Body Composition and Adipose Tissue in HIV Lipodystrophy: Effects of Tesamorelin Therapy

NCT03226821

IRB approval date: Nov 10, 2021

BACKGROUND

Study Purpose and Rationale

HIV lipodystrophy is increasingly recognized as a common and clinically significant long-term sequelae of HIV treatment. In the HIV lipodystrophy lipohypertrophy phenotype, which we will study, VAT is increased and this is associated with reduced GH secretion (1, 2). Mounting evidence also links this phenotype with dyslipidemia, insulin resistance, subclinical atherosclerosis and CV disease in patients with HIV disease (3-8). The etiology of HILVD with central adiposity is unclear, but this phenotype is increasingly common with newer, less lipotoxic combination anti-retroviral therapy (cART) use (9). VAT and hepatic lipid accumulation, are important health concerns for HIVLD patients (10-12).

GH modulates fat metabolism and is known to be lipolytic (13-15), especially in VAT (16-21). Other evidence also suggests that GH reduces hepatic fat content, which seems to track with VAT mass in other clinical settings. Rodent and GH deficiency (GHD) data suggest that reduced GH action in liver increases hepatic lipid. Mice with impaired GH signaling or receptor deletion have increased hepatic lipid and steatosis (22, 23) and in case reports hepatic lipid was increased in GHD patients (24-26). This is a clinically important, but under-recognized feature of GHD, which may progress to NASH. A few small studies found that GH therapy reduced VAT (27-29), but liver fat changes were inconclusive (29, 30). GH therapy effects on liver fat require further study.

This project will test our primary hypothesis that GH is a negative regulator of liver fat in HIVLD patients by testing for a reduction of liver fat with GHRH analogue treatment. A GHRH analogue (tesamorelin) stimulates GH secretion and reduces VAT in HIVLD patients (31, 32). Tesamorelin therapy stimulates pituitary GH secretion and restores the deficient GH secretion seen in HIVLD. This is demonstrated by a rise of IGF-1 levels, a marker of overall GH secretion, in patients with HIVLD. Tesamorelin therapy of HIVLD with central adiposity has been shown to reduce VAT (31, 32). However, studies to date used only single slice abdominal CT and DXA to assess body composition (32-34). Recently, in a 1H MRS study tesamorelin reduced liver fat(32). Our study adds to this work with its total body MRI assessments combined with direct examination of biopsied adipose tissue before and after tesamorelin. Whole-body scanning is advantageous and

more accurate than single-slice imaging (35-37). Our study will provide novel data on tesamorelin's effects on HIVLD and liver fat accumulation, both increasingly recognized as important long-term complications of HIV infection (10-12). Our findings will help elucidate GH's role in liver fat accumulation, a precursor to hepatic steatosis and NASH (38), a significant health problem in the general population.

The actions of GH in adipose tissue are central to its effects to reduce adipose tissue mass. GH may have effects both on adipocytes as well as inflammatory cells in adipose tissue. Thus GH effects in adipose tissue may importantly relate to adipose tissue and thus circulating inflammation in disorders of GH secretion. In our ongoing study examining the effects of GH excess on adipose tissue changes in acromegaly, our preliminary identified that GH produces a unique adipocyte-immune cell phenotype that contributes to circulating inflammation and insulin resistance and may alter adipose tissue energy metabolism. Our hypothesis stems from our identification in preliminary work of a novel and unexpected GH-induced dissociation of inflammatory & immune phenotypes. Specifically, we found a pro-inflammatory pattern of adipocyte activation yet suppression of macrophage inflammation that reverses with acromegaly treatment.GH-induced increased lipolysis may initiate these effects. GH increases HSL, reduces LPL activity and activates the –adrenergic 3 receptor (18, 39-44). GH also inhibits lipogenesis and reduces AT mass (41, 45). In other models, disordered lipid metabolism promotes lipolysis and FFA release leading to pro-inflammatory cytokine release. This inflammatory response then promotes insulin resistance in adipose tissue (46-55). Thus, cytokines released by inflamed adipose tissue may play a role in GH induced insulin resistance. The effect of GH excess on AT inflammation had not been examined before our study. By contrast, in other models, activation of inflammatory (adipocyte cytokine) and immune (macrophage) components of AT parallel each other. Our findings are supported by recent in vitro data that GH induces pro-inflammatory cytokines in pre-adipocytes yet suppresses inflammatory cytokines in macrophages (56). This study examined isolated cells (56, 57), ours provides evidence of this effect in vivo. In this study, we will test the effect of restoration of GH secretion through administration of GHRH analogue therapy on AT inflammation in HIVLD groups. In patients with GHD with central obesity, as shown in a cross-sectional study, SAT phenotype may be similar to that of general obesity, namely increased adipocyte size and expression of pro-inflammatory factors and macrophage infiltration (58).

Effects of GH therapy are unknown, but it may reduce both adipocyte and macrophage inflammation.

