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GENOME WIDE EXPRESSION ANALYSIS AND METABOLIC MECHANISMS PREDICTING BODY WEIGHT MAINTENANCE

Dissertation
zur Erlangung des akademischen Grades
"doctor rerum naturalium"
(Dr. rer. nat.)

eingereicht an der
Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Potsdam

von
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Potsdam, Dezember 2015

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Published online at the
Institutional Repository of the University of Potsdam:
URN urn:nbn:de:kobv:517-opus4-100767
http://nbn-resolving.de/urn:nbn:de:kobv:517-opus4-100767

1 SUMMARY

Obesity is a major health problem for many developing and industrial countries. Increasing rates reach almost 50% of the population in some countries and related metabolic diseases including cardiovascular events and T2DM are challenging the health systems. Adiposity, an increase in body fat mass, is a major hallmark of obesity. Adipose tissue is long known not only to store lipids but also to influence whole-body metabolism including food intake, energy expenditure and insulin sensitivity. Adipocytes can store lipids and thereby protect other tissue from lipotoxic damage. However, if the energy intake is higher than the energy expenditure over a sustained time period, adipose tissue will expand. This can lead to an impaired adipose tissue function resulting in higher levels of plasma lipids, which can affect other tissue like skeletal muscle, finally leading to metabolic complications. Several studies showed beneficial metabolic effects of weight reduction in obese subjects immediately after weight loss. However, weight regain is frequently observed along with potential negative effects on cardiovascular risk factors and a high intra-individual response.

We performed a body weight maintenance study investigating the mechanisms of weight maintenance after intended WR. Therefore we used a low caloric diet followed by a 12-month life-style intervention. Comprehensive phenotyping including fat and muscle biopsies was conducted to investigate hormonal as well as metabolic influences on body weight regulation. In this study, we showed that weight reduction has numerous potentially beneficial effects on metabolic parameters. After 3-month WR subjects showed significant weight and fat mass reduction, lower TG levels as well as higher insulin sensitivity. Using RNA-Seq to analyse whole fat and muscle transcriptome a strong impact of weight reduction on adipose tissue gene expression was observed. Gene expression alterations over weight reduction included several cellular metabolic genes involved in lipid and glucose metabolism as well as insulin signalling and regulatory pathways. These changes were also associated with anthropometric parameters assigning body composition. Our data indicated that weight reduction leads to a decreased expression of several lipid catabolic as well as anabolic genes. Long-term body weight maintenance might be influenced by several parameters including hormones, metabolic intermediates as well as the transcriptional landscape of metabolic active tissues. Our data showed that genes involved in biosynthesis of unsaturated fatty acids might influence the BMI 18-month after a weight reduction phase. This was further supported by analysing metabolic parameters including RQ and FFA levels. We could show that subjects maintaining their lost body weight had a higher RQ and lower FFA levels, indicating increased metabolic flexibility in subjects.

Using this transcriptomic approach we hypothesize that low expression levels of lipid synthetic genes in adipose tissue together with a higher mitochondrial activity in skeletal muscle tissue might be beneficial in terms of body weight maintenance.

2 ZUSAMMENFASSUNG

Die Adipositas hat sich in den letzten Jahren zu einem deutlichen Gesundheitsproblem in Industrie- und Entwicklungsländern entwickelt. So sind in einigen Länder bis zu 50% der Bevölkerung übergewichtig und Begleiterkrankungen wie Herzkreislauferkrankungen und Typ 2 Diabetes belasten das Gesundheitssystem. Ein Anstieg der Körperfettmasse spielt bei der Adipositas eine große Rolle. Mittlerweile ist bekannt, dass Fettgewebe nicht nur Lipide speichert, sondern auch den Gesamtmetabolismus wie Nahrungsaufnahme, Energieumsatz und Insulinsensitivität beeinflusst. Lipide werden in Adipozyten gespeichert und verhindern so eine vermehrte Fetteinlagerung in andere Gewebe. Somit stellt das Fettgewebe ein wichtiges Organ dar, das andere periphere Gewebe vor dem toxischen Effekt erhöhter Lipidspiegel schütz. Ist über einen längeren Zeitraum die Energiezufuhr höher als der Energieverbrauch, kommt es zu einer Expansion des Fettgewebes. Dies kann im weiteren Verlauf zu einer Dysfunktion der Adipozyten und des Fettgewebes führen. Erhöhte Lipidspiegel können dann nicht mehr im Fettgewebe gespeichert werden und es kommt zu einer Anreicherung in der Peripherie. Vor allem das Muskelgewebe und die Leber sind hiervon betroffen, was zu weiteren metabolischen Komplikationen führt. Eine Gewichtsreduktion führt in adipösen Personen zu einer Verbesserung zahlreicher metabolischer Parameter. Diverse Studien zeigten jedoch, dass nur ein geringer Anteil dieser Personen in der Lage waren, das reduzierte Körpergewicht zu erhalten. Diese Wiederzunahme des Körpergewichts führt unter anderem zu einer Erhöhung des kardiovaskulären Risikos. Im Allgemeinen ist eine hohe Variabilität bei der Gewichtsreduktion und der Wiederzunahme zu beobachten.

Diese Arbeit basiert auf Daten einer Studie, die Effekte einer Gewichtsreduktion auf den Gewichtserhalt untersucht. Nach einer 3-monatigen Gewichtsreduktion mittels einer niederkalorischen Diät wurden die Probanden in Kontroll- und Interventionsgruppe eingeteilt. Anthropometrische sowie metabolische Parameter inklusive Muskel- und Fettgewebsbiopsien wurden erfasst. In dieser Studie konnte gezeigt werden, dass eine Gewichtsreduktion verschiedenste positive Auswirkungen auf den Metabolismus der Teilnehmer hat. Die Probanden zeigten nach 3-monatiger Gewichtsreduktion eine signifikante Reduktion des Körpergewichts und der Fettmasse, erniedrigte Triglyzerid Spiegel und eine verbesserte Insulinsensitivität. Mittels RNA-Seq konnten wir zusätzlich zeigen, dass eine Gewichtsreduktion deutliche Auswirkungen auf das Transkriptom des Fettgewebes besitzt. Unter anderem wurden Genexpressionsveränderungen im Bereich zell-metabolischer Gene wie Lipid- und Glukosestoffwechsel als auch im Bereich des Insulinsignalweges und regulatorischer Gene ermittelt. Diese Expressionsveränderungen zeigten auch einen Zusammenhang mit dem BMI. Unsere Daten weisen darauf hin, dass eine Gewichtsreduktion zu einer Erniedrigung der Expression von Genen im Fettstoffwechsel führt. Ein langfristiger Gewichtserhalt wird durch zahlreiche Parameter wie Hormone,

Stoffwechselintermediate und vermutlich auch den transkriptionellen Zustand im metabolisch aktiven Gewebe beeinflusst. Die hier gezeigten Daten deuten darauf hin, dass Gene beteiligt in der Biosynthese von ungesättigten Fettsäuren den BMI 18 Monate nach einer Gewichtsreduktion beeinflussen. Weitere Analysen in Bezug auf den RQ und die FFA Spiegel bestätigen diese Daten. Wir konnten zeigen, dass der Gewichtserhalt mit einem erhöhten RQ und niedrigen FFA Spiegel korrelierten. Dies könnte auf eine erhöhte metabolische Flexibilität in Personen mit Gewichtserhalt hinweisen. Aufgrund dieser Daten spekulieren wir, dass eine niedrige Expression von Lipidsynthese-Genen im Fettgewebe zusammen mit einer erhöhten mitochondrialen Aktivität im Skeletmuskel einen positiven Einfluss auf einen langfristigen Gewichtserhalt besitzt.

3 INTRODUCTION

3.1 Obesity

Obesity and overweight have already reached an epidemic dimensions in many countries. In 2008 about 502 million people were considered obese and rates are still rising (Wang et al. 2011). According to the WHO obesity is defined by a body mass index (BMI) above 30 kg/m^2. Epidemiological analyses showed a preponderance of obesity rates in groups with a high socioeconomic status. By now, increasing levels are also visible in lower socioeconomic status groups including developing countries (Swinburn et al. 2011). It is hypothesized that the excessive supply of food accompanied by reducing level of physical activity is a major reason for the increase of obesity rates (Flier 2004). Inter-individual genetic differences could explain the susceptibility to environmental factors and why some individuals are prone to develop obesity. In general, obesity is caused by an imbalance between energy intake and expenditure, when more calories are consumed than utilized (De Ferranti and Mozaffarian 2008). This spare energy is stored in lipid droplets in adipose tissue. Adipocytes in overweight and obese patients show a higher rate of proliferation (hyperplasia) but also elevated cellular volume (hypertrophy). Changes in cellular function are associated with alterations in mitochondrial and ER metabolism not only leading to a different growth pattern, but also to insulin resistance, higher production of adipokines and increased release of free fatty acids (FFA) (De Ferranti and Mozaffarian 2008). Dysfunctions of adipocytes lead to systemic effects like accumulation of fatty acids (FA) in liver and other internal organs (visceral fat accumulation) (Després and Lemieux 2006). Frequently lipid accumulation in skeletal muscle is observed, which in turn leads to insulin insensitivity, dysfunction in endothelial cells, β-cells and liver cells (de Ferranti & Mozaffarian, 2008). Taken together, obesity is associated with increased higher risk of several diseases including cardiovascular diseases, type 2 diabetes (T2DM), airway diseases, other metabolic diseases and certain cancers (De Ferranti and Mozaffarian 2008; Després and Lemieux 2006; Després et al. 2008; Gregor and Hotamisligil 2011). Theses obesity-associated diseases are linked to a decreased life expectancy and premature death (Apostolopoulou et al. 2012).

3.2 Body Weight Regulation

Body weight regulation is comprised of complex networks of organ crosstalk and different signalling circuits. To maintain a stable body weight over a long time period, energy intake and expenditure need to be balanced. In humans, adipose tissue is thought to be highly important for this process as seen in studies with imbalanced energy intake and expenditure leading to an increase of adipose tissue (Wells and Siervo 2011; Jéquier and Tappy 1999). Regulatory processes

involve signals arising from adipose tissue acting on hypothalamus and the autonomic nervous system. There is a feedback loop for weight regulation in humans involving three major steps: 1) a sensor for energy levels, 2) hypothalamic centres which receive and integrate these signals through the action of leptin, 3) effector systems, influencing the two determinants of energy balance (intake and expenditure) (Jéquier and Tappy 1999).

