**Upregulated Lipolysis Correlates with Exacerbated Metabolic Outcomes in Response to Elevated Glucocorticoids in Obesity**

**Abstract**

Obesity and chronically elevated glucocorticoids are common in the US and other developed countries and result in similar metabolic syndrome-associated co-morbidities, but the combination of these ailments on metabolic outcomes has not been studied. Measures of glucose homeostasis and markers of hepatic lipid accumulations were assessed to determine whether obesity exaggerated the effects of glucocorticoid-induced metabolic disturbances. The combination of obesity and glucocorticoids resulted synergistic elevations in hepatic steatosis and insulin resistance and this was matched with synergistic elevations in markers of lipolysis. Lipolysis may be a key player in glucocorticoid-induced insulin resistance and fatty liver.

**Introduction**

Obesity has become an epidemic in the US and other developing countries, and though Cushing’s disease is rare, it is estimated that 1-3% of US, UK and Denmark populations are prescribed corticosteroids at any given time (1–4). Comparing this staggering statistic with the fact that many Americans are overweight or obese, it is likely that obesity and glucocorticoid excess are combined in many instances. Given the similar co-morbidities associated with obesity and chronically elevated glucocorticoids, we hypothesized that the combinations of these two conditions would lead to even worse metabolic outcomes than either of them alone. This is supported by studies in rats showing that corticosterone and high-fat diets combine to cause worsened insulin resistance and NAFLD (5,6).

Obesity is often accompanied by a multitude of metabolic complications, such as insulin resistance (7–9) and non-alcoholic fatty liver disease (NAFLD; (10,11)). Similarly, chronically elevated glucocorticoids, such as is seen in Cushing’s disease or corticosteroid treatment, is often matched with impaired glucose tolerance or type 2 diabetes (12,13) and is associated with fatty liver (14,15), and changes in fat mass and distribution (16). Though these diseases and their co-morbidities are well characterized, underlying mechanisms caused by the disease subsequently leading to these metabolic complications have not been identified. Moreover, to our knowledge, the combination of obesity and elevated glucocorticoids on the severity of the aforementioned outcomes has yet to be investigated.

There is an array of physiological changes that occur as a result of elevated glucocorticoids including decreased lean mass (17–19), increased fat mass (18,20,21) and increased lipolysis (22–24), all of which have been associated with decreased insulin sensitivity (25–27). Likewise, obesity, insulin resistance and increased fatty acid flux are associated with NAFLD (10,28–30). Recent tissue-specific knockouts of glucocorticoid signaling molecules have implicated adipose tissue as a central node linking glucocorticoid action and lipolysis to systemic insulin resistance and NAFLD (31–34). Lipolysis is the breakdown of triglyceride into fatty acids and glycerol. It has also been demonstrated that inhibition of lipolysis promotes insulin sensitivity (25,35–37).

Here we show that chronically elevated glucocorticoids in the presence of obesity have synergistic effects on lipolysis, insulin resistance and fatty liver disease. Obese glucocorticoid-treated mice have reduced fat mass compared to all other groups, yet have hyperglycemia and severe insulin resistance; therefore, we speculate that it is the lipolysis drives insulin resistance in obese animals.

**Methods**

**Patient Recruitment and data collection**: Patients were recruited and data was collected as described in Hochberg et al. (18).

**Animal Procedures:** C57BL/6J adult male mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) at nine weeks of age. Following a one-week acclimation period, mice were treated as described previously (18) or were either kept on normal chow (NCD) or given high fat diet (45% fat; 35% carbohydrate; 20% protein; Research Diets, New Brunswick, NJ; ID D12451) for 12 weeks, depending on the experiment. Mice stayed on their respective diets and were treated with 1 mg/kg per day of dexamethasone (Sigma–Aldrich) in their drinking water (*n*=22) or used as controls (*n*=32) for six weeks. All animal procedures were approved by the University of Tennessee Health Science Center Institutional Animal Care and Use Committee. Animal body weight and composition was determined weekly using an echoMRI 2100. Food was weighed weekly, with food intake determined as the decrease in food weight per mouse per week per cage. All mice were provided with access to food and water *ad libitum* throughout the study, unless otherwise noted. At the end of treatment, mice were fasted for 16 h and were sacrificed by cervical dislocation at ZT3 after isoflurane anesthesia. Following cervical dislocation, a sagittal incision was made along the medioventral surface of each mouse and the skin was carefully pulled back to expose the subcutaneous fat depots. The incision was extended along the anterior surface of each hind limb to allow careful dissection of the inguinal fat pads. A small incision was then made into the rectus abdominus muscle to expose the abdominal cavity. The epididymal fat pads were identified and carefully dissected out. The right fat pads from each mouse were weighed and snap frozen in liquid nitrogen for later analysis, along with a section of the large lobe of the liver. Small pieces of tissues were placed in 10% formalin for histology.