This study will determine the effects of GHRH analogue therapy on adipose

tissue inflammation in HIVLD. In patients with GHD and central obesity, as shown in a cross-sectional study, SAT phenotype may be similar to that of general obesity, namely increased adipocyte size and expression of pro-inflammatory factors and macrophage infiltration (58). Effects of GH therapy are unknown, but it may reduce both adipocyte and macrophage inflammation. In the few studies that directly examined SAT in patients with the HIVLD and the central lipohypertrophy phenotype, induction of pro-inflammatory cytokines such as TNF, macrophage infiltration and impaired mitochondrial function were present in both abdominal SAT and VAT (59-61). However, VAT was hypertrophied, but central SAT may not be, possibly reflecting greater sensitivity of SAT to toxic inflammation(59, 62, 63). Our study is unique since the direct effects of tesamorelin on adipose tissue have not been studied. We will focus on how tesamorelin modulates the adipose tissue inflammatory/immune phenotype. Animal models suggest that GH sufficiency is needed to maintain an anti-inflammatory phenotype in adipose tissue (57, 64). Thus, restoration of GH action in adipose tissue in HIVLD may be important to reducing inflammation in adipose tissue and thus systemically and reducing these patients' long-term CV risk.

We will also test for new mechanisms by which GH supplementation with tesamorelin may alter lipid and energy metabolism in adipose tissue in HIVLD. Genes implicated to mediate these processes have never before been studied in paired adipose tissue samples from before and after GHRH analog treatment in HIVLD patients. Utilizing our unique tissue set, we will examine expression of genes for enzymes believed to mediate GH-induced reduction in adipose tissue lipid stores and others recently found altered by GH therapy including CIDEA (lipolysis and energy balance regulator) and PLPLA3 (lipid hydrolase) (41). We will examine expression of genes for fatty acid desaturases. These are linked to diabetes (65) and increased in GH transgenic mice (66) and acromegaly in a small cross sectional study (67). We will study the gene for PIK3R1 (p85), an enzyme important to insulin's metabolic actions and that was induced by GH in mouse adipose tissue (68), and the gene for TCF7L2, a transcription factor insulin resistance and upregulated in SAT in patients with insulin resistance(69) and acromegaly(67). We will explore the possible effect of HIVLD and its treatment with GHRH analog therapy on the transformation of white adipose

tissue (WAT) to the more energetically favorable brown adipose tissue (BAT). GH deficiency may have a negative impact on the WAT to BAT transition. Emerging evidence links immune cell phenotype, in particular that of macrophages with M2 characteristics, to regulation of WAT "browning" (70-72). With GHRH analog therapy, alternative M2 macrophage recruitment into adipose tissue may be associated with a relative "browning" of WAT. To test this hypothesis we will examine relative expression of genes associated with this process before and after GHRH treatment. These studies will provide valuable new insights into the mechanisms by which GH deficiency its therapy with tesamorelin disrupt adipose tissue health in HIVLD.

GH effects on circulating inflammatory cells in GH disorders may relate to their associated CV risk. Thus, we test our Hypothesis that the inflammatory profile of circulating monocytes mirrors that of adipose tissue macrophages (ATMs) before and after tesamorelin therapy. A significant portion of ATMs are derived from circulating monocytes(73). Thus, we hypothesize that GH's anti-inflammatory effect on ATMs will be detectable in circulating monocytes. We will examine circulating monocytes for our adipocyte-immune cell pattern before and after tesamorelin treatment. GHD and HIVLD increase CV risk. In a prior study, GH therapy in patients with GH deficiency due to pituitary disease had reduced peripheral monocyte TNF and IL-6 expression (74), supporting our hypothesis that GH suppresses monocyte and macrophage inflammation. Since monocytes and macrophages play key roles in atherosclerosis development (75, 76), an activated, pro-inflammatory monocyte profile in HIVLD with central adiposity could lead to circulating inflammation and increase CV risk in these patients. GH supplementation with GHRH therapy could help reverse this pattern. These data may help elucidate the nature of the relationship between circulating immune cells and atherosclerosis in disorders of GH secretion or action.

Thus, the purpose of this project is to examine the effect of GHRH analog therapy (tesamorelin) on liver fat and central adiposity in patients with HIV lipodystrophy. This project also examines adipose tissue macrophage infiltration, pro-inflammatory cytokine, adipokine and macrophage activation marker gene expression as well as circulating adipokine and inflammatory cytokine levels before and after GHRH analog therapy.

With the collaboration and expertise of Dr. Ferrante, this project will be the first to directly examine the effect of HIV lipodystrophy with central adiposity and its treatment with the GHRH analog tesamorelin on adipose tissue macrophage infiltration and inflammatory cytokine and

adipokine expression, which are now recognized to have a central role in insulin resistance, diabetes, vascular disease and fatty liver, all relevant to morbidities of HIV lipodystrophy.

Research Aims & Abstracts

The primary hypothesis to be tested is that therapy with the GHRH analog tesamorelin, which augements GH secretion), will reduce hepatic lipid and visceral adipose tissue mass in patients with HIV lipodystrophy and central adiposity. The second hypothesis to be tested is the tesamorelin therapy will reduce adipose tissue inflammation and circulating pro-inflammatory markers on macrophages in these patients.

STUDY DESIGN

The CORE study is an open-label, single arm prospective study of tesamorelin therapy of patients with HIV lipodystrophy.