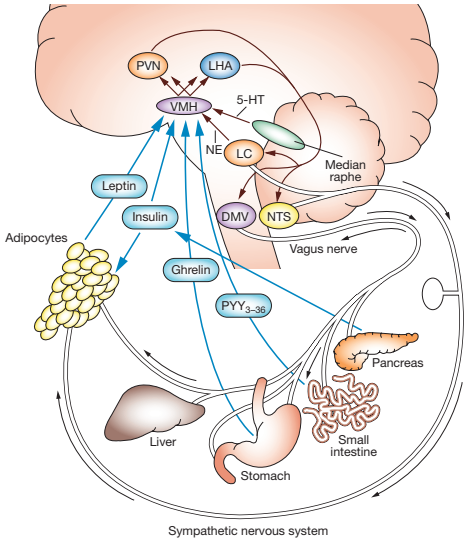

Figure 1: Model of regulatory pathways in body homeostasis. Central signals = brown, afferent signals = blue, efferent signals = white. 5-HT = serotonin (5-hydroxytryptamine), DMV = dorsal motor nucleus of the vagus, LC = locus coeruleus, LHA = lateral hypothalamic area, NE = norepinephrine, NTS = nucleus tractus solitaries, PVN = paraventricular nucleus, PYY3–36 = peptide YY3–36, VMH = ventromedial hypothalamus (Lustig 2006).

As also environmental aspects influence body weight, regulatory systems are more complex and need to adapt to changes in environment and behaviour. However, these processes seem to be highly individual and depending on genetic factors (Maclean et al. 2011).

The hypothalamic neurons are under control of afferent signals from adipose tissue, gastrointestinal system, liver and skeletal muscle. This includes neuronal and hormonal signals from different systems.

3.2.1 Hormonal Regulation

Body weight control comprise of a complex network of a variety of different hormones. This includes gut-derived hormones including ghrelin, peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), central-derived hormones like pro-opiomelanocortin (POMC), neuropeptide Y (NPY) and melanin-concentrating hormone (MCH) and pancreatic-derived ones including insulin and

pancreatic polypeptide (PP). This work will further concentrate on the adipose tissue-derived hormone leptin and the β-cell secreted insulin.

3.2.1.1 Leptin

Adipokines like leptin are involved in this signal cascade. Leptin was identified as the product of the obese gene (*ob*) in mice (Y. Zhang et al. 1994). Obese gene knockout mice (*ob/ob*) show alterations in the feeding behaviour (increased food intake, hyperphagia) and energy expenditure leading to obesity and insulin resistance. This outcome can completely be reversed by leptin application in *ob/ob* mice and in leptin deficient humans (I. Farooqi et al. 1999; I. S. Farooqi et al. 2002; Schwartz et al. 1996). Another obese mouse model harbouring a mutated leptin receptor (*db/db*) shows a similar phenotype (Coll, Farooqi, and O'Rahilly 2007; Zhou and Rui 2014). These data highlights the central role of leptin within the neuroendocrine regulation of energy metabolism.

Levels of secreted leptin are correlated with adipose tissue mass. Leptin is known to act in the hypothalamus where it regulates food intake by activating POMC-expressing neurons and inhibiting neurons expressing NPY. Thereby it activates catabolic pathways leading to reduced appetite and increased energy expenditure (Wisse 2004). Likewise, it directly acts on the skeletal muscle and affects insulin sensitivity by influencing glucose transporter class 4 (GLUT4) content and activity level of AKT, class 1A phosphaditylinositide 3-kinase (PI3K) and protein kinase C (PKC) (Yaspelkis et al. 2002; Yaspelkis et al. 2004). There are reports indicating that leptin prevents excess lipid accumulation by decreasing FFA uptake, increasing levels of lipolysis and FFA oxidation. This might also contribute to increased insulin sensitivity. However, the later one is discussed controversial (Havekes and Sauerwein 2010). Leptin directly affects adipose tissue insulin responsiveness and lipid metabolism. *In vivo* administration of leptin on adipocytes results in an increase of FA oxidation, most likely via activation of proliferator-activated receptor alpha (PPARα). On the contrary, it leads to inhibition of lipid synthesis (Harris 2014).

Interestingly, in obese patients high levels of plasma leptin are detectable in correlation with increasing fat mass, however these patients seem to show leptin resistance in the hypothalamus (Wisse 2004; Könner and Brüning 2012). In the periphery, leptin resistant adipocytes were also reported, concordant with a reduced expression of leptin receptor in morbidly obese women (Harris 2014).

3.2.1.2 Insulin

Besides leptin other hormones like insulin are involved in the regulation of body homeostasis. Insulin is a peptide hormone released form pancreatic β-cells in response to glucose. It plays an important role in regulation of blood glucose levels and lipolysis. It acts on the peripheral tissue

where it binds to its specific receptor and promotes glucose uptake by regulation of glucose transporters and specific enzymes of glucose oxidation.

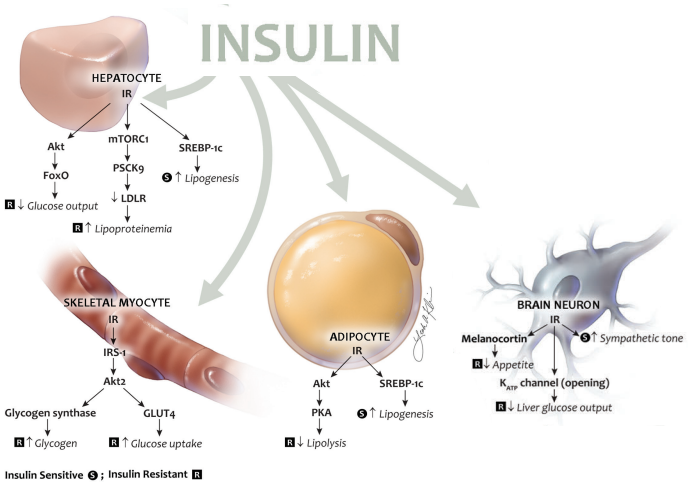

Figure 2: Overview of insulin signalling in hepatocyte, adipocyte, skeletal myocyte and neuron. AdipoIR, =insulin receptor, LDLR = low–density receptor, MHC = myosin heavy chain, mTORC1 = mammalian target of rapamycin complex 1, PCSK9 = proprotein convertase subtilisin/kexin type 9, PKA = protein kinase A, IRS-1 = insulin receptor substrate-1, SREBP-1c = sterol regulatory element binding protein-1c, FoxO = Forkhead box, class O (adapted from Rask-Madsen & Kahn, 2012).

Insulin suppresses hepatic glucose output by inhibiting gluconeogenesis and increasing glycogen synthesis (Figure 2). It is therefore one of the critical parts in whole-body glucose homeostasis (Taniguchi, Emanuelli, and Kahn 2006). The insulin receptor-signalling cascade consists of numerous downstream targets and is highly regulated. The best-studied down-stream targets are the insulin receptor substrates (IRS) and the following PI3K – AKT – mammalian target of rapamycin (mTOR) axis (Cheng, Tseng, and White 2010).

In addition insulin modifies central-nervous brain activity by suppression of appetite via stimulation of POMC-neuron activity (Könner and Brüning 2012). Moreover it leads to activation of the sympathetic nervous system (Taniguchi, Emanuelli, and Kahn 2006; Rask-Madsen and Kahn 2012).

Insulin resistance is defined by a decreased efficacy of insulin function. The cellular outcome is an alteration in insulin signalling like phosphorylation of the IRS protein family (Saltiel 2012). This results in hyperglycaemia and subsequently hyperinsulinaemia. Insulin resistance is associated with obesity and frequently observed in T2DM (Alemany 2011; Saltiel 2012). In an insulin resistant state, levels of blood glucose are high due to impaired uptake in metabolic active tissues, mainly liver and skeletal muscle. Furthermore hepatocytes show enhanced gluconeogenesis and glucose is shuttled into the blood stream. Similar observation can be made for lipogenesis. In adipose tissue impairment of insulin signalling leads to higher levels of triglyceride (TG) hydrolysis. Taken

together, insulin resistant subjects have elevated blood glucose, insulin, FFA and TG levels (Meshkani and Adeli 2009) as well as ectopic lipid accumulation in organs like liver and muscle tissue (Galgani, Moro, and Ravussin 2008).

3.3 Weight reduction

Weight reduction (WR) is known to have beneficial effects on the health status of obese subjects, including decreasing risks for different associated diseases like T2DM. It is associated with decrease of fat and to some extent also lean mass, increase of insulin sensitivity and aerobic capacity. It also has a positive effect on circulating markers of inflammation, showing lower values for CRP, IL-6, IL-8 and MCP-1 and simultaneously increasing concentration of adiponectin. Accordingly expression levels in adipose tissue show reduced values of IL-6, IL-8, TNF-α and increased levels of adiponectin. Adipose mRNA levels of macrophage markers CD68 and CD14, which are increased in obesity, are lower after WR. In contrast, mRNA of macrophage specific makers CD68 and CD14 are low in skeletal muscle and reveal no difference expression after WR. On cytokine level, predominantly IL-6 shows reduced levels in skeletal muscle after WR (Bruun 2006a).

Unfortunately the majority of subjects tends to regain weight after an intended WR and only around 20% are able to maintain the lost weight (Franz et al. 2007; Maclean et al. 2011). Even if successful WR shows a high variability, the amount of initial WR is one of the major determinants of long-term success (Neiberg et al. 2012). Therefore understanding mechanisms underlying the initial WR may also help to identify potential determinants of long-term success.

3.4 Adipose Tissue

Adipose tissue can be separated in several distinct fat depots (Figure 3). The subcutaneous and visceral fat depots are extensively studied in terms of metabolic syndrome (Muller 2011). About 80-90% fat is stored in the subcutaneous adipose tissue. Moreover, it is well known for its important role in whole-body homeostasis by secreting factors (Karastergiou et al. 2012; Wisse 2004). In several studies visceral adipose tissue was found to be linked to the metabolic syndrome (Wajchenburg 2014; Muller 2011; Després and Lemieux 2006). However, abdominal subcutaneous adipose tissue showed a high rate of postprandial FFA release in obese subjects. This might in turn lead to metabolic complications like ectopic lipid accumulation and hepatic insulin resistance (Z. Guo et al. 1999).