**Insulin Tolerance Tests and Euglycemic Clamp Experiments:** Insulin tolerance was assessed following five weeks of treatment (27 weeks of age). Mice were given i.p. injections of insulin (Humulin R, Lilly, Indianapolis, IN, USA) at a concentration of 2.5 mU/g following a six hour fast. Blood glucose was determined using a One Touch Ultra Glucometer (Lifescan) prior to and every 15 minutes post injection. For the Clamp experiment C57BL/6J adult (70d) male mice were fed the same HFD as above for 8 weeks and treated with dexamethasone for 3 weeks prior to the experiment. Conscious glucose clamps were performed as described in

**Cell culture:** 3T3-L1 fibroblasts (pre-adipocytes) were cultured in 10% newborn calf serum, high glucose Dulbecco's Modification of Eagle's Medium (DMEM) with 1% penicilin, streptomycin and glutamine until confluence. A differentiation cocktail including 250nM dexamethasone, 3-isobutyl-1-methylxanthine and insulin in 10% fetal bovine serum, high glucose DMEM with 1% PSG at two days post confluence for four days (38). Media was then replaced including only insulin in the cocktail for an additional three days. For the following three days, cells remained in FBS media with no additional treatment. To assess lipolysis, cells remained in FBS media and were treated with ethanol (vehicle) or 250nM dexamethasone for five days before lysing.

**Assessment of Triglycerides from Cells and Tissue:** 3T3-L1 ells were grown and treated as described above and a triglyceride assay was performed at the end of the treatment period. Cells were lysed in homogenization buffer (50 mM Tris pH 8, 5 mM EDTA, 30 mM Mannitol, PI inhibitor) undergoing three freeze thaw cycles. Frozen liver tissue was homogenized with 5mm stainless steel pellets in this buffer using a TissueLyser II (Qiagen) set to 30Hz for a duration of 5 minutes. Lipids were extracted using KOH and Chloroform:methanol mixture and left to evaporate overnight. A mixture of butanol, methanol and triton were then used to get the lipids into a solution. Triglyceride content was assessed using the Triglyceride Assay Kit (Sigma) and absorbance was detected as described in (39).

**Histology:** Tisssues were kept in 10% formalin for 24 hours and then stored in 70% ethanol until further processing. Following a series of wash steps, tissues were embedded in paraffin wax and sent to either the University of Michigan University of Michigan Comprehensive Cancer Center Tissue Core or the Unit for Laboratory Animal Medicine In-vivo Animal Core (University of Michigan, Ann Arbor) where they were processed and stained with H&E to assess cell morphology/inflammation and collagen formation, respectively.

**Analysis of mRNA:** Cells and tissues were lysed in TRIzol using the TissueLyser II, as decribed above, and RNA was extracted using the PureLink RNA mini kit (Life Technologies). cDNA was synthesized from 0.5-1g of RNA using the High Capacity Reverse Transcription Kit (Life Technologies). Primers, cDNA and Power SYBR Green PCR Master Mix (Life Technologies) were combined in accordance with the manufacturer’s guidelines and quantitative real-time PCR was performed as previously described (40). mRNA expression level was normalized to *Actb* after evaluation of several reference genes (Table 1).

**Protein Analysis:** Cells and tissues were lysed in RIPA buffer (50 mM Tris, pH 7.4, 0.25% sodium deoxycholate, 1% NP40, 150 mM sodium chloride, 1 mM EDTA, 100 uM sodium orthovanadate, 5 mM sodium fluoride and 10 mM sodium pyrophosphate) on ice then centrifuged for 15 minutes at 13 000 RPM at 4°C. Clarified lysates were loaded on SDS-PAGE gels, transferred and blotted using antibodies raised against ATGL (Cell Signaling Technologies), HSL, pHSL, CGI-58, GAPDH, and Actin. Antibody complexes were detected by anti-mouse and anti-rabbit fluorescent conjugated antibodies and visualized using an Odyssey image scanner and blots were quantified using the Odyssey software version 2.1 (LiCOR).