Enrolled subjects will have a screening visit and 6 visits - a baseline visit before starting tesamorelin, a visit at 1 month, 3 months, 6 months, 9 months and at 1 year of tesamorelin (GHRH analogue) therapy. Blood sampling for safety labs and clinical examinations will be performed at each visit. At the baseline, 6 month and 1 year visits subjects will also undergo body composition testing by total body MRI/MRS, adipose tissue biopsy, indirect calorimetry, FSIGT and additional blood sampling. Tesamorelin is being used in this study as per its FDA approved indication and dosing for treatment of HIV lipodystrophy with central adiposity. Tesamorelin, 2 mg, will be taken daily by patients by subcutaneous self-injection

Subject Inclusion Criteria:

- HIV-infected subjects with HIV lipodystrophy (HIVLD) and abdominal fat accumulation.
- Males and females
- Ages 18-65 years
- Waist Circumference (WC): 102 cm for men, 88 cm for women (ethnicities except East/South Asians) 90 cm for men, 80 cm for women (East/South Asians)
- CD4 count >100 cells/mm3.
- HIV RNA load < 400 copies/mL

- Fasting glucose <150 mg/dL
    - Patients with mild diabetes (HbA1c<7%) that is well controlled with diet and/or oral diabetic agents besides TZDs are eligible.
- Stable antiretroviral therapy for 8 weeks (any regimen).

Exclusion criteria:

- Diabetes mellitus that is not well controlled
- History of malignancy

Study procedures

Patients who are interested in the study will first undergo screening. Screening which consist of a telephone interview to further explain the study and confirm the subjects' interest and eligibility and a review of medical records for the above entry inclusion and exclusion criteria. If needed to obtain information on entry criteria not available from the medical record, subjects will have a brief screening visit in person to obtain additional information. If subjects meet all entry criteria and agree they will be enrolled.

Enrolled subjects will have 6 visits - a baseline visit before starting tesamorelin, a visit at 1 month, 3 months, 6 months, 9 months and at 1 year of tesamorelin (GHRH analogue) therapy Blood sampling for safety labs and clinical examinations will be performed at each visit. At the baseline, 6 month and 1 year visits subjects will also undergo body composition testing by total body MRI/MRS, adipose tissue biopsy, indirect calorimetry, FSIGT and additional blood sampling.

Study Drug Tesamorelin is being used in this study as per its FDA approved indication and dosing for treatment of HIV lipodystrophy with central adiposity. Tesamorelin, 2 mg, will be taken daily by patients by subcutaneous self-injection at rotating sites on the abdomen. Safety assessments including measurements of fasting blood glucose and liver function tests and clinical examinations for signs and symptoms of GH excess or local injection site reactions will be performed at 1 month and every 3 months of therapy.

At the baseline, 6 month and 1 year visits subjects will undergo body composition testing, adipose tissue biopsy, indirect calorimetry for measurement of REE (resting energy expenditure), FSIGT and fasting blood sampling.

Body composition testing: Anthropometrics and total body MRI and 1H-MRS after a 12 hour fast.

Anthropometric measurements: Subjects will have measurements of waist circumference with a tape measure and height and body weight will be measured.

Total body MRI: Quantification of total and regional body composition by whole body multi-slice MRI, liver fat by Modified DIXON method (35, 79), and 1H-MRS (magnetic resonance spectroscopy) assessment of intra-hepatic lipid (IHL) and IMCL of the anterior tibialis muscle will be performed at the Columbia Hatch MR Research Center as previously described (80, 81). Analyses will be done in the NY Obesity Research and Nutrition Center Image Analysis Lab on the CUMC campus and under the direction of Dr. Shen(80, 81). The analysis will include measurement of inter-muscular AT (IMAT)(AT between muscle groups and beneath the fascia (82, 83), which is distinct from intra-myocellular lipid (IMCL)(lipid in myocytes). MRIs will be analyzed by the same experienced reader blinded to study conditions using SliceOmatic software (TomoVision) (82, 84). Liver fat will also be determined by Modified DIXON method performed during the total body MRI sequences. It has advantages of not requiring ROI selection during acquisition and measures fat for the entire liver. DIXON is validated against MRS (35, 79).

Adipose tissue biopsy: After a 12-hour fast, subjects will undergo abdominal subcutaneous AT biopsy by the PI at the umbilicus level. Alternating sides of the abdomen will be samples on serial biopsies. After local anesthesia, 2-4g of AT will be using aspirated using a 15g needle. Pieces will be immediately frozen in liquid nitrogen & stored at –80°C, others are fixed for immunohistochemistry.

Indirect Calorimetry (TrueOne2400, ParvoMedics) will measure resting energy expenditure (REE) after an overnight fast (12hr.) and 30 min. of rest with subjects still, awake and supine in a thermoneutral environment. REE will be determined from the last 20 minutes of 30 minutes of gas exchange results (85). The cart is calibrated prior to each test (86).

Insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) with reduced sample number (n=12)(87) will be performed to assess insulin sensitivity. In this protocol, a 300 mg/kg bolus of glucose will be administered after an overnight fast followed by an infusion of 0.03 units/kg of insulin 20 minutes later. In the reduced sample model, 12 blood samples (insulin and glucose) will be drawn at times 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 minutes (22). The data will be analyzed by Bergman Minimal Model to calculate our outcome measures, insulin

sensitivity index (Si) and disposition index (DI) to estimate B-cell function (87-92). FSIGT is used extensively to assess insulin sensitivity and provides estimates that correlate well with those of the clamp in non-diabetics (87, 91, 93).

Blood Sampling: Fasting serum or plasma samples will also be obtained for IGF-1, GH, leptin, FFA, glucose, TNF, IL-6, hsCrp, adiponectin (high molecular weight & total), leptin and lipid panel.