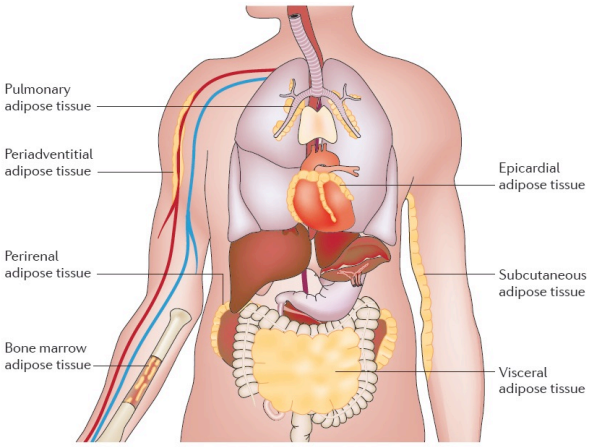

Figure 3: Adipose tissue depots in the human body (Ouchi et al. 2011).

Depot specific differences including visceral and subcutaneous as well as accumulation of abdominal fat seem to have a huge impact on the development of associated diseases (You et al. 2014; Després and Lemieux 2006). For example, visceral adipose tissue shows higher rates of lipolytic activity compared to subcutaneous depots. Moreover, due to its location, mobilisation of FFA will lead to a direct release into the portal vein and therefore has a direct effect on the liver (Wajchenburg 2014). Nevertheless several studies showed dysregulation in abdominal subcutaneous adipose tissue contributes to the development of obesity associated complications. This includes release of adipokines, lipid species and hyperplasia (Tchoukalova et al. 2008; Samaras et al. 2010; Gaidhu et al. 2010).

In general, adipose tissue consists of a complex mixture of cells: stroma vascular cells (SVCs), preadipocytes, adipocytes, fibroblasts, endothelial cells and macrophages. Adipocytes are the main fraction of cells in adipose tissue. They are specified to store and handle lipids. In these unique cells lipids are stored in one or a few large lipid droplets that almost fill the whole cell and therefore determine the cell size. In times of excessive nutrient supply, lipid storages and adipocyte size will increase. It is hypothesized that as long as adipose tissue can efficiently store the oversupply of lipids, non-adipose tissues like internal organs are not affected by lipid accumulation (Muller 2011). Thus impaired adipose tissue function may be detrimental for diminished function of these non-adipose tissues.

3.5 Skeletal Muscle Tissue

Muscle movement during exercise is a process which is highly energy consuming and a major contributor to energy homeostasis. Skeletal muscle tissue is the major insulin-sensitive organ; in fact 80-85% of insulin-stimulated glucose uptake takes place in muscle. Insulin resistance in

skeletal muscle is thereby highly relevant for the development of metabolic diseases like T2DM (Eckardt, Sell, and Eckel 2008; Havekes and Sauerwein 2010). Skeletal muscles consist of different fibre types, namely type I "slow-twitch" and type II "fast-twitch" fibres. They differ in expression of myosin heavy chain (MYH) protein isoforms, metabolic enzymes and show different contractile properties and endurance capacity. Furthermore, expression of the transcription factors myoD and myogenin are known to correlate with type I and II muscles. Whereas myoD is highly expressed in type II fibres, myogenin is prevalent in type I. Type I fibres are not only slow-twitch but also show higher insulin-sensitivity. In diabetic patients a switch between type I and type II (less oxidative) was observed together with down-regulation of myogenin, decreased succinate dehydrogenase activity and IL-6 levels (mainly expressed in type I fibres) (Schiaffino and Reggiani 2011; Eckardt, Sell, and Eckel 2008).

Recent research demonstrated secretory function of myocytes, which is also of interest regarding the development of insulin resistance and the metabolic syndrome. According to adipokines myocyte secreted factors are called "myokines". Like adipokines, they are important for the muscle-organ crosstalk and may modulate the lean-to-fat mass ratio. They included several different peptides like cytokines (interleukines 6, 7, 8 and 15; IL-6, IL-7, IL-8 and IL-15) or growth hormones like the growth differentiation factor 8 (GDF8) better known as myostatin (Trayhurn, Drevon, and Eckel 2011). Myostatin is involved in skeletal muscle development and is known to negatively regulate skeletal muscle mass. Additionally, it was found to be expressed in low levels in adipocytes where it induces adipogenesis and fat accumulation (Gonzalez-Cadavid and Bhasin 2004; T. Guo et al. 2009). Increased myostatin levels are observed in obese subjects and associated with insulin resistance. Myostatin inhibition or knockdown results in increased muscle mass, a reduction in fat mass and insulin resistance (C. Zhang et al. 2011; Lin et al. 2002).

Some known adipokines are also described to be myokines like IL-6, IL-8 and monocyte chemotactic protein (MCP) 1, which are involved in myogenesis, exercise response, inflammation and insulin sensitivity (Eckardt, Sell, and Eckel 2008).

Besides glucose, skeletal muscle uses FFA as fuel source. It is well known that muscle tissue can adapt to the availability of energy source by switching between carbohydrate and fat metabolism. This process is best described as the glucose FA cycle or Randle cycle (Kelley and Mandarin 2000). In obese and T2DM patients impaired FA metabolism was observed in skeletal muscle. This leads to a reduction in metabolic flexibility and lipid accumulation in skeletal muscle tissue (Blaak 2004; Holloway, Bonen, and Spriet 2009).

3.6 Muscle-Fat Crosstalk

Previous studies have demonstrated a tissue crosstalk between adipose tissue and skeletal muscle. In fact, a negative crosstalk between excess body fat and skeletal muscle can lead to disturbances in

insulin signalling and to insulin resistance (Sell, Eckel, and Dietze-Schroeder 2006; Eckardt, Sell, and Eckel 2008).

To mimic an *in vivo* situation or at least a co-culture condition it is possible to use adipocyte-conditioned medium, which contains virtually all secreted adipokines. Skeletal muscle cells cultured in adipocyte-conditioned medium show a similar expression patter as in a direct co-culture model, which was also observed in muscle cells from T2DM patients (Eckardt, Sell, and Eckel 2008). Using this model, a study from Kovalik et al. showed that myocytes have a switch in substrate preference from glucose to FA. This was also observed in muscle cells from obese subjects. However, accumulation of lipid intermediates indicates a limited metabolic flux in obese muscle cells. In the myocytes elevated mRNA levels of pyruvate dehydrogenase kinase 4 (PDK4), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), very long-chain acyl-CoA dehydrogenase (VLCAD), and adipose triglyceride lipase (ATGL) were detected, all promoting FA catabolism. The strongest effect was observed using lipolytically active adipocytes. This indicates an underlying role of FA and their metabolites in altering muscle expression profiles towards oxidation acting on the PPAR transcription factor family (Kovalik et al. 2011).

3.7 Metabolic flexibility

For living organisms/systems (e.g. cells, whole organisms, organs) the availability for a potential adaptation to different fuel is important. The term "metabolic flexibility" was defined by Kelly and Mandarino as "the capacity to switch from predominantly lipid oxidation and high rates of FA uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions" (Kelley and Mandarin 2000). In fed state insulin secretion is triggered by glucose and leads to anabolic action including glucose uptake in muscle tissue and suppression of lipolysis in adipocytes. In liver, glucose and its metabolites are converted by *de novo* lipogenesis to FA, which is shuttled to white adipose tissue for storage. Vice versa fasting results in a drop of blood glucose and insulin leading to activation of lipolysis in white adipocytes and release of FA (Liu, Alexander, and Lee 2014). In obese subjects a less flexible and diminished efficiency to switch between glucose and lipid substrates has been reported (Liu, Alexander, and Lee 2014; Wells and Siervo 2011).

Respiratory quotient (RQ) is regarded as a surrogate of fuel oxidation and hereby the metabolic flexibility. A drop in RQ depicts a switch from carbohydrate to lipid oxidation for example after an overnight fast. In obese and diabetic subjects an elevated fasting RQ is observed, indication inflexibility to switch to lipid oxidation during fasting (Galgani, Moro, and Ravussin 2008). The underlining mechanisms are not yet understood.

3.8 Cellular energy metabolism

Whole-body metabolism is regulated by the interplay of different organs and their specialised cells. For most cells adenosine triphosphate (ATP) is the primary energy source driving all cellular processes. ATP generation mainly takes place in the mitochondria by the ATP synthase as part of the oxidative phosphorylation. Therefore, preserving a constant ATP concentration is highly important for survival. This includes regulation of ATP producing and consuming processes. Consuming processes lead to a decrease in ATP and an increase in adenosine di-/monophosphate (ADP/AMP). On cellular level ADP/ATP and AMP/ATP ratios are monitored by AMP-activated protein kinase (AMPK) (Hardie, Ross, and Hawley 2012).

To maintain a certain level of energy cells need to take up nutrients and convert them into energy. Main metabolites are glucose, lipids and amino acids, which can be taken up by cells and shuttled into different metabolic pathways (Buchakjian and Kornbluth 2010).

All metabolic networks are connected and influence each other's activity. Carbohydrate and lipid pathways share intermediates that either can be metabolised or act as inhibitor. For example acetyl-CoA, produced by the glycolysis, can be used by lipogenesis to build up FA or by the tricarboxylic acid cycle (TCA) for ATP production (Figure 4).

3.8.1 Carbohydrate metabolism

Glucose is an important energy source for the human body as some tissues including the brain and red blood cells show an obligatory need for it (Turner et al. 2014). Glucose enters the cells via glucose transporters (GLUT), which exhibit a family of 14 different transporters. GLUT proteins are expressed tissue-dependent. GLUT4, GLUT10 and GLUT11 are transporters for glucose, fructose and galactose expressed in muscle tissue. In adipose tissue GLUT4 and GLUT13 are expressed (Mueckler and Thorens 2013). The main glucose transporter in skeletal muscle and adipose tissue is the insulin-dependent GLUT4. Under high levels of insulin GLUT4 is translocated to the plasma membrane and glucose is taken up as described above (Mueckler and Thorens 2013; Saltiel 2012). In liver the main transporter for carbohydrate substrates is GLUT2, which works insulin-independent and has a low affinity to glucose.