**Determination of Glycerol and Fatty Acid Levels:** Twenty-one-week-old *ad libitum* chow fed C57BL/6J male mice were briefly anesthetized with isoflurane at 11 weeks post dexamethasone treatment and blood was taken via retro orbital bleed at baseline and 15 minutes following an i.p. injection of 10mg/kg isoproterenol (Sigma-Aldrich) in PBS. Additionally, serum was taken from these mice as well as from another cohort of twenty-eight-week old mice on HFD or chow six weeks post dexamethasone treatment following an overnight fast, just prior to sacrifice. Glycerol and free fatty acids were assessed via Serum Triglyceride Determination Kit (Sigma-Aldrich) and HR Series NEFA-HR(2) (Wako Diagnostics), respectively, in accordance with manufacturer’s guidelines.

**Statistics**: For animal studies, two-way ANOVA analyses were performed to test for significance of diet and treatment, as well as their interaction. For cell culture experiments a Student’s *t*-test was used after confirming equal variance and normality. P-values below p=0.05 were considered significant.

**Results**

# Dexamethasone-Induced Insulin Resistance is Worsened in the Presence of Obesity

Our group has previously published data that suggested different and gene expression in adipose tissue between those with Cushing’s disease (ACTH-secreting pituitary adenoma) and controls (non-secreting pituitary adenoma; (22)). Based on this, we speculated that the glucocorticoid responses may vary according to obesity status. Here we have re-analyzed the data stratifying the Cushingoid and control groups by BMI (Figure 1A), classifying these individuals as “Not obese” (BMI < 30) and “Obese” (BMI ≥ 30). There were no significant differences in BMI in the controls compared to the Cushing’s however, were near-significant interaction between obesity status and Cushing’s diagnosis on HOMA-IR score (p=0.057; Figure 1A-B). We observed only a 17% increase in HOMA-IR score comparing non-obese subjects with and without Cushing’s disease, but a 3.4 fold increase in obese patients.

To investigate if obesity status influences insulin sensitivity in the presence of elevated glucocorticoids we performed an insulin tolerance test (ITT) on lean (NCD) and diet-induced obese (HFD) mice that were untreated (Control) or treated with glucocorticoids (Dexamethasone; Figure 1C). HFD-fed, dexamethasone-treated mice were significantly more resistant to insulin-stimulated glucose uptake when compared to all other groups (Figure 1D. Additionally, HFD dexamethasone-treated mice exhibited dramatic fasting hyperglycemia, with a significant interaction between diet and drug (p=0.00009; Figure 1E). While HFD animals had a 24% increase in fasting glucose when compared to NCD animals, in the presence of Dexamethasone, HFD-fed animals had a 122% increase in fasting glucose relative to NCD controls not treated with dexamethasone. In the lean, NCD-fed animals, dexamethasone caused a 18% decrease in fasting glucose.

To evaluate glucose homeostasis in more detail we performed a hyperinsulinemic euglycemic clamp in obese mice (11 weeks of HFD) treated with dexamethasone for the final three weeks. This shorter HFD/dexamethasone exposure still caused dramatic insulin resistance, hyperglycemia and reductions in lean mass, but no differences in fat mass between the groups (Supplementary Figures 1A-F). During the hyperinsulinemic phase, the glucose infusion rate was 39% lower in obese dexamethasone-treated mice when compared to obese controls indicating insulin resistance at euglycemia (Figure 1E). Basal endogenous glucose production (EGP) was 37% higher in the dexamethasone treated group (p=0.026). Moreover, In the dexamethasone group, EGP was reduced to near zero by insulin but only 70s% in the control group (p=0.0091) resulting in EGP being 5-fold higher during the insulin phase in dexamethasone treated mice (p=0.014) when compared to controls (Figure 1F). Overall glucose turnover was slightly decreased in the presence of insulin (p=0.141; Figure 1G). There were significant reductions in 2-deoxyglucose uptake in subcutaneous white adipose (p=0.019), heart (p=0.0003) and gastrocnemius tissues (p=0.00001; Figure 1H). These data suggest that increased EGP and impaired suppression of EPG by insulin are the primary causes of the observed insulin resistance and hyperglycemia in obese, dexamethasone treated animals.

# HFD-Induced Liver Steatosis in Dexamethasone Treated mice

Obesity and chronic elevations in glucocorticoids have been associated with increased liver fat and even NAFLD (10,14). We observed slight but non-significant increases in plasma ALT, a liver enzyme associated with liver disease, in obese Cushing’s patients (38% increase in non-obese subjects versus a 2.8 fold increase in obese subjects, p=0.13 for the interaction of disease and obesity status; Figure 2A). In our mouse model of HFD-fed, dexamethasone treated mice we observed drastically elevated liver triglycerides when compared to all other groups with a significant interaction of drug and diet (p=0.0000677; Figure 2B). In support of this, H&E staining of hepatic tissue clearly depicts exacerbated lipid levels in the obese, glucocorticoid-treated group when compared to HFD-fed or dexamethasone-treated controls (Figure 2C).