Peripheral blood mononuclear cell collection: A sample (2 tbsp.) of heparinized blood will be obtained. Peripheral blood mononuclear cells will be isolated by density centrifugation using Ficoll (Life Technologies). Recovery of highly purified monocytes will be performed by flow cytometry with florophore conjugated anti-CD14 primary antibody. Cells will be analyzed on a FACSCalibur and analysis performed using CellQuest software (Becton Dickinson). Monocytes will be separated using a FACSAria cell sorter (BD Biosciences). CD14+ cells will be centrifuged and immediately frozen for later gene expression analysis. PCR will be performed in batches for gene expression of TNF, IL6, MCP-1, IL1-B, IL-4. A complete blood count will be performed from this sample.

The total amount of blood drawn at baseline, 6 month and 1 year visits will be 4 tablespoons and at the other visits it will be ½ tablespoon.

Blood sampling for safety labs and physical examinations: At each visit, blood will be drawn for liver function tests (AST, ALT), and fasting blood sugar. Subjects will also have a physical examination including measurement of vital signs, temperature and examination for signs and symptoms of GH excess such as edema and local skin reactions.

Adipose Tissue Analyses: Frozen and fixed adipose tissue samples will be later analyzed in batches.

Immuno-histochemical analysis: Macrophage antigen, CD68, expressing cells fraction/sample will be calculated to determine macrophage content (94). Adipocyte size will be measured (Image-Pro Plus, Media Cybernetics, Bethesda, MD) (95) and size distribution will be quantified (96). Adipocyte count, counts of crown-like structure and other immune (T, NK) cells and fibrosis analysis will be conducted (97). Sample processing and analyses will be uniform and paired samples will be directly compared. Quantitative RT PCR will be performed on total RNA extracted from frozen AT as described (94). Relative expression values will be determined for: adipocyte-specific genes adiponectin (ADIPOQ) and leptin (LEP), proinflammatory genes (TNF,

IL6), genes of macrophage activation markers (CD11c, CD68, MCP-1, IL1- Genes associated with AT "browning" (UCP-1, DIO2, CIDEA, PGC-1, PRDM-16), and lipid metabolism, (CIDEA, PLPLA3, SCD, PIK3R1, TCF7L2). We will use control genes to normalize data (94). Samples are analyzed in labs of Dr. Ferrante and NYONRC AT core.

HORMONE ASSAYS: Hormones and other markers will be measured with the following assays: IGF-1 (WHO 02/254) & GH (WHO 98/574, 22kDa GH specific antibody), IDS-iSYS chemiluminescence assay; IGF-1 & GH (WHO 98/574, 22kDa & 20kDa antibody), Insulin, IGFBP-3, hsCRP - chemiluminescent immunometric (Immulite,Siemens); HbA1C, glucose, lipids (Cobas Integra, Roche); Adiponectin (total & HMW)- ELISA (Millipore); Leptin- RIA (Millipore); FFA - enzymatic colorimetric (WAKO); IL-6 & TNF -ultrasensitive ELISA R & D.

STATISTICAL PROCEDURES

The primary outcome to be examined will be change in hepatic lipid content and the secondary outcome will be change in VAT mass with tesamorelin therapy. Additional outcomes examined will be changes in relative gene expression of adipose tissue genes, change in Si, REE and changes in other hormones and markers with tesamorelin therapy.

The general analytic approach will be to compare baseline to post-therapy parameters in each subject by paired t-test. Repeated measures ANOVA will test adipose tissue mass change over time. Correlations between variables will be tested by Pearson's correlation. Non-normally distributed data will be log-transformed or nonparametrics used. Multiple comparisons corrections and post-hoc tests will be used. Macrophage content (% CD68 positive cell per sample) and average adipocyte cross-sectional area will be compared in HIV lipodystrophy patients adipose tissue before vs. after tesamorelin therapy by paired t-test. Adipocyte area and macrophage content will be correlated with BMI and fat mass. Correlations between gene expression data and body mass or AT mass will be compared by a nonparametric approach. Ratios of macrophage content or relative gene expression per kg AT will be compared in patients before & after treatment by appropriate t-tests. For analysis of the blood samples differences between baseline and final study visit for each marker will be compared by paired t-test. Patterns and time course of change in serum and adipose tissue inflammation will be descriptively compared. Matching serum/gene expression marker changes will be correlated.

Sample Size is based on the number of subjects needed to detect a significant decrease in hepatic lipid with tesamorelin therapy. Preliminary data from our acromegaly treatment study were used for this estimation. Sample size of 24 pairs has 88% power to detect a hepatic lipid change of 2% with a SD of 3% at alpha= .05 and 12 pairs of each gender has 80% power to detect this hepatic lipid change within gender group with a within-gender SD of 2% at alpha= .05 using a two-sided paired t-test.