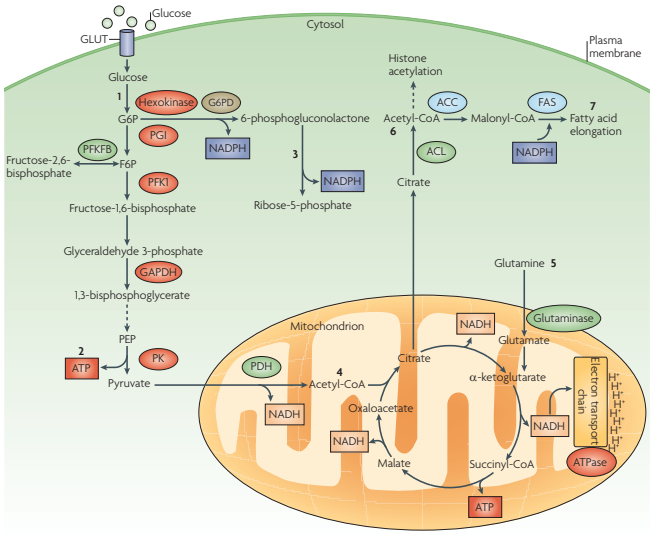

Figure 4: Schematic overview of central metabolic processes. GAPDH = glyceraldehyde-3-phosphate dehydrogenase, GLUT = glucose transporter, F6P = fructose-6-phosphate, FAS, =FA synthase, PDH =pyruvate dehydrogenase, PEP = phosphoenolpyruvate, PFK1 = phosphofructokinase 1, PFKFB = 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, PGI = phosphoglucose isomerase, PK = pyruvate kinase, G6P = Glucose-6-phosphate, G6PD = G6P dehydrogenase, ACL = ATP citrate lyase, ACC = acetyl-CoA carboxylase, NADH/NADPH = nicotineamide adenine dinucleotide/phosphate (Buchakjian and Kornbluth 2010).

This low affinity is important for glucose uptake even under low plasma glucose and high intracellular glucose levels (Mueckler and Thorens 2013). Intracellular glucose can be shuttled into different metabolic processes, energy generation or storage in form of glycogen. Energy producing processes include glycolysis, in which pyruvate and ATP is produced, followed by the TCA, where the pyruvate is used to produce ATP and NADH. In the pentose phosphate pathways ribose-5-phosphate and NADPH is produced, which can be used for nucleotide synthesis and as coenzyme in other metabolic processes (Buchakjian and Kornbluth 2010).

3.8.2 Lipid metabolism

Adipose tissue is the main storage side for lipids in form of TG. They are stored in big lipid droplets, which represent a large area in mature adipocyte. TG are generated in an enzymatic process called lipogenesis. Acyl groups either produced from FA or glucose are re-esterified with glycerol. Different enzymes including acetyl-CoA synthases, FA synthases, FA elongases and FA desaturases play an important role in this process (Sethi and Vidal-Puig 2007a; Sethi and Vidal-Puig 2007b).

In times of nutrient deprivation TG are stepwise hydrolysed by three enzymes, leading to release of FFA and glycerol (Lafontan and Langin 2009). Lipolysis starts with ATGL breaking TG into diacylglycerides, which are further processed into monoacylglycerides by hormone sensitive lipase (HSL). The last step is performed by the monacylglycerol lipase (MGL) producing free FA and

glycerol. Lipolysis is highly controlled by different hormonal pathways (Figure 5) (Lafontan and Langin 2009; Langin 2006; Zechner et al. 2012).

Insulin leads to inhibition of lipolysis via decreasing levels of cAMP via PDE-3B activation. This is followed by deactivation of protein kinase A (PKA) and thus a lower phosphorylation state of HSL and its inhibition (Saltiel and Kahn 2001).

Catecholamines like adrenalin and noradrenalin act via alpha- and beta-adrenoreceptors to either activate or inhibit lipolytic enzymes. Beta-adrenoreceptor activation leads to increasing cAMP concentrations, activation of PKA and HSL. In contrast, alpha-adrenoreceptor stimulation leads to inhibition of lipolysis by decreases in cAMP. In general, a higher amount of alpha-adrenoreceptors is found in subcutaneous adipose tissue and may therefore explain the lower lipolytic potential (Langin 2006).

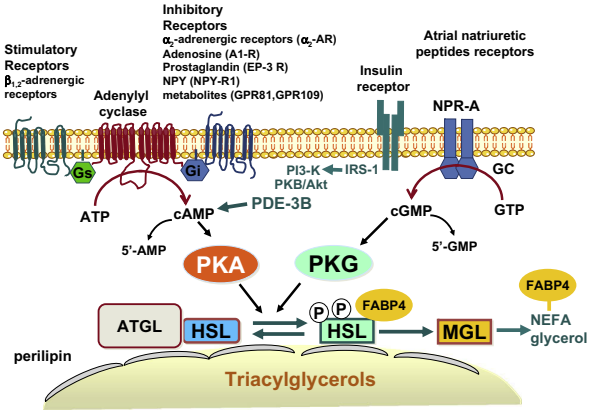

Figure 5: Lipolysis regulation in white adipocytes. ATGL = adipose triglyceride lipase, FABP4 = adipocyte FA binding protein 4, GC = guanylyl cyclase; Gi, inhibitory GTP-binding protein, Gs = stimulatory GTP-binding protein, HSL = hormone-sensitive lipase, MGL = monoacylglycerol lipase, NEFA = nonesterified FA, NPR-A = type A natriuretic peptide receptor (Lafontan and Langin 2009).

Protein kinase G (PKG) can be activated by natriuretic peptides (ANP) via the natriuretic receptor A (NPRA). Activation of NPRA leads to increasing levels of cGMP and therefore activation of PKG. HSL is known to be activated by phosphorylation of PKA as well as PKG. Hence, ANP leads to activation of HSL and increasing levels of lipolysis independent of adrenergic and insulin stimulation (Langin 2006).

3.8.2.1 Free fatty acids

FA can be classified into different groups due to the their carbon chain length; short-chain FA (2-6 carbon atoms), medium-chain FA (8-12 carbon atoms), long-chain FA (14-18 carbon atoms) and very long-chain FA (20-26 carbon atoms). In the circulation, the most abundant FA species are long

and very long-chain FA. They differ not only in chain length but in the degree of saturation (double bond) and include palmitic acid (C16:0), palmitoylic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) (Turner et al. 2014).

FFA are mobilised from adipocytes and shuttled to effector organs like skeletal muscle. Muscle cells highly depend on FA oxidation for energy regeneration, especially under exercise and after a prolonged period of fasting. FFA are taken up from the circulation, prepared for mitochondrial transport and undergo β-oxidation. Increasing levels of β-oxidation intermediates like acetyl-CoA will finally result in inhibition of glycolysis (Jeukendrup 2002).

3.8.2.2 Acylcarnitines

Long-chain FA are transported via the carnitine shuttle into the mitochondria. In the cytoplasm long-chain acyls are esterified to CoA for the transport process. The carnitine palmitoyl transferase 1 (CPT1) exchanges the CoA for carnitine resulting in acylcarnitine (AC), which are then transported by carnitine acylcarnitine translocase (CACT) into the mitochondrial matrix. In the matrix CPT2 converts AC back into free carnitine and long-chain acyl-CoAs which than enter β-oxidation (Schooneman et al. 2013).

AC can be measured in the plasma, indicating a potential transport mechanism into the circulation. They are markers for mitochondrial oxidative capacity (M. Mai et al. 2013) and plasma levels can be linked to defects in FA oxidation (Schooneman et al. 2013). More recently several studies linked elevated AC levels to metabolic diseases including T2DM (Adams et al. 2009; M. Mai et al. 2013; Koves et al. 2008).

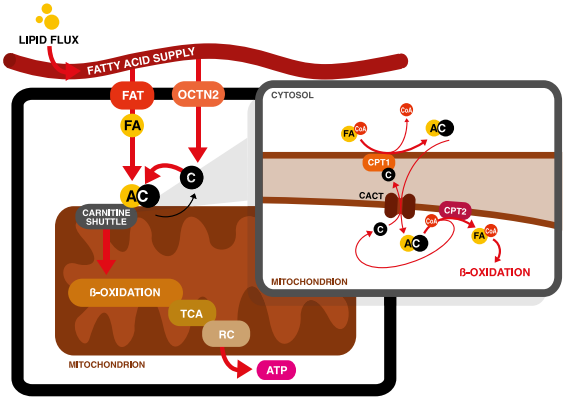

Figure 6: FA transport via the carnitine shuttle. FA are transported into the cell by FA transporters (FAT/OCTN2) and esterified to CoA. CPT1 exchanges the CoA moiety for carnitine (C). CACT transports the acylcarnitine (AC) into the mitochondrial matrix where CPT2 reconverts AC to acyl-CoA and carnitine. The acyl-CoA is used for β-oxidation and production of ATP by the respiratory chain (RC) (Schooneman et al. 2013).

3.9 Expression analysis in obesity

After using gene wide association studies (GWAS) to identify certain genes involved in obesity or the metabolic syndrome, researchers started to investigate expressional differences linked to disease models. The aim is to identify potential candidates predicting the outcome of either a disease or an intervention/treatment (Riedmaier and Pfaffl 2013). Concentrating on metabolic disease several studies investigated the effect of WR and low caloric diet on expressional changes (Mutch et al. 2010; Marquez-Quinones et al. 2010; Mutch et al. 2007; Grant et al. 2013). Several of these data showed clear effects of WR on the adipose tissue transcriptome and stated the potential to predict the outcome of WR using transcriptomic data (Mutch et al. 2010; Mutch et al. 2007). Different method can be used to analyse the expression profile and most studies are using microarrays. However, this work will apply the more advanced and sophisticated RNA sequencing.