Genes involved in hepatic *de novo* lipogenesis, *Srebf1* and *Fasn*, were assessed via qPCR in these liver lysates (Figure 2D). There was a significant effect of diet and drug on *Fasn* expression (p=0.014). Though both transcripts were somewhat elevated in response to HFD alone, there was no significant synergism with dexamethasone. This finding indicates that lipid accumulation resulting from dexamethasone treatment is likely occurring via a different mechanism than accelerated glucocorticoid-dependent upregulation activation of *de novo* lipogenesis.

# Dexamethasone Causes Decreased Fat Mass in HFD-Fed Mice

We evaluated body mass in mice via EchoMRI. Contrary to expectations, we observed reductions in fat mass in the HFD-fed dexamethasone treated group (Figure 3A,B). It is These reductions were not depot specific, as they were observed in both inguinal and epididymal adipose tissue (Figure 3C). There was no change in fat mass in response to dexamethasone treatment in the chow-fed group (Figure 3B). We observed no significant differences in food consumption throughout this study (Figure 3D). These data suggested that glucocorticoids may promote a lipodystrophy-like phenotype in obese animals.

Dexamethasone Treatment Results in Increased Lipolysis

One potential mechanism of these alterations in lipid deposition is accelerated adipocyte lipolysis. Lipolysis has previously been associated with insulin resistance, is a known cause of NAFLD, and has been shown to increase with glucocorticoid treatment. We first assessed whether there was a direct effect of dexamethasone on adipocyte lipolysis in cultured adipocytes. 3T3-L1 fibroblasts were either kept in media alone (pre-adipocytes), differentiated (mature adipocytes) or treated with dexamethasone following differentiation (mature adipocytes +dexamethasone) over a 15-day period. Dexamethasone treatment following differentiation lead to decreased lipid content (p=) and increased glycerol release into the media (p=), indicating increased lipolysis (Figure 4 A-B). To assess this further, we measured lipolytic enzyme mRNA and protein expression levels in these cells (Figure 4 C,D). Expression of ATGL (encoded by *Pnpla2*) was enhanced following dexamethasone treatment in 3T3-L1 cells at both the transcript (p=; XX fold) and protein (p=; XX fold) level. These data show that glucocorticoids directly stimulate both ATGL levels and lipolysis in cultured adipocytes.

To assess the effects of glucocorticoid-induced lipolysis *in vivo,* we measured the by-products of triglyceride breakdown, glycerol and free fatty acids in basal and stimulated conditions (Figure 4 E). Stimulation of lipolysis was achieved via isoproterenol, a -adrenergic receptor agonist, or by a 16-hour fast. Serum free fatty acids and glycerol were measured for each of these conditions. Dexamethasone treatment led to significant increases in basal (p<0.01; XX fold), fasted (p=0.01; XX fold) and isoproterenol-stimulated (p<0.01; XX fold) glycerol as well as basal (p<0.01; XX fold) and stimulated (p<0.01; XX fold) free fatty acids, indicating dexamethasone enhances basal and stimulated lipolysis *in vivo* in lean mice. Consistent with these findings, qPCR analysis in the inguinal white adipose tissue (iWAT) showed an upregulation of *Pnpla2* transcripts in the dexamethasone-treated mice compared to controls (p=; XX fold ; Figure 4F).

Since the HFD-fed, dexamethasone-treated mice are subject to more severe insulin resistance and hepatic lipid accumulation, we tested whether lipolysis was also elevated to a further extent that is seen in the chow-fed mice. To assess lipolysis, we measured serum glycerol following a 16-hour fast (Figure 5A). As expected, serum glycerol was elevated in dexamethasone treated animals, but this effect was even more robust in the obese animals and there was a significant interaction between drug and diet (p=0.017).

We quantified mRNA and protein expression of the lipolytic enzymes, ATGL and HSL, in the iWAT of these mice (5B,C). Consistent with the above findings, expression of ATGL was elevated in the dexamethasone-treated groups and with a significant synergistic effect of glucocorticoids and obesity at both the transcript (p=0.02) and protein (p=0.003) level. These data support the hypothesis that glucocorticoid-stimulated lipolysis is augmented in the context of obesity, potentially via increased transactivation of Pnpla2/ATGL.