REFERENCES

1. Rietschel P, Hadigan C, Corcoran C, Stanley T, Neubauer G, Gertner J and Grinspoon S (2001) Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. J Clin Endocrinol Metab 86: 504-10.
2. Koutkia P, Meininger G, Canavan B, Breu J and Grinspoon S (2004) Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy. Am J Physiol Endocrinol Metab 286: E296-303.
3. Stanley TL and Grinspoon SK (2012) Body composition and metabolic changes in HIV-infected patients. The Journal of infectious diseases 205 Suppl 3: S383-90. 3349298.
4. Wohl D, Scherzer R, Heymsfield S, Simberkoff M, Sidney S, Bacchetti P, Grunfeld C and Investigators FS (2008) The associations of regional adipose tissue with lipid and lipoprotein levels in HIV-infected men. J Acquir Immune Defic Syndr 48: 44-52. 3156607.
5. Guaraldi G, Stentarelli C, Zona S, Orlando G, Carli F, Ligabue G, Lattanzi A, Zaccherini G, Rossi R, Modena MG, Alexopoulos N, Palella F and Raggi P (2010) Lipodystrophy and anti-retroviral therapy as predictors of sub-clinical atherosclerosis in human immunodeficiency virus infected subjects. Atherosclerosis 208: 222-7.
6. Grunfeld C, Rimland D, Gibert CL, Powderly WG, Sidney S, Shlipak MG, Bacchetti P, Scherzer R, Haffner S and Heymsfield SB (2007) Association of upper trunk and visceral adipose tissue volume with insulin resistance in control and HIV-infected subjects in the FRAM study. J Acquir Immune Defic Syndr 46: 283-90. 3164883.
7. Hadigan C, Meigs JB, Corcoran C, Rietschel P, Piecuch S, Basgoz N, Davis B, Sax P, Stanley T, Wilson PW, D'Agostino RB and Grinspoon S (2001) Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 32: 130-9.
8. Grinspoon S and Carr A (2005) Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N Engl J Med 352: 48-62.
9. Domingo P, Estrada V, Lopez-Aldeguer J, Villaroya F and Martinez E (2012) Fat redistribution syndromes associated with HIV-1 infection and combination antiretroviral therapy. AIDS reviews 14: 112-23.
10. Sherman KE, Rockstroh J and Thomas D (2015) HIV and Liver Disease: An Update. Hepatology.

11. Scherzer R, Heymsfield SB, Lee D, Powderly WG, Tien PC, Bacchetti P, Shlipak MG, Grunfeld C, Study of Fat R and Metabolic Change in HIVI (2011) Decreased limb muscle and increased central adiposity are associated with 5-year all-cause mortality in HIV infection. Aids 25: 1405-
12. Orlando G, Guaraldi G, Zona S, Carli F, Bagni P, Menozzi M, Cocchi S, Scaglioni R, Ligabue G and Raggi P (2012) Ectopic fat is linked to prior cardiovascular events in men with HIV. J Acquir Immune Defic Syndr 59: 494-7.
13. Freda PU, Reyes CM, Nuruzzaman AT, Sundeen RE and Bruce JN (2003) Basal and glucose-suppressed GH levels less than 1 μg/L in newly diagnosed acromegaly. Pituitary 6: 175-80.
14. Ho KK, O'Sullivan AJ and Hoffman DM (1996) Metabolic actions of growth hormone in man. Endocr J 43 Suppl: S57-63.
15. Russell-Jones DL and Weissberger AJ (1996) The role of growth hormone in the regulation of body composition in the adult. Growth Regul 6: 247-52.
16. Engelson ES, Glesby MJ, Mendez D, Albu JB, Wang J, Heymsfield SB and Kotler DP (2002) Effect of recombinant human growth hormone in the treatment of visceral fat accumulation in HIV infection. J Acquir Immune Defic Syndr 30: 379-91.
17. Johannsson G, Marin P, Lonn L, Ottosson M, Stenlof K, Bjorntorp P, Sjostrom L and Bengtsson BA (1997) Growth hormone treatment of abdominally obese men reduces abdominal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure. J Clin Endocrinol Metab 82: 727-34.
18. Richelsen B (1997) Action of growth hormone in adipose tissue. Horm Res 48 Suppl 5: 105-10.
19. Lafontan M and Berlan M (2003) Do regional differences in adipocyte biology provide new pathophysiological insights? Trends Pharmacol Sci 24: 276-83.
20. Hoffstedt J, Arner P, Hellers G and Lonnqvist F (1997) Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. J Lipid Res 38: 795-804.
21. Richelsen B, Pedersen SB, Moller-Pedersen T and Bak JF (1991) Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E2. Metabolism 40: 990-6.
22. Fan Y, Menon RK, Cohen P, Hwang D, Clemens T, DiGirolamo DJ, Kopchick JJ, Le Roith D, Trucco M and Sperling MA (2009) Liver-specific deletion of the growth hormone receptor reveals essential role of growth hormone signaling in hepatic lipid metabolism. J Biol Chem 284: 19937-44. 2740419.
23. Barclay JL, Nelson CN, Ishikawa M, Murray LA, Kerr LM, McPhee TR, Powell EE and Waters MJ (2011) GH-dependent STAT5 signaling plays an important role in hepatic lipid metabolism. Endocrinology 152: 181-92.
24. Ichikawa T, Nakao K, Hamasaki K, Furukawa R, Tsuruta S, Ueda Y, Taura N, Shibata H, Fujimoto M, Toriyama K and Eguchi K (2007) Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. Hepatology international 1: 287-94. 2716823.
25. Takahashi Y, Iida K, Takahashi K, Yoshioka S, Fukuoka H, Takeno R, Imanaka M, Nishizawa H, Takahashi M, Seo Y, Hayashi Y, Kondo T, Okimura Y, Kaji H, Kitazawa R, Kitazawa S and Chihara K (2007) Growth

hormone reverses nonalcoholic steatohepatitis in a patient with adult growth hormone deficiency. Gastroenterology 132: 938-43.