3.9.1 RNA Sequencing (RNA-Seq)

Sequencing is a well-established method used for genomic sequencing or epigenomic studies. Transcriptome analyses are mainly done using microarrays. However, it is not possible to detect new transcripts with this method or investigate expression pattern without a known genome (Costa et al. 2010). Therefore, the development of a new sequencing technique, in which RNA is used as template, led to improvements in transcriptomic analysis. So called RNA sequencing has some advantage compared to microarrays; 1) potentially unlimited dynamic range of expression, 2) increased sensitivity, 3) improved possibility to discriminate regions of high sequence identity, 4) unbiased approach and therefore profiling transcription without prior assumption of which regions might be expressed (Cloonan et al. 2009).

RNA-Seq starts with a cDNA library consisting of small mRNA fragments (lengths of about 100 base pairs). These fragments are ligated to adaptors and sequenced (Marguerat and Bähler 2010).

However, sequencing the mammalian transcriptome bears some technical challenges. Due to intron splicing transcripts are not contiguous. Especially near exon-exon boundaries there are tags problematic to map directly to the genome due to missing introns. Repetitive sequences are also challenging, because they are not unique and can be located to several different sequences. Analysis of large and complex genomes as the mouse or human one therefore requires considerable computational resources (Mortazavi et al. 2008).

As mentioned before, RNA-Seq reads are small and large in number; a fact leading to several new approaches for mapping these reads to the genome (Garber et al. 2011). There are different algorithms with distinctive functions like assigning reads that map uniquely to sites of genome origin, assigning multireads (match equally well to several sites) to their most likely site or detection of splice-crossing sites (Mortazavi et al. 2008). Final data evaluation includes investigation of differentially expressed genes, alternative exon usage, single nucleotide

polymorphisms (SNP) and pathway analysis (Eswaran et al. 2012; Sultan et al. 2008; Cloonan et al. 2008).

4 AIM

Adipose and skeletal muscle tissue have a substantial impact on substrate storage, utilisation and energy expenditure. All these processes are involved in body weight regulation and maintenance. In obesity several hormonal and cellular circuits appear to be dysregulated finally leading to metabolic diseases.

In this work, a randomized controlled human trial was performed to study hormonal regulators of body weight maintenance after intended WR. Genome-wide gene expression of adipose and skeletal muscle tissue was investigated to better understand WR induced effects on cellular metabolic networks and to investigate whether specific molecular pathways predict body weight maintenance. To address this, obese subjects, who successfully underwent a WR, were randomized into a control group and a group receiving a multimodal life-style intervention. This clinical trail focused on effects of WR and the prediction of long-term weight maintenance. Beside a comprehensive phenotypic approach, adipose and skeletal muscle tissue biopsies were taken and the gene expression pattern was analysed by RNA-Seq. The main hypothesis was that alteration in the transcriptomic landscape of adipose and skeletal muscle tissue, induced by WR, may affect long-term body weight control.

The main questions of this work were:

1) Which genes and pathways are linked to parameters of body composition and metabolism in obese subjects?
2) How does WR affect the transcriptome of adipose and skeletal muscle tissue?
3) Can the gene expression pattern after WR predict long-term weight maintenance?

5 RESULTS

5.1 Quality control of RNA-Seq data

Adipose and skeletal muscle biopsies were taken before and after WR. Tissue pieces were processed, deep RNA-sequencing was performed and reads were aligned to the human genome (7.2.4).

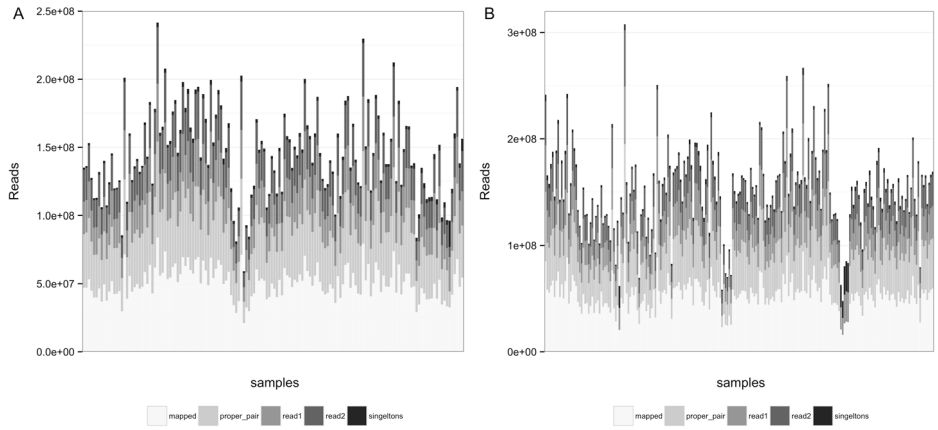

Figure 7: Histogram of tophat2 output. **A** Total reads from adipose tissue samples. **B** Total reads from skeletal muscle tissue samples. mapped = reads mapped to potential genomic side, proper_paired = pairs of read 1 and read 2 mapped correctly together, read 1/read 2 = both reads of a paired-end sequencing analysis, singletons = reads without a mate-pair.

We checked the alignment quality of the tophat2 output by plotting the mapped and proper-paired reads, total read1 and read2 and the singletons (Figure 7).

Samples with read depth below $20x10^7$ and a high number of singletons were filtered out. This led to a final sample number of 150 in adipose and 174 in skeletal muscle tissue. For further analysis only proper-paired reads were used.

Counts per exon were extracted using the HTSeq algorithm, resulting in datasets with over 15,000 genes. We further reduced the list by filtering out genes with a mean count below 20 between all subjects.

Data was normalized for library size and dispersion was estimated using the DESeq2 package. The effect of the normalization is clearly visible, as data without normalization shows a high variance between samples. This variation is depicted in Figure 8 as variance of the mean (log conversion).

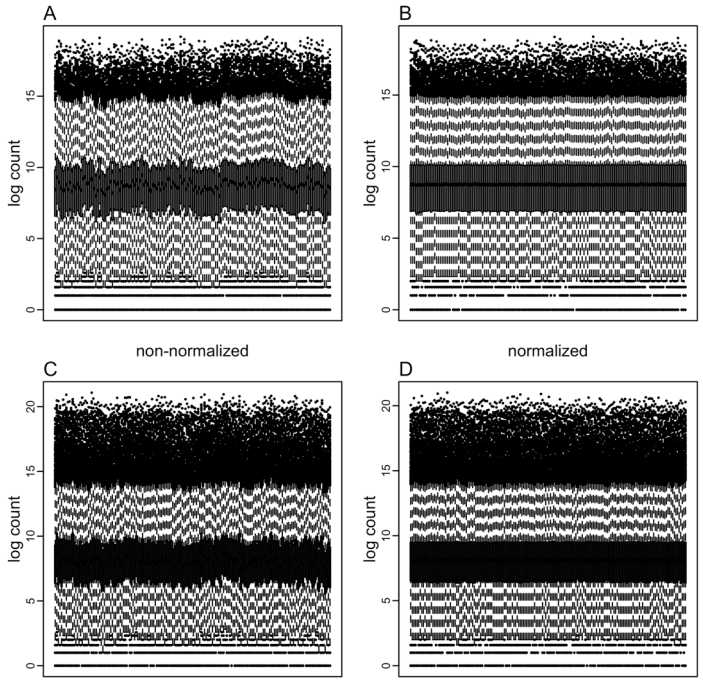

Figure 8: Count data before and after normalization for library size and dispersion of all samples used for further analysis. **A** Non-normalized count data from adipose tissue samples. **B** Normalized count data from adipose tissue samples. **C** Non-normalized count data from skeletal muscle tissue samples. **D** Normalized count data from skeletal muscle tissue samples, n= 150 for adipose tissue, n = 174 for skeletal muscle tissue.

We used the *plotDispEsts* function embedded in the DESeq2 package to plot the intra-sample dispersion (Figure 9). The dispersion shows the variability between all samples and therefore between the individuals. It is anti-proportional to the read count length giving a lower dispersion in longer reads. The algorithm uses the dispersion to calculate fitted values. Therefore, all values below the threshold (Figure 9, red line) are moved towards the red line. A higher dispersion between samples will result in less significant hits, because larger differences between samples would be needed. As seen in Figure 9 the dispersion in both tissue is comparable. However in terms of genes with a higher count, the dispersion in adipose tissue might be slightly higher (Figure 9 A).

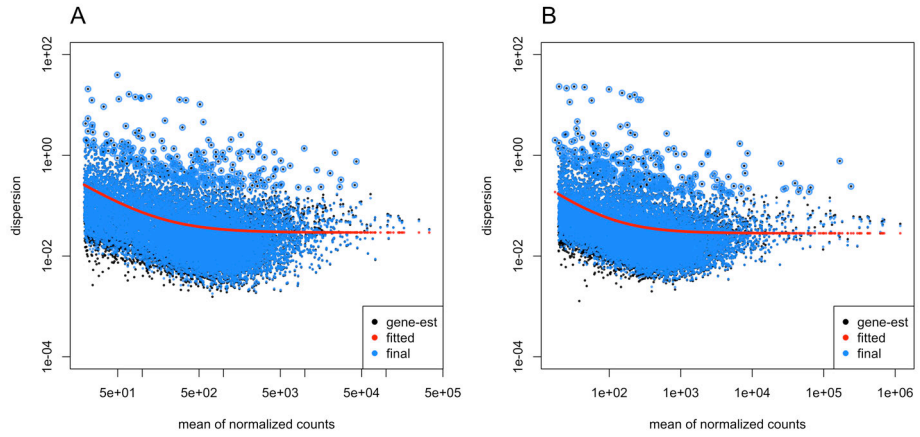

Figure 9: Smearplots show the dispersion between all samples used in further analysis. **A** Dispersion between 150 adipose tissue samples. **B** Dispersion between 174 skeletal muscle tissue samples. Gene-est = gene-wise dispersion estimates (per gene); fitted = distribution of gene-est; final = final dispersion values.

To further control the quality of the RNA-Seq data we used quantitative real-time PCR (qPCR) for a selected number of genes. The majority of the measured genes showed similar expression changes in both methods. However, the qPCR measurements showed a higher variability (Figure 10).