**Discussion**

Chronic glucocorticoid elevations are associated with many co-morbidities such as increased fat mass (18,20,21), decreased muscle mass (17–19), insulin resistance (12,13) and non-alcoholic fatty liver disease (NAFLD; (14,15)), all of which can have a negative effect on health (41–44). These side effects are similar those seen in obesity; however, the combination of chronically elevated glucocorticoids in the context of pre-existing obesity has not been assessed. Here we show that glucocorticoid-induced insulin resistance and NAFLD are exacerbated when paired with obesity, more than the sum of their individual effects.

Obese patients with Cushing’s disease were found to have a trend toward increases in HOMA-IR score, indicating increased insulin resistance as well as increases in the liver enzyme ALT, a marker of liver disease. In line with these findings, increased in central adiposity, such as is seen in people with obesity, has been previously associated with enhanced fatty acid flux (i.e. lipolysis) when compared to lower body fat stores (45), which is thought to contribute to insulin resistance and fatty liver (25,46). There are two limitations to this human study, one is the small sample size and the second is that it was not possible to determine the physiological status of the patients before they developed a tumor; therefore, we could discern whether obesity was present prior to or after development of Cushing’s disease. For this reason, we designed a mouse study to investigate whether being obese prior to glucocorticoid treatment leads to worsened outcomes.

We found that HFD-fed, dexamethasone-treated mice exhibited hyperglycemia and severe insulin resistance. This was primarily due to increased endogenous glucose production in these animals. Significant elevations in liver fat was also observed in HFD-fed, dexamethasone-treated mice, consistent with a trend towards elevated ALT levels seen in the obese Cushing’s patients. Cushing’s disease is often paired with increased fat mass, which has been proposed to contribute to fatty liver (47,48). Indeed, obesity is a known risk factor of NAFLD (10,11). Previous work from our lab shows increased fat mass, specifically in the iWAT, following 12 weeks of dexamethasone treatment (18) in chow-fed mice, and increased fat mass has also been reported by others using various glucocorticoids. However, to our surprise, the glucocorticoid treatment in obese mice led to an overall reduction in adiposity. Therefore, when comparing HFD control mice to HFD dexamethasone-treated mice, increased fat mass is not cause of the observed exacerbations in insulin resistance and increased liver fat. Lipolysis has been linked to increased gluconeogenesis by several studies. One potential mechanism is that the increased flux of fatty acids, oxidized in the liver to acetyl-CoA, activate pyruvate carboxylase and gluconeogenesis (49).

Lipolysis has been implicated in insulin resistance (25,35) and NAFLD (28) and there is evidence to support it is enhanced in the obese state (50). Glucocorticoids are known to stimulate lipolysis (22–24). There is some debate as to which genes glucocorticoids are acting on to promote lipolysis. Downregulation of *Pde3b* (51) and upregulation of -adrenergic receptors (52) and lipase transcripts (53) have been proposed as possible mechanisms. We assessed all of the previously proposed targets and found ATGL to be synergistically activated by obesity and glucocorticoid-treatment. Consistent with this, the products of lipolysis were greater in obese, dexamethasone-treated mice than diet or glucocorticoid-alone controls. The mechanisms by which obesity and glucocorticoids synergize to activate ATGL expression are not clear at this time.

Further research is needed to determine whether the insulin resistance observed is due to obesity or the high fat content of the diet. We evaluated glucocorticoid treatment in obesity; however, Riddell and colleagues have reported similar findings when giving HFD and glucocorticoids in concert to rats, prior to the onset of obesity (5,6,54). It is possible that HFD lends to elevated FFAs, leading to a similar phenotype as elevated adipose tissue lipolysis. Further studies are needed to determine whether diet or obesity status or both are the source of this elevated metabolic risk.

In summary, glucocorticoids are a commonly prescribed drug used to treat a multitude of health issues but are known to induce a variety of metabolic side effects. Their actions in persons with obesity are not yet clear, in spite of a huge number of obese individuals on prescription glucocorticoids. The data presented here shows that obesity does in fact pose a greater risk for the harmful co-morbidities associated with chronically elevated glucocorticoids and this might be considered when determining treatment options. More work is required in the area to assess whether blocking glucocorticoid/lipolytic action in the fat tissue would be beneficial to prevent or enhance recovery of the afore-mentioned glucocorticoid-induced comorbidities.

# Acknowledgements

This study was supported by funds from NIH Grant R01-DK107535 (DB). This study also utilized the University of Michigan Metabolism, Bariatric Surgery and Behavior Core (U2C-DK110768), the Michigan Nutrition Obesity Research Center (P30-DK089503) and the University of Michigan Comprehensive Cancer Center Core. We would like to thank the other members of the Bridges laboratory, Thurl Harris (University of Virginia) and Edwards Park (UTHSC) for insights on this work.