26. Takano S, Kanzaki S, Sato M, Kubo T and Seino Y (1997) Effect of growth hormone on fatty liver in panhypopituitarism. Archives of disease in childhood 76: 537-8. 1717220.
27. Snel YE, Brummer RJ, Doerga ME, Zelissen PM, Bakker CJ, Hendriks MJ and Koppeschaar HP (1995) Adipose tissue assessed by magnetic resonance imaging in growth hormone-deficient adults: the effect of growth hormone replacement and a comparison with control subjects. Am J Clin Nutr 61: 1290-4.
28. Egger A, Buehler T, Boesch C, Diem P, Stettler C and Christ ER (2011) The effect of GH replacement therapy on different fat compartments: a whole-body magnetic resonance imaging study. Eur J Endocrinol 164: 23-9.
29. Sato T, Katabami T, Furukawa K, Narimatsu H, Hashimoto T, Nakajima Y, Ohta A, Sasaoka T and Tanaka Y (2012) Intracellular lipid content of liver and skeletal muscle in patients with adult growth hormone deficiency without diabetes mellitus. Obesity research & clinical practice 6: e263-346.
30. Gardner CJ, Irwin AJ, Daousi C, McFarlane IA, Joseph F, Bell JD, Thomas EL, Adams VL, Kemp GJ and Cuthbertson DJ (2012) Hepatic steatosis, GH deficiency and the effects of GH replacement: a Liverpool magnetic resonance spectroscopy study. Eur J Endocrinol 166: 993-1002.
31. Falutz J, Mamputu JC, Potvin D, Moyle G, Soulban G, Loughrey H, Marsolais C, Turner R and Grinspoon S (2010) Effects of tesamorelin (TH9507), a growth hormone-releasing factor analog, in human immunodeficiency virus-infected patients with excess abdominal fat: a pooled analysis of two multicenter, double-blind placebo-controlled phase 3 trials with safety extension data. J Clin Endocrinol Metab 95: 4291-304.
32. Stanley TL, Feldpausch MN, Oh J, Branch KL, Lee H, Torriani M and Grinspoon SK (2014) Effect of tesamorelin on visceral fat and liver fat in HIV-infected patients with abdominal fat accumulation: a randomized clinical trial. JAMA 312: 380-9. 4363137.
33. Koutkia P, Canavan B, Breu J, Torriani M, Kissko J and Grinspoon S (2004) Growth hormone-releasing hormone in HIV-infected men with lipodystrophy: a randomized controlled trial. JAMA 292: 210-8.
34. Falutz J, Potvin D, Mamputu JC, Assaad H, Zoltowska M, Michaud SE, Berger D, Somero M, Moyle G, Brown S, Martorell C, Turner R and Grinspoon S (2010) Effects of tesamorelin, a growth hormone-releasing factor, in HIV-infected patients with abdominal fat accumulation: a randomized placebo-controlled trial with a safety extension. J Acquir Immune Defic Syndr 53: 311-22.
35. Shen W, Gong X, Weiss J and Jin Y (2013) Comparison among T1-weighted magnetic resonance imaging, modified dixon method, and magnetic resonance spectroscopy in measuring bone marrow fat. Journal of obesity 2013: 298675. 3628209.
36. Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J, Heymsfield SB and Heshka S (2004) Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. Journal of applied physiology 97: 2333-8.
37. Tsai W, Shen W, St-Onge MP. (2012) Change in single-slice visceral adipose tissue area in relation to change in total volume in men and women during weight loss. Int J Body Comp Res 10(3): 87-92.

38. Satapathy SK and Sanyal AJ (2015) Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. Seminars in liver disease 35: 221-35.
39. Nielsen S, Moller N, Christiansen JS and Jorgensen JO (2001) Pharmacological antilipolysis restores insulin sensitivity during growth hormone exposure. Diabetes 50: 2301-8.
40. Richelsen B, Pedersen SB, Kristensen K, Borglum JD, Norrelund H, Christiansen JS and Jorgensen JO (2000) Regulation of lipoprotein lipase and hormone-sensitive lipase activity and gene expression in adipose and muscle tissue by growth hormone treatment during weight loss in obese patients. Metabolism 49: 906-11.
41. Zhao JT, Cowley MJ, Lee P, Birzniece V, Kaplan W and Ho KK (2011) Identification of novel GH-regulated pathway of lipid metabolism in adipose tissue: a gene expression study in hypopituitary men. J Clin Endocrinol Metab 96: E1188-96.
42. Johansen T, Richelsen B, Hansen HS, Din N and Malmlof K (2003) Growth hormone-mediated breakdown of body fat: effects of GH on lipases in adipose tissue and skeletal muscle of old rats fed different diets. Horm Metab Res 35: 243-50.
43. Simsolo RB, Ezzat S, Ong JM, Saghizadeh M and Kern PA (1995) Effects of acromegaly treatment and growth hormone on adipose tissue lipoprotein lipase. J Clin Endocrinol Metab 80: 3233-8.
44. Oscarsson J, Ottosson M and Eden S (1999) Effects of growth hormone on lipoprotein lipase and hepatic lipase. J Endocrinol Invest 22: 2-9.
45. Chaves VE, Junior FM and Bertolini GL (2013) The metabolic effects of growth hormone in adipose tissue. Endocrine 44: 293-302.
46. Ferrante AW, Jr. (2013) Macrophages, fat, and the emergence of immunometabolism. J Clin Invest 123: 4992-3. 3859415.
47. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ and Ferrante AW, Jr. (2013) Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. Cell Metab 18: 816-30. 3939841.
48. Olarescu NC, Ueland T, Godang K, Lindberg-Larsen R, Jorgensen JO and Bollerslev J (2014) Inflammatory adipokines contribute to insulin resistance in active acromegaly and respond differently to different treatment modalities. Eur J Endocrinol 170: 39-48.
49. Ferrante AW, Jr. (2013) The immune cells in adipose tissue. Diabetes Obes Metab 15 Suppl 3: 34-8. 3777665.
50. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R and Ferrante AW, Jr. (2010) Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 120: 3466-79.
51. Capel F, Klimcakova E, Viguerie N, Roussel B, Vitkova M, Kovacikova M, Polak J, Kovacova Z, Galitzky J, Maoret JJ, Hanacek J, Pers TH, Bouloumie A, Stich V and Langin D (2009) Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization. Diabetes 58: 1558-67.
52. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H and Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116: 3015-25. 1616196.

53. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y and Ogawa Y (2007) Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol 27: 84-91.
54. Osborn O and Olefsky JM (2012) The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 18: 363-74.
55. Cullberg KB, Larsen JO, Pedersen SB and Richelsen B (2014) Effects of LPS and dietary free fatty acids on MCP-1 in 3T3-L1 adipocytes and macrophages in vitro. Nutrition & diabetes 4: e113. 3974034.
56. Kumar PA, Chitra PS, Lu C, Sobhanaditya J and Menon R (2014) Growth hormone (GH) differentially regulates NF-kB activity in preadipocytes and macrophages: implications for GH's role in adipose tissue homeostasis in obesity. Journal of physiology and biochemistry 70: 433-40.
57. Benencia F, Harshman S, Duran-Ortiz S, Lubbers ER, List EO, Householder L, Al-Naeeli M, Liang X, Welch L, Kopchick JJ and Berryman DE (2015) Male bovine GH transgenic mice have decreased adiposity with an adipose depot-specific increase in immune cell populations. Endocrinology 156: 1794-803. 4398765.
58. Ukropec J, Penesova A, Skopkova M, Pura M, Vlcek M, Radikova Z, Imrich R, Ukropcova B, Tajtakova M, Koska J, Zorad S, Belan V, Vanuga P, Payer J, Eckel J, Klimes I and Gasperikova D (2008) Adipokine protein expression pattern in growth hormone deficiency predisposes to the increased fat cell size and the whole body metabolic derangements. J Clin Endocrinol Metab 93: 2255-62.
59. Gallego-Escuredo JM, Villarroya J, Domingo P, Targarona EM, Alegre M, Domingo JC, Villarroya F and Giralt M (2013) Differentially altered molecular signature of visceral adipose tissue in HIV-1-associated lipodystrophy. J Acquir Immune Defic Syndr 64: 142-8.
60. Sevastianova K, Sutinen J, Greco D, Sievers M, Salmenkivi K, Perttila J, Olkkonen VM, Wagsater D, Lidell ME, Enerback S, Eriksson P, Walker UA, Auvinen P, Ristola M and Yki-Jarvinen H (2011) Comparison of dorsocervical with abdominal subcutaneous adipose tissue in patients with and without antiretroviral therapy-associated lipodystrophy. Diabetes 60: 1894-900. 3121420.
61. Domingo P, Gutierrez Mdel M, Gallego-Escuredo JM, Torres F, Mateo MG, Villarroya J, Lamarca K, Domingo JC, Vidal F, Villarroya F and Giralt M (2014) A 48-week study of fat molecular alterations in HIV naive patients starting tenofovir/emtricitabine with lopinavir/ritonavir or efavirenz. J Acquir Immune Defic Syndr 66: 457-65.
62. Bastard JP, Caron M, Vidal H, Jan V, Auclair M, Vigouroux C, Luboinski J, Laville M, Maachi M, Girard PM, Rozenbaum W, Levan P and Capeau J (2002) Association between altered expression of adipogenic factor SREBP1 in lipoatrophic adipose tissue from HIV-1- infected patients and abnormal adipocyte differentiation and insulin resistance. Lancet 359: 1026-31.
63. Jan V, Cervera P, Maachi M, Baudrimont M, Kim M, Vidal H, Girard PM, Levan P, Rozenbaum W, Lombes A, Capeau J and Bastard JP (2004) Altered fat differentiation and adipocytokine expression are inter-related and linked to morphological changes and insulin resistance in HIV-1-infected lipodystrophic patients. Antiviral therapy 9: 555-64.

64. Odle AK, Drew PD and Childs GV (2015) Giant mice reveal new roles for GH in regulating the adipose immune microenvironment. Endocrinology 156: 1613-5. 4398772.
65. Kroger J and Schulze MB (2012) Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. Current opinion in lipidology 23: 4-10.
66. Oberbauer AM, German JB and Murray JD (2011) Growth hormone enhances arachidonic acid metabolites in a growth hormone transgenic mouse. Lipids 46: 495-504.
67. Hochberg I, Tran QT, Barkan AL, Saltiel AR, Chandler WF and Bridges D (2015) Gene Expression Signature in Adipose Tissue of Acromegaly Patients. PloS one 10: e0129359. 4472931.
68. del Rincon JP, Iida K, Gaylinn BD, McCurdy CE, Leitner JW, Barbour LA, Kopchick JJ, Friedman JE, Draznin B and Thorner MO (2007) Growth hormone regulation of p85alpha expression and phosphoinositide 3-kinase activity in adipose tissue: mechanism for growth hormone-mediated insulin resistance. Diabetes 56: 1638-46.
69. Ahlzen M, Johansson LE, Cervin C, Tornqvist H, Groop L and Ridderstrale M (2008) Expression of the transcription factor 7-like 2 gene (TCF7L2) in human adipocytes is down regulated by insulin. Biochem Biophys Res Commun 370: 49-52.
70. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM and Chawla A (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 480: 104-8. 3371761.
71. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD and Chawla A (2014) Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157:1292-308. 4129510.
72. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA and Spiegelman BM (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157: 1279-91. 4131287.
73. Nagareddy PR, Kraakman M, Masters SL, Stirzaker RA, Gorman DJ, Grant RW, Dragoljevic D, Hong ES, Abdel-Latif A, Smyth SS, Choi SH, Korner J, Bornfeldt KE, Fisher EA, Dixit VD, Tall AR, Goldberg IJ and Murphy AJ (2014) Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity. Cell Metab 19: 821-35. 4048939.
74. Serri O, St-Jacques P, Sartippour M and Renier G (1999) Alterations of monocyte function in patients with growth hormone (GH) deficiency: effect of substitutive GH therapy. J Clin Endocrinol Metab 84: 58-63.
75. Swirski FK and Nahrendorf M (2013) Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 339:161-6. 3891792.
76. Mestas J and Ley K (2008) Monocyte-endothelial cell interactions in the development of atherosclerosis. Trends in cardiovascular medicine 18:228-32. 2650852.
77. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F, American Heart A, National Heart L and Blood I (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112: 2735-52.