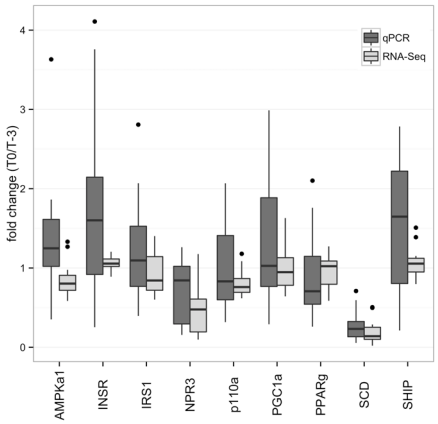

Figure 10: Boxplot showing the expression of target genes measured in a qPCR and RNA-Seq approach. Expression is depicted as fold change over the WR phase.

5.1.1 Principle component analysis (PCA) of count data

To investigate potential clusters in the expression data, we used PCA as an unbiased approach. As expected expression pattern could be clearly separated by gender in muscle and adipose tissue (Figure 11 A and B). This was not possible using age as factor (data not shown).

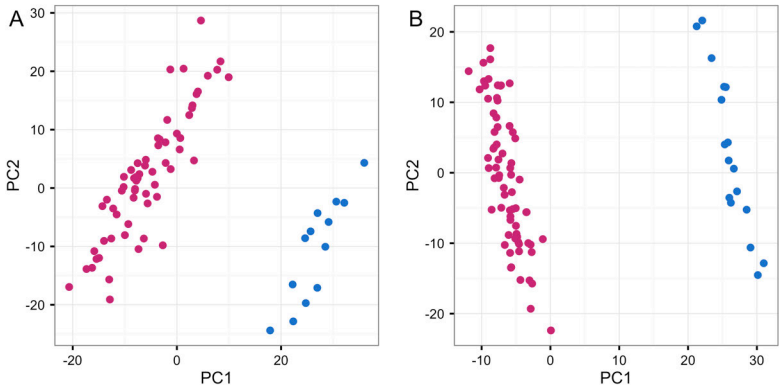

Figure 11: First two principle components (PC) of a PCA using the normalized expression dataset of adipose and skeletal muscle tissue before WR. Colours indicate the gender, purple = females, blue = males. Separation of subjects in **A** adipose tissue, **B** skeletal muscle tissue.

5.2 Before weight reduction

5.2.1 Phenotypic parameters

223 obese subjects were initially screened for participation. 156 underwent a 3-month WR by a very low caloric diet. In total 143 showed a successful WR of 8% and were included into the study. Anthropometric and metabolic parameters at baseline are listed in Table 1.

Table 1: Phenotypic parameters of 143 participants before WR.

	Before WR		
	Mean	±	SD
Age [yr]	50.55	±	12.59
Females	122		
BMI [kg/m]	37.35	±	6.11
Waist circumference	108.16	±	13.21
Waist-to-hip ratio	0.89	±	0.09
Total body fat	41.04	±	3.14
Lean body mass	64.08	±	17.68
ISI_{clamp}	0.06	±	0.03
HOMA-IR	2.88	±	2.65
fasting glucose [mg/dl]	90.86	±	24.77
120 min-glucose [mg/dl]	129.24	±	49.95
FFA [mmol/l]	0.61	±	0.23
TG [mg/dl]	141.98	±	108.32
RQ	0.81	±	0.06

BMI = Body mass index. Results are depicted as mean ± SD.

Significant differences between male and female subjects were observed. In general, males had a lower TBF (females = 41.94 kg vs. males = 38.96 kg, $p < 0.001$) and a higher waist-to-hip ratio (WHR) (females = 0.84 vs. males = 1.02, $p < 0.001$).

5.2.2 Differentially expressed genes between male and female subjects before weight reduction

Differentially regulated genes between males and females could by clustered into functional terms using DAVID (Huang, Lempicki, and Sherman 2009). In adipose tissues the gender-specific differences in gene expression seemed to be stronger as a higher amount of differentially expressed genes could be detected in adipose tissue (see Table 2).

Table 2: Functional cluster analyses of differentially expressed genes in adipose and skeletal muscle tissue between male and female subjects.

Tissue	DE genes	Cluster
Adipose tissue	1359	40
Skeletal muscle tissue	275	4

Number of cluster and respectively involved differently expressed genes (before WR) are listed.

Interestingly, genes differentially expressed between male and female subjects were highly connected to each other in adipose tissue (Figure 12A).

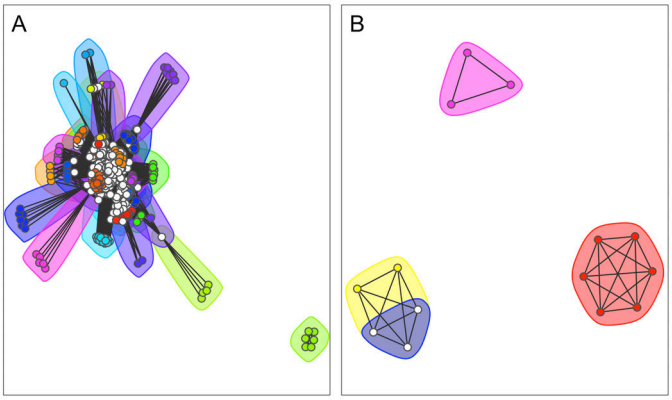

Figure 12: Functional networks of differentially expressed genes comparing males vs. females. Colours indicate different functional clusters. Circles represent transcripts; black lines are the edges between single transcripts and depict connections between functional clusters and transcripts. Clusters of altered transcripts compared **A** in adipose tissue and **B** in skeletal muscle tissue.

Only a small amount of genes could be mapped to a single cluster (depicted in green in Figure 12A). Genes showing a different expression pattern between males and females in skeletal muscle tissue were not connected and seemed to be mapped to more distinct pathways (Figure 12B).

To get an in-depth view of involved genes, we analysed their function based on GO-terms, Swiss-Prot/UniProt protein knowledgebase as well as KEGG derived keywords using DAVID.

Above identified genes could be grouped into several clusters including metabolic relevant processes like TCA cycle, TG synthesis and FA β-oxidation (Table 3).

Table 3: Metabolic relevant functional categories of identified genes differentially expressed between males and females in adipose tissue before WR.

Category	Term	Number of genes
SP_PIR_KEYWORDS	mitochondrion	94
KEGG_PATHWAY	valine, leucine and isoleucine degradation	15
KEGG_PATHWAY	citrate cycle (TCA cycle)	11
GOTERM_BP_FAT	triglyceride biosynthetic process	6
COG_ONTOLOGY	lipid metabolism	15
GOTERM_BP_FAT	glucose metabolic process	22
GOTERM_BP_FAT	fatty acid β-oxidation	8
GOTERM_BP_FAT	response to steroid hormone stimulus	24
GOTERM_BP_FAT	steroid hormone receptor signalling pathway	10
KEGG_PATHWAY	PPAR signalling pathway	12

Number of genes indicates the number of genes differentially expressed between males and females and involved in this pathway. SP_PIR = protein information resource, BP = biological process.

In detail, genes involved in TCA cycle, FA β-oxidation and the PPAR signalling pathway were higher expressed in women (Table 4). Similar results could be found for the steroid hormone receptor signalling pathway and the TG biosynthetic process (Table 4). In contrast, we observed a higher number of glucose metabolic genes to be lower expressed in women (Table 4). Nevertheless, the majority of involved glucose metabolic genes seem to be higher expressed in women.

Table 4: Metabolic relevant functional categories with regard to up- and down-regulation of included genes in women.

Term	Included Genes	Higher expressed in women	Lower expressed in women
citrate cycle (TCA cycle)	11	11	-
glucose metabolic process	22	16	6
fatty acid β-oxidation	8	8	-
steroid hormone receptor signalling pathway	10	8	2
PPAR signalling pathway	12	12	-
triglyceride biosynthetic process	6	5	1

In contrast, differentially expressed genes in skeletal muscle do not show any metabolic clusters.

5.2.3 Differentially expressed genes between old and young subjects before weight reduction

Next we investigated potential differences between young and old subjects. We used a similar approach as in 5.2.2.

Differences in expression profiles between old and young subjects were less prominent in adipose tissue, whereas skeletal muscle tissue demonstrated stronger age dependent differences (Table 5 and Figure 13).

Table 5: Functional cluster analyses of differentially expressed genes in adipose and skeletal muscle tissue between old and young subjects.

Tissue	DE genes	Cluster
Adipose tissue	6	0
Skeletal muscle tissue	957	28

Number of clusters and respectively involved differently expressed genes (before WR) are listed.

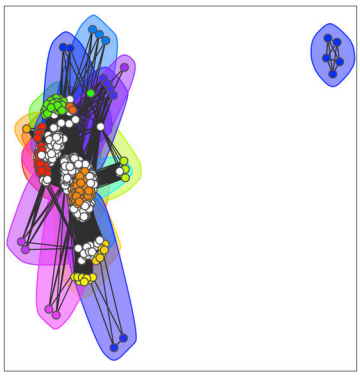

Figure 13: Functional networks of differentially expressed genes in skeletal muscle tissue comparing old vs. young subjects. Colours indicate different functional clusters. Circles represent transcripts; black lines are the edges between single transcripts and depict connections between functional clusters and transcripts.

Table 6: Metabolic relevant functional categories of identified genes differentially expressed between old and young subjects in adipose tissue before WR.

Category	Term	Number of genes
SP_PIR_KEYWORDS	mitochondrion	144
KEGG_PATHWAY	oxidative phosphorylation	45
KEGG_PATHWAY	cardiac muscle contraction	17
KEGG_PATHWAY	citrate cycle (TCA cycle)	9
GOTERM_BP_FAT	pyruvate metabolic process	9
GOTERM_BP_FAT	glucose catabolic process	9

Number of genes indicate the number of differentially expressed genes between young and old and involved in this pathway. SP_PIR = protein information resource, BP = biological process.

In-depth analysis revealed clusters of mitochondrial genes, including genes involved in oxidative phosphorylation and TCA cycle (Table 6).

The majority of identified metabolic clusters showed a lower expression in old subjects (Table 7).

Table 7: Metabolic relevant functional categories with regard to up- and down-regulation of included genes in old subjects.