1. Overman R a., Yeh JY, Deal CL. Prevalence of oral glucocorticoid usage in the United States: A general population perspective. Arthritis Care Res. 2013;65:294–8.

2. Fardet L, Petersen I, Nazareth I. Original article Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. 2011;

3. Hsiao C, Ph D, Cherry DK, Beatty PC, Ph D, Rechtsteiner EA, Care H. National Ambulatory Medical Care Survey : 2007 Summary. 2010;

4. Laugesen K, Otto J, Jørgensen L, Sørensen HT, Petersen I. Systemic glucocorticoid use in Denmark : a population-based prevalence study. 2017;1–6.

5. Beaudry JL, Anna MD, Teich T, Tsushima R, Riddell MC. Exogenous Glucocorticoids and a High-Fat Diet Cause Severe Hyperglycemia and Hyperinsulinemia and Sprague-Dawley Rats. 2013;154:3197–208.

6. Shpilberg Y, Beaudry JL, Souza AD, Campbell JE, Peckett A, Riddell MC. A rodent model of rapid-onset diabetes induced by glucocorticoids and high-fat feeding. 2012;680:671–80.

7. Karam JH, Grodsky GM, Ph D, Forsham PH. Excessive Insulin Response to Glucose in Obese Subjects as Measured by Immunochemical Assay.

8. Bagdadea JD, Bierman EL, Porte D, Ii JR, Nih W, Presented GF-. The Significance of Basal Insulin Levels in the Evaluation of the Insulin Response to Glucose in Diabetic and Nondiabetic Subjects \*. 1967;46.

9. Steffensen C, Pereira AM, Dekkers OM. Prevalence of hypercortisolism in type 2 diabetes patients : a systematic review and. 2016;

10. Wanless I, Lentz J. Fatty Liver Hepatitis ( Steatohepatitis ) and Obesity : An Autopsy Study with Analysis of Risk Factors. Hepatology. 1990;12:1106–10.

11. Youssef WI, Mccullough AJ. Steatohepatitis in obese individuals. 2002;16:733–47.

12. Pivonello R, Martino MC De, Leo M De, Lombardi G, Colao A. Cushing’s Syndrome. 2008;37:135–49.

13. Resmini E, Minuto ÆF, Colao A, Ferone ÆD. Secondary diabetes associated with principal endocrinopathies : the impact of new treatment modalities. 2009;85–95.

14. Rockall A, Sohaib S, Evans D, Kaltsas G, Isidori A, Monson J, Besser G, Grossman A, Reznek R. Hepatic steatosis in Cushing’s syndrome: a radiological assessment using computed tomography. Eur J Endocrinol [Internet]. 2003;149:543–8. Available from: http://www.eje-online.org/cgi/doi/10.1530/eje.0.1490543

15. Cerda J, Fardella CE, Arrese M. Overexpression of 11 b -hydroxysteroid dehydrogenase type 1 in visceral adipose tissue and portal hypercortisolism in non-alcoholic fatty liver disease. 2012;392–9.

16. Rutters F, Nieuwenhuizen AG, Lemmens SGT, Born JM, Westerterp-plantenga MS. Hypothalamic – Pituitary – Adrenal ( HPA ) axis functioning in relation to body fat distribution. 2010;738–43.

17. Dardevet D, Somet C, Taillandier D, Savary I, Attaix D, Grizard J. Sensitivity and Protein Turnover Response to Glucocorticoids Are Different in Skeletal Muscle from Adult and Old Rats Lack of Regulation of the Ubiquitin-Proteasome Proteolytic Pathway in Aging. J Clin Invest. 1995;96:2113–9.

18. Hochberg I, Harvey I, Tran QT, Stephenson EJ, Barkan AL, Saltiel AR, Chandler WF, Bridges D. Gene expression changes in subcutaneous adipose tissue due to Cushing’s disease. J Mol Endocrinol [Internet]. 2015;55:81–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26150553

19. Schakman O, Kalista S, Barbé C, Loumaye A, Thissen JP. Glucocorticoid-induced skeletal muscle atrophy ଝ. Int J Biochem Cell Biol [Internet]. Elsevier Ltd; 2013;45:2163–72. Available from: http://dx.doi.org/10.1016/j.biocel.2013.05.036

20. Abad V, Chrousos GP, Reynolds JC, Nieman LK, Hill SC, Weinstein RS, Leong GM. Glucocorticoid Excess During Adolescence Leads to a Major Persistent Deficit in Bone Mass and an Increase in Central Body Fat. 2001;16:1879–85.

