67	0.06
Large HDL	9.2 ± 0.6	6.4 ± 0.6 [‡]	4.7 ± 0.4 [‡]	<0.0001	<0.0001
Medium HDL	3.5 ± 0.5	2.9 ± 0.5	4.1 ± 0.5	0.28	0.29
Small HDL	18.2 ± 0.9	20.5 ± 1.0	21.4 ± 0.8 [‡]	0.04	0.01
Mean Particle Size: (nm)					
Mean VLDL Particle Size	49.0 ± 1.5	49.4 ± 1.4	55.4 ± 1.9 [‡]	0.007	0.08
Mean LDL Particle Size	21.6 ± 0.1	21.0 ± 0.1 [‡]	20.6 ± 0.1 [‡]	<0.0001	<0.0001
Mean HDL Particle Size	9.3 ± 0.08	8.9 ± 0.1 [‡]	8.6 ± 0.05 ^{‡‡}	<0.0001	<0.0001
Oxidized LDL (mU/l)	2394 ± 607	2196 ± 451	3783 ± 1966	0.61	0.93
Fasting GH (µg/l)	0.8 ± 0.2	0.1 ± 0.04 [‡]	0.1 ± 0.04 [‡]	0.0002	<0.0001
Peak Stimulated GH (µg/l)	44.7 ± 4.9	13.4 ± 1.4 [‡]	4.2 ± 0.2 [‡]	<0.0001	<0.0001
IGF-1 (µg/l)	93.1 ± 7.6	85.6 ± 6.1	60.1 ± 4.0 [‡]	0.001	<0.0001

* P value for overall ANOVA adjusted for age, gender, race and BMI

† indicates significantly different from normal weight subjects.

‡ indicates significantly different from obese subjects with GH sufficiency.

Table 2

Univariate analyses of LDL particle size with various metabolic parameters among all subjects.

	r	P
Age:	−0.10	0.31
BMI:	−0.48	<0.0001
Waist Circumference:	−0.47	<0.0001
% Body fat by DXA:	−0.36	0.0002
VAT	−0.51	<0.0001
SAT	−0.36	0.0004
Total Cholesterol:	−0.12	0.23
Triglycerides:	−0.60	<0.0001
HDL Cholesterol:	0.68	<0.0001
LDL Cholesterol:	−0.26	0.007
HbA1c	−0.31	0.001
Fasting glucose:	−0.27	0.006
2-hour glucose:	−0.43	<0.0001
Fasting insulin:	−0.37	0.0001
HOMA:	−0.37	0.0002
Fasting GH:	0.27	0.006
Peak stimulated GH:	0.50	<0.0001
IGF-1:	0.25	0.02

Table 3

Univariate analyses of HDL particle size with various metabolic parameters among all subjects.

	r	P
Age:	−0.15	0.12
BMI:	−0.44	<0.0001
Waist Circumference:	−0.51	<0.0001
% Body fat by DXA:	−0.33	0.0007
VAT	−0.58	<0.0001
SAT	−0.39	<0.0001
Total Cholesterol:	−0.16	0.11
Triglycerides:	−0.49	<0.0001
HDL Cholesterol:	0.81	<0.0001
LDL Cholesterol:	−0.41	<0.0001
HbA1c	−0.31	0.002
Fasting glucose:	−0.24	0.02
2-hour glucose:	−0.38	<0.0001
Fasting insulin:	−0.38	<0.0001
HOMA:	−0.35	0.0003
Fasting GH:	0.23	0.02
Peak stimulated GH:	0.57	<0.0001
IGF-1:	0.24	0.02

Table 4

Univariate analyses of lipoprotein particle size with peak stimulated GH on standard stimulation test among all subjects.

	r	P
VLDL & Chylomicron:		
Total VLDL & Chylomicron	−0.27	0.005
Large VLDL & Chylomicron	−0.30	0.003
Medium VLDL	−0.20	0.05
Small VLDL	−0.21	0.04
IDL Particles:	−0.27	0.007
LDL Particles:		
Total LDL	−0.44	<0.0001
Large LDL	0.27	0.007
Small LDL	−0.48	<0.0001
Medium Small LDL	−0.50	<0.0001
Very Small LDL	−0.47	<0.0001
HDL Particles:		
Total HDL	−0.08	0.41
Large HDL	0.49	<0.0001
Medium HDL	−0.18	0.07
Small HDL	−0.31	0.002
Mean Particle Size:		
Mean VLDL Particle Size	−0.19	0.06
Mean LDL Particle Size	0.50	<0.0001
Mean HDL Particle Size	0.57	<0.0001
Oxidized LDL	0.02	0.86

Table 5

Multivariate regression analyses among all subjects for (A) LDL particle size and (B) HDL particle size with peak stimulated GH, age, gender, race, ethnicity, tobacco use, use of lipid lowering medication, BMI, HOMA, and cholesterol.

A. Multivariate model for LDL particle size. Overall R² for the model = 0.43, P<0.0001.			
	Beta Estimate	SEM	P
Age	0.01	0.008	0.21
Gender (male or not)	0.05	0.08	0.54
Race (Caucasian or not)	−0.16	0.07	0.03
Ethnicity (Hispanic or not)	−0.36	0.14	0.01
Tobacco (pack years)	−0.004	0.006	0.51
Lipid lowering med	−0.67	0.29	0.02
LDL cholesterol	−0.003	0.002	0.19
BMI	−0.02	0.01	0.07
HOMA	−0.06	0.04	0.09
Peak stimulated GH	0.01	0.004	0.02

B. Multivariate model for HDL particle size. Overall R² for the model = 0.73, P<0.0001.			
	Beta Estimate	SEM	P
Age	0.002	0.003	0.55
Gender (male or not)	−0.01	0.03	0.76
Race (Caucasian or not)	−0.01	0.03	0.70
Ethnicity (Hispanic or not)	−0.03	0.06	0.55
Tobacco (pack years)	−0.002	0.002	0.31
Lipid lowering med	−0.15	0.12	0.22
HDL cholesterol	0.02	0.002	<0.0001
BMI	0.0007	0.005	0.88
HOMA	−0.004	0.01	0.79
Peak stimulated GH	0.006	0.002	0.0009