Term	Included Genes	Higher expressed in old subjects	Lower expressed in old subjects
oxidative phosphorylation	45	-	45
cardiac muscle contraction	17	2	15
citrate cycle (TCA cycle)	9	-	9
glucose catabolic process	9	2	7

5.2.4 Functional networks and anthropometric parameters

Further, we analysed associations between adipose and muscle gene expression and phenotypic parameters, at baseline. Looking at correlations before WR might give an idea of phenotype-gene associations in obese subjects.

The data was first analysed at baseline to assess associations of gene expression and obesity (BMI).

5.2.4.1 Anthropometric parameters – before weight reduction

Adipose and skeletal muscle tissue are known to have an important role in body weight regulation. In both tissues, we found several genes associated with the BMI. Functional annotation analysis of the associated genes using DAVID revealed several functional process including metabolic pathways.

Table 8: Summary of functional annotation analysis of identified genes correlating with BMI in adipose tissue using DAVID.

Category	Term	Number of genes
GOTERM_BP_FAT	inflammatory response	64
SP_PIR_KEYWORDS	immune response	45
GOTERM_BP_FAT	response to insulin stimulus	26
KEGG_PATHWAY	iInsulin signalling pathway	33
GOTERM_BP_FAT	fatty acid metabolic process	39
GOTERM_MF_FAT	lipid binding	73
GOTERM_BP_FAT	response to estrogen stimulus	23
KEGG_PATHWAY	adipocytokine signalling pathway	16

Terms were selected according to possible metabolic relevance. Number of genes indicates the number of genes correlating with BMI and involved in this pathway. SP_PIR = protein information resource, BP = biological process, MF = molecular function.

Basal association between gene expression and phenotype in adipose tissue included several transcripts involved in insulin signalling pathways, lipid metabolism as well as inflammatory response (Table 8).

Associations of basal gene expression in skeletal muscle and BMI revealed a number of genes involved in mitochondrial function (Table 9).
We found clusters of mitochondrial genes, oxidative phosphorylation as well as genes coding for muscle proteins and insulin signalling pathway.

Table 9: Summary of functional annotation analysis of identified genes correlating with BMI in skeletal muscle tissue using DAVID.

Category	Term	Number of genes
GOTERM_CC_FAT	mitochondrion	161
GOTERM_CC_FAT	mitochondrial envelope	75
GOTERM_CC_FAT	mitochondrial inner membrane	60
GOTERM_CC_FAT	mitochondrial matrix	39
SP_PIR_KEYWORDS	muscle protein	17
SP_PIR_KEYWORDS	oxidative phosphorylation	13
KEGG_PATHWAY	insulin signalling pathway	24
KEGG_PATHWAY	pyruvate metabolism	11
GOTERM_BP_FAT	glucose catabolic process	10

Terms were selected according to possible metabolic relevance. Number of genes indicates the number of genes correlating with BMI and involved in this pathway. SP_PIR = protein information resource, BP = biological process, CC = cellular compartment.

To further investigate associations between gene expression and phenotypic parameters we repeated this analysis with waist circumference and TBF. In general we found similar results as shown here using the BMI (data not shown). This further supports our results showing gene-phenotype associations.

5.2.5 Functional networks and lipid metabolic parameters

As our results particularly supported an association of lipid metabolism and estimates of obesity, we further analysed the relationship to lipid metabolites and substrate utilization (either fat or carbohydrates). Latter can be assessed by respiratory quotient (RQ).

5.2.5.1 Lipid metabolic parameters – before weight reduction

FFA are released from adipocytes and can be used as energy source of metabolic active tissue like skeletal muscle. We investigated associations between FFA levels and gene expression in adipose tissue to look for a possible link to higher metabolic active tissue.
Associations of FFA and gene expression before WR in adipose tissue showed a cluster involved in lipid transport (data not shown). However, our analysis revealed no additional lipid metabolic genes in adipose tissue correlating with circulating FFA.

As FFA are metabolized in skeletal muscle we looked for associations of skeletal muscle gene expression and circulating FFA. Functional annotation analysis of muscular transcripts correlating with FFA using DAVID resulted in several clusters. Two clusters involved in lipid and NADP metabolism were of specific interest (Table 10).

Table 10: Summary of functional annotation analysis of identified genes correlating with FFA in skeletal muscle tissue using DAVID.

Category	Term	Number of genes
SP_PIR_KEYWORDS	NADP	15
GOTERM_BP_FAT	lipid biosynthetic process	18
GOTERM_BP_FAT	enzyme linked receptor protein signalling pathway	18

Terms were selected according to possible metabolic relevance. Number of genes indicates the number of genes correlating with FFA and involved in this pathway. SP_PIR = protein information resource, BP = biological process.

The RQ is a variable indicating the preference in energy source usage. Healthy, non-obese subjects are able to switch between energy sources, e.g. changing to lipid oxidation after fasting (drop in RQ). As it was shown that obese subjects have a higher fasting RQ (Galgani, Moro, and Ravussin 2008), we investigated possible associations between gene expression and RQ.

Table 11: Summary of functional annotation analysis of identified genes correlating with RQ in adipose tissue using DAVID.

Category	Term	Number of genes
SP_PIR_KEYWORDS	mitochondrion	190
GOTERM_CC_FAT	mitochondrial inner membrane	97
KEGG_PATHWAY	Oxidative phosphorylation	55
GOTERM_CC_FAT	mitochondrial matrix	52
GOTERM_BP_FAT	glucose metabolic process	26
GOTERM_BP_FAT	lipid catabolic process	26
GOTERM_BP_FAT	lipid biosynthetic process	41
GOTERM_BP_FAT	fatty acid metabolic process	24

Terms were selected according to possible metabolic relevance. Number of genes indicates the number of genes correlating with RQ and involved in this pathway. SP_PIR = protein information resource, BP = biological process, CC = cellular compartment.

We found several mitochondrial genes associated with RQ before WR in adipose tissue (Table 11). However, we did not reveal an association to metabolic involved transcripts. We found the majority of transcripts associated with RQ in muscle tissue to be involved in transcription, transcriptional regulation or apoptosis (data not shown).

5.3 Weight reduction phase

The following sections will concentrate on the 3-month weight reduction phase.

An overview of the study design is depicted in Figure 14.

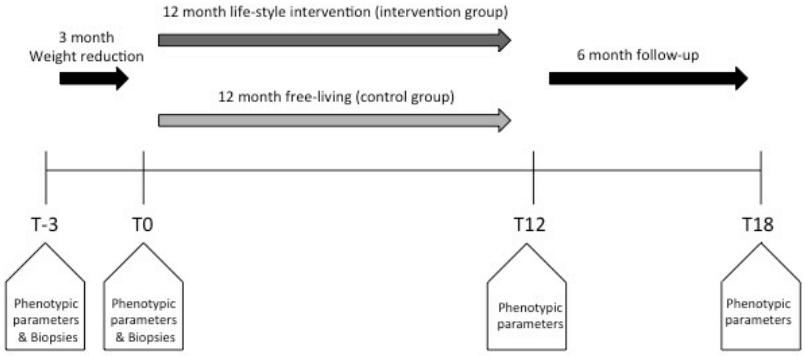

Figure 14: Schematic overview of the study design.

5.3.1 Phenotypic changes over the 3-month weight reduction phase

As described in section 5.2.1 in total 143 individuals were randomized into the intervention or control group and could be included in the subsequent analysis.

Metabolic and anthropometric parameters of these randomized participants before and after WR period are presented in Table 12.

Table 12: Phenotypic parameters of 143 participants in the WR trail. Results are shown before and after 3-month weigh reduction phase.

	before WR		after WR		
	Mean	± SD	Mean	± SD	
BMI [kg/m²]	37.35	± 6.11	32.68	± 5.70	***
Waist circumference [cm]	108.16	± 13.21	98.77	± 12.74	***
Waist-to-hip ratio	0.89	± 0.09	0.87	± 0.08	**
Total body fat [kg]	41.04	± 3.14	37.83	± 4.34	***
Lean body mass [kg]	64.08	± 17.68	60.34	± 15.17	**
ISI_{clamp}	0.06	± 0.03	0.08	± 0.04	***
HOMA-IR	2.88	± 2.65	1.58	± 1.00	***
fasting glucose [mg/dl]	90.86	± 24.77	78.93	± 16.60	***
120 min-glucose [mg/dl]	129.24	± 49.95	121.04	± 37.17	**
FFA [mmol/l]	0.61	± 0.23	0.64	± 0.25	
TG [mg/dl]	141.98	± 108.32	93.47	± 40.55	***
RQ	0.81	± 0.06	0.77	± 0.07	***

Values depicted as mean ± SD. *** P < 0.001, ** P < 0.01, vs. before WR.

In summary an expected WR of -4.67 ± 1.47 kg/m² (-12.6 ± 3.7%) was observed after 3-month of dietary intervention. WR was not different between male and female subjects (Figure 15).

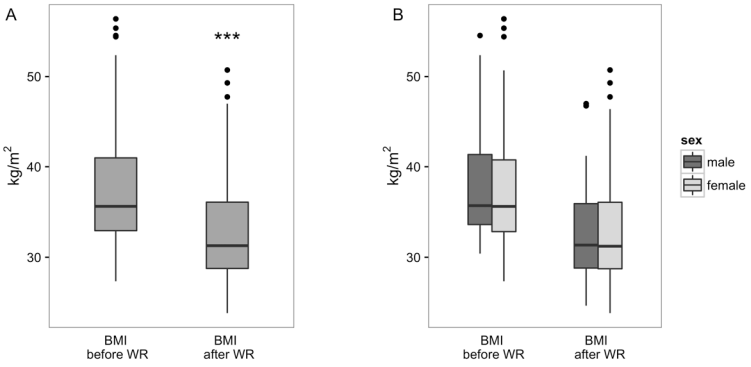

Figure 15: BMI reduction over 3 month WR in the **A** entire cohort, **B** between male and female subjects. (*** p < 0.001 vs. before WR).

WR was accompanied by a significant reduction of TBF and WHR. Improvements of TBF or BMI did not show gender-specific differences. However, male subjects showed a higher reduction in WHR over the WR (p = 0.04) (Figure 16). Though, this was not adjusted for baseline WHR and might be due to gender-specific differences in WHR before WR.