21. Geer EB, Shen W, Gallagher D, Punyanitya M, Looker HC, Post KD, Freda PU. Female Patients with Cushing ’ s Disease. 2011;73:469–75.

22. Djurhuus CB, Gravholt CH, Nielsen S, Pedersen SB, Møller N, Schmitz O. Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. 2004;488–94.

23. Kršek M, Rosická M, Nedvídková J, Ková HKČ, Hána V, Marek J, Haluzík M, Lai EW, Pacák K. Increased Lipolysis of Subcutaneous Abdominal Adipose Tissue and Altered Noradrenergic Activity in Patients with Cushing ‘ s Syndrome : An In-vivo Microdialysis Study. 2006;421–8.

24. Djurhuus CB, Gravholt CH, Nielsen S, Mengel a, Christiansen JS, Schmitz OE, Møller N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab. 2002;283:E172–7.

25. Rebrin K, Steil GM, Mittelman SD, Bergman RN. Causal Linkage between Insulin Suppression of Lipolysis and Suppression of Liver Glucose Output in Dogs. 1996;98:741–9.

26. Zhang M, Hu T, Zhang S, Zhou L. Associations of Different Adipose Tissue Depots with Insulin Resistance : A Systematic Review and Meta-analysis of Observational Studies. Nat Publ Gr [Internet]. Nature Publishing Group; 2015;1–6. Available from: http://dx.doi.org/10.1038/srep18495

27. Dirks ML, Wall BT, Valk B Van De, Holloway TM. One Week of Bed Rest Leads to Substantial Muscle Atrophy and Induces Whole-Body Insulin Resistance in the Absence of Skeletal Muscle Lipid Accumulation. 2016;65:2862–75.

28. Gastaldelli A, Harrison SA, Belfort-aguilar R, Hardies LJ, Balas B, Schenker S, Cusi K. Importance of Changes in Adipose Tissue Insulin Resistance to Histological Response During Thiazolidinedione Treatment of Patients with Nonalcoholic Steatohepatitis. 2009;

29. Westerbacka J, Rvi AS, Halavaara J, Yki-ja H. Fat Accumulation in the Liver Is Associated with Defects in Insulin Suppression of Glucose Production and Serum Free Fatty Acids Independent of Obesity in Normal Men. 2002;87:3023–8.

30. Bugianesi E, Gastadelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease : sites and mechanisms. Diabetologia. 2005;48:634–42.

31. Mueller KM, Hartmann K, Kaltenecker D, Vettorazzi S, Bauer M, Mauser L, Amann S, Jall S, Fischer K, Esterbauer H, et al. Adipocyte Glucocorticoid Receptor De fi ciency Attenuates Aging- and HFD-Induced Obesity and Impairs the Feeding-Fasting Transition. 2017;66:272–86.

32. Shen Y, Roh HC, Kumari M, Rosen ED. Adipocyte glucocorticoid receptor is important in lipolysis and insulin resistance due to exogenous steroids , but not insulin resistance caused by high fat feeding. Mol Metab [Internet]. Elsevier GmbH; 2017; Available from: http://dx.doi.org/10.1016/j.molmet.2017.06.013

33. Morgan SA et al. 11β-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess: Supporting Information. 2011;1–10.

34. Wang Y, Yan C, Liu L, Wang W, Du H, Fan W, Lutfy K, Jiang M, Friedman TC, Liu Y. 11 -Hydroxysteroid dehydrogenase type 1 shRNA ameliorates glucocorticoid-induced insulin resistance and lipolysis in mouse abdominal adipose tissue. AJP Endocrinol Metab [Internet]. 2014;308:E84–95. Available from: http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.00205.2014

35. Edgerton DS, Kraft G, Smith M, Farmer B, Williams PE, Coate KC, Printz RL, Brien RMO, Cherrington AD. Insulin ’ s direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. 2017;2:1–14.

36. Corbit KC, Camporez JPG, Tran JL, Wilson CG, Lowe DA, Nordstrom SM, Ganeshan K, Perry RJ, Shulman GI, Jurczak MJ, et al. Adipocyte JAK2 mediates growth hormone – induced hepatic insulin resistance. 2017;2:1–14.