78. Rosenzweig JL, Ferrannini E, Grundy SM, Haffner SM, Heine RJ, Horton ES, Kawamori R and Endocrine S (2008) Primary prevention of cardiovascular disease and type 2 diabetes in patients at metabolic risk: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 93: 3671-89.
79. Ma X, Holalkere NS, Kambadakone RA, Mino-Kenudson M, Hahn PF and Sahani DV (2009) Imaging-based quantification of hepatic fat: methods and clinical applications. Radiographics : a review publication of the Radiological Society of North America, Inc 29: 1253-77.
80. Freda PU, Shen W, Heymsfield SB, Reyes-Vidal CM, Geer EB, Bruce JN and Gallagher D (2008) Lower visceral and subcutaneous but higher intermuscular adipose tissue depots in patients with growth hormone and insulin-like growth factor I excess due to acromegaly. J Clin Endocrinol Metab 93: 2334-43. 2435633.
81. Freda PU, Shen W, Reyes-Vidal CM, Geer EB, Arias-Mendoza F, Gallagher D and Heymsfield SB (2009) Skeletal muscle mass in acromegaly assessed by magnetic resonance imaging and dual-photon x-ray absorptiometry. J Clin Endocrinol Metab 94: 2880-6. 2730874.
82. Gallagher D, Kuznia P, Heshka S, Albu J, Heymsfield SB, Goodpaster B, Visser M and Harris TB (2005) Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. Am J Clin Nutr 81: 903-10.
83. Song MY, Ruts E, Kim J, Janumala I, Heymsfield S and Gallagher D (2004) Sarcopenia and increased adipose tissue infiltration of muscle in elderly African American women. Am J Clin Nutr 79: 874-80.
84. Kim J, Heshka S, Gallagher D, Kotler DP, Mayer L, Albu J, Shen W, Freda PU and Heymsfield SB (2004) Intermuscular adipose tissue-free skeletal muscle mass: estimation by dual-energy X-ray absorptiometry in adults. J Appl Physiol 97: 655-60.
85. Weir JB (1949) New methods for calculating metabolic rate with special reference to protein metabolism. The Journal of physiology 109: 1-9. 1392602.
86. ParvoMedics (2008) TrueOne 2400 Metabolic Measurement System, Operator's Guide. Operator's Guide Version 4.3.
87. Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen YD, Sands RE, Pei D, Savage PJ and Bergman RN (1994) A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study. Diabetes 43: 1114-21.
88. Cobelli C, Toffolo GM, Dalla Man C, Campioni M, Denti P, Caumo A, Butler P and Rizza R (2007) Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. Am J Physiol Endocrinol Metab 293: E1-E15.
89. Bergman RN and Ader M (2000) Free fatty acids and pathogenesis of type 2 diabetes mellitus. Trends Endocrinol Metab 11: 351-6.
90. Steil GM, Volund A, Kahn SE and Bergman RN (1993) Reduced sample number for calculation of insulin sensitivity and glucose effectiveness from the minimal model. Suitability for use in population studies. Diabetes 42: 250-6.
91. Bergman RN, Prager R, Volund A and Olefsky JM (1987) Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest 79: 790-800.

92. Pacini G and Bergman RN (1986) MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23: 113-22.
93. Muniyappa R, Lee S, Chen H and Quon MJ (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 294: E15-26.
94. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW, Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112: 1796-808. 2966995.
95. Guo K, Mogen J, Struzzi S and Zhang Y (2009) Preadipocyte transplantation: an in vivo study of direct leptin signaling on adipocyte morphogenesis and cell size. American journal of physiology. Regulatory, integrative and comparative physiology 296: R1339-47. 2689822.
96. McLaughlin T, Lamendola C, Coghlan N, Liu TC, Lerner K, Sherman A and Cushman SW (2014) Subcutaneous adipose cell size and distribution: relationship to insulin resistance and body fat. Obesity (Silver Spring) 22: 673-80. 4344365.
97. Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, Basdevant A, Guerre-Millo M, Poitou C, Zucker JD, Bedossa P and Clement K (2010) Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. Diabetes 59: 2817-25. 2963540.
98. Oh DY, Morinaga H, Talukdar S, Bae EJ and Olefsky JM (2012) Increased macrophage migration into adipose tissue in obese mice. Diabetes 61: 346-54. 3266418.