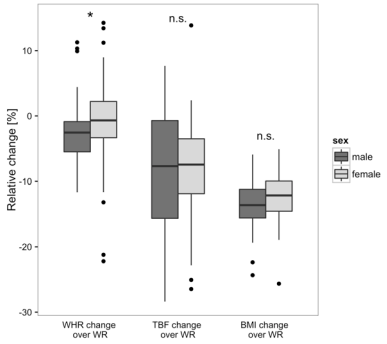

Figure 16: Changes of WHR and TBF over WR in male and female subjects. (* P < 0.05 vs. males).

WR also has beneficial effects on lipid and glucose metabolism. In fact, WR leads to a significant improvement in whole-body insulin sensitivity assessed by an increase of insulin sensitivity index (ISI_{clamp}) and a decrease in HOMA-IR (Table 12). The RQ as an estimate of substrate utilization decreased significantly during WR.

Intra-individual differences in response to WR are described already in (Dina 2008; Lissner et al. 1991). A high variability of weight changes as well as reduction of hormonal parameter, like leptin,

was seen during WR, even if a standardized intervention was performed in all subjects (Figure 17). Interestingly changes in plasma leptin levels differed highly in subjects with comparable WR/change in BMI (Figure 17 C).

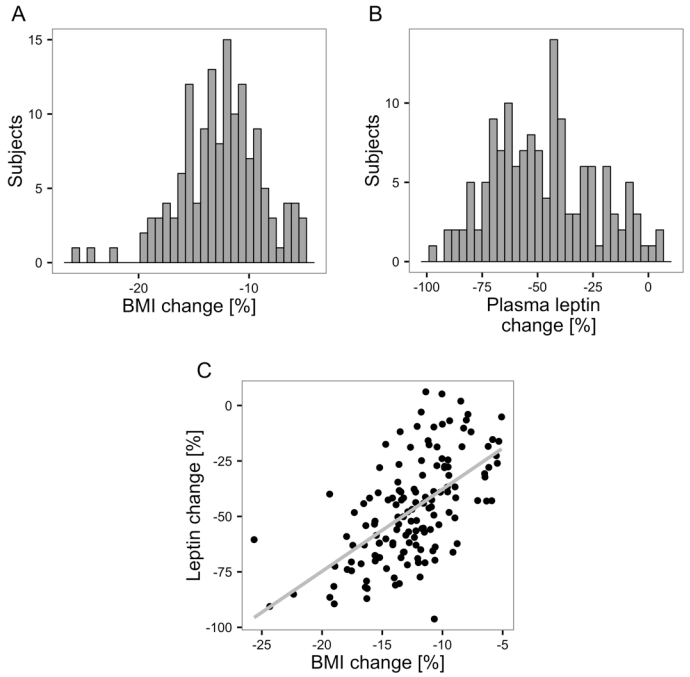

Figure 17: BMI and plasma leptin changes over WR. **A** Individual changes of BMI, **B** individual changes of plasma leptin **C** Changes of plasma leptin levels in relation to BMI changes.

5.3.2 Differentially regulated genes

As mentioned above, all subjects showed improvements of several metabolic and anthropometric parameters after the 3-month WR. As skeletal muscle and adipose tissue play a crucial role in metabolism we investigated the effects of WR on adipose and skeletal muscle gene expression.

5.3.2.1 Intra-individual variability

In accordance to the high variability of anthropometric parameters during WR, we found a substantial variability of changes in gene expression. To visualize this variability, we plotted the changes of 500 randomly selected genes in all subjects in adipose tissue and skeletal muscle (Figure 18).

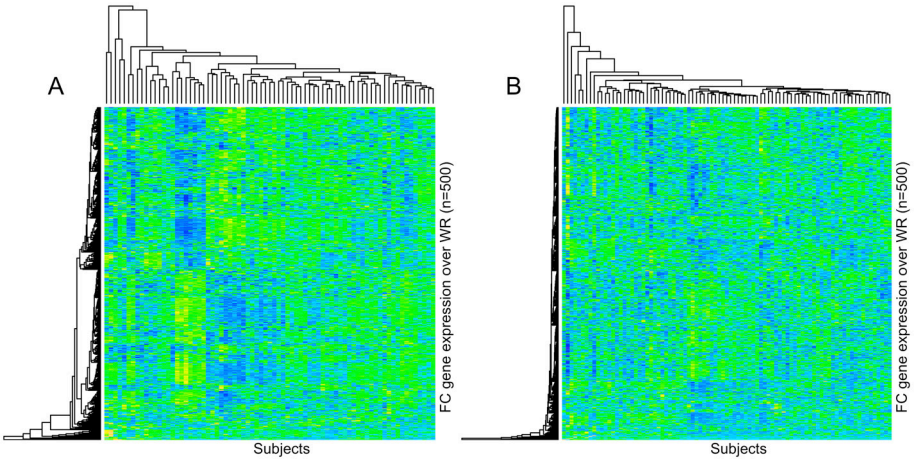

Figure 18: Heatmap of 500 randomly selected genes. Changes of expression over WR in **A** adipose tissue, **B** skeletal muscle tissue, are depicted.

5.3.2.2 Expression changes over weight reduction

In total 8388 transcripts in adipose tissue and 134 transcripts in skeletal muscle were identified as differentially expressed (Figure 19).

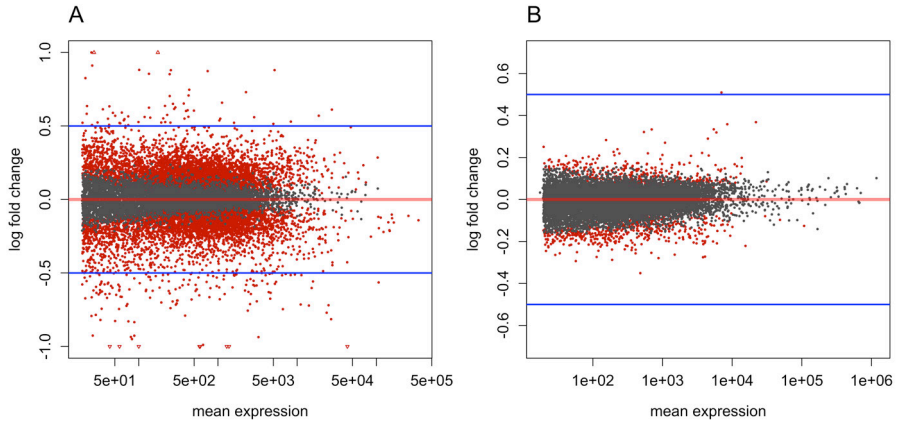

Figure 19: Differentially expressed genes in RNA-Seq data. Red dots indicate significantly altered transcripts (adj. for multiple testing). The blue line shows the log fold change threshold of +/- 0.5. Expression changes in **A** adipose tissue, **B** skeletal muscle tissue.

We used the expression of well-known adipokines (Raucci et al. 2013) to further approve the data. The change in expression levels of several well-known adipokines over the WR phase was calculated.

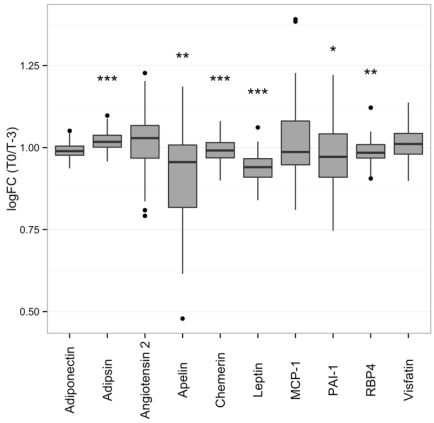

Figure 20: LogFC expression of well-known adipokines in adipose tissue. LogFC is calculated between time point T0 and T-3. Values under 1 indicate a down-regulation and over 1 indicate an up-regulation over WR. (*** P < 0.001, ** P < 0.01, * P < 0.05 , adjusted for multiple testing)

Thereby we could confirm most of the already described changes of several adipokines like adipsin, apelin, leptin, PAI-1 and RBP4 (Rasouli and Kern 2008; Raucci et al. 2013), although the response of adiponectin was different from some previous reports (Bruun 2006b; You et al. 2014)(Figure 20).

To further reduce the list of identified genes to a list with a substantial expression change we choose only highly altered transcripts (logFC over/below +/- 0.5), thereby generating a sub-list of 222 transcripts for adipose tissue (further referred to as highly altered transcripts). In contrast, we could not detect a single transcript in skeletal muscle tissue with a log fold change over/below +/- 0.5. Even selecting a lower threshold of +/- 0.3 did not yield any transcripts with substantial changes (in total only 7 transcripts with unknown function appeared, data not shown).

Only the upper 10% of highly altered genes in adipose tissue are depicted in Table 13.

Out of these 22 transcripts around 45% are involved in lipid and carbohydrate metabolism (Table 13). The predominant negative logFC indicates that the majority of these genes were down-regulated by WR.

Table 13: Upper 10% of highly altered transcripts in adipose tissue.

Name	baseMean	logFC	P-value
fatty acid desaturase 1 •	1291.39	-1.28	4.65E-27
stathmin-like 2 •	57.19	-1.26	2.24E-18
Unknown	174.46	1.25	1.08E-18
stearoyl-CoA desaturase (delta-9-desaturase) •	43053.38	-1.18	3.25E-17
hypothetical protein LOC286411	100.12	-1.13	6.68E-23
fatty acid desaturase 2 •	1378.40	-1.10	4.99E-23
ELOVL family member 6, elongation of long-chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast) •	596.36	-1.09	5.96E-15
ribosomal protein L19 pseudogene 9	27.27	1.07	9.46E-14
hypothetical protein FLJ37543	43.10	-1.04	1.62E-17
UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6 •	584.21	-1.00	2.12E-16
Unknown	25.30	1.00	1.26E-12
Unknown	649.09	-0.99	8.51E-17
Unknown	82.15	-0.95	9.47E-17
aldolase C, fructose-bisphosphate •	3246.46	-0.94	4.61E-19
Unknown	79.63	-0.94	3.15E-15
collagen, type XI, alpha 