37. Schoiswohl G, Stefanovic-Racic M, Menke MN, Wills RC, Surlow B a., Basantani MK, Sitnick MT, Cai L, Yazbeck CF, Stolz DB, et al. Impact of Reduced ATGL-Mediated Adipocyte Lipolysis on Obesity-Associated Insulin Resistance and Inflammation in Male Mice. Endocrinology [Internet]. 2015;156:3610–24. Available from: http://press.endocrine.org/doi/10.1210/en.2015-1322

38. Chiang S-H, Chang L SA. TC10 and Insulin ‐ Stimulated Glucose Transport. 2002;406:1257–62.

39. Lu B, Bridges D, Yang Y, Fisher K, Cheng A, Chang L, Meng Z, Lin J, Downes M, Yu RT, et al. Metabolic Crosstalk: molecular links between glycogen and lipid metabolism in obesity. Diabetes [Internet]. 2014;63:1–49. Available from: http://dx.doi.org/10.2337/db13-1531

40. Lu B, Bridges D, Yang Y, Fisher K, Cheng A, Chang L, Meng ZX, Lin JD, Downes M, Yu RT, et al. Metabolic crosstalk: Molecular links between glycogen and lipid metabolism in obesity. Diabetes. 2014;63:2935–48.

41. Jaruvongvanich V, Wirunsawanya K, Sanguankeo A. Nonalcoholic fatty liver disease is associated with coronary artery calcification : A systematic review and meta-analysis. Dig Liver Dis [Internet]. Editrice Gastroenterologica Italiana; 2016;48:1410–7. Available from: http://dx.doi.org/10.1016/j.dld.2016.09.002

42. Kramer C, Zinman B, Retnakaran R. Are Metabolically Healthy Overweight and Obesity Benign Conditions ? Ann Intern Med. 2013;159:758–69.

43. Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, Rosenfeld R, Chen Q, Boone T, Simonet WS, et al. Reversal of Cancer Cachexia and Muscle Wasting by ActRIIB Antagonism Leads to Prolonged Survival. Cell [Internet]. Elsevier Ltd; 2010;142:531–43. Available from: http://dx.doi.org/10.1016/j.cell.2010.07.011

44. Roper N, Bilous R, Kelly W, Unwin N, Connolly V. Cause-Specific Mortality in a Population. Diabetes Care. 2002;25.

45. Manolopoulos KN, Karpe F, Frayn KN. Marked resistance of femoral adipose tissue blood flow and lipolysis to adrenaline in vivo. 2012;3029–37.

46. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. 2005;115:1343–51.

47. Yan Y, Hou D, Zhao X, Liu J. Childhood Adiposity and Nonalcoholic Fatty Liver Disease in Adulthood. 2017;139.

48. Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Publ Gr [Internet]. Nature Publishing Group; 2017;49:842–7. Available from: http://dx.doi.org/10.1038/ng.3855

49. Perry RJ, Camporez JG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, Jurczak MJ, Abudukadier A, Han S, Zhang X, et al. Hepatic Acetyl CoA Links Adipose Tissue Inflammation to Hepatic Insulin Resistance and Type 2 Diabetes. Cell. 2015;160:745–58.

50. Gaidhu MP, Anthony NM, Patel P, Hawke TJ, Ceddia RB. Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. Am J Physiol - Cell Physiol [Internet]. 2010;298:C961–71. Available from: http://ajpcell.physiology.org/content/298/4/C961%5Cnhttp://ajpcell.physiology.org/content/298/4/C961.short%5Cnhttp://ajpcell.physiology.org/content/ajpcell/298/4/C961.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/20107043

51. Xu C, He J, Jiang H, Zu L, Zhai W, Pu S, Xu G. Direct effect of glucocorticoids on lipolysis in adipocytes. Mol Endocrinol [Internet]. 2009;23:1161–70. Available from: http://mend.endojournals.org/content/23/8/1161.full%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/19443609

52. Lacasa D, Agli B, Giudicelli Y. PERMISSIVE ACTION OF GLUCOCORTICOIDS ON CATECHOLAMINE-INDUCED LIPOLYSIS : DIRECT “IN VITRO” EFFECTS ON THE FAT CELL ~-ADRENORECEPTOR-COUPLED-ADENYLATE CYCLASE SYSTEM Dani~le. Biochem Biophys Res Commun. 1988;153:489–97.

53. Campbell JE, Peckett AJ, D’souza AM, Hawke TJ, Riddell MC. Adipogenic and lipolytic effects of chronic glucocorticoid exposure. Am J Physiol Cell Physiol [Internet]. 2011;300:C198-209. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20943959

54. D’souza AM, Beaudry JL, Szigiato AA, Trumble SJ, Snook LA, Bonen A, Giacca A, Riddell MC. Consumption of a high-fat diet rapidly exacerbates the development of fatty liver disease that occurs with chronically elevated glucocorticoids. Am J Physiol Gastrointest Liver Physiol. 2012;302:850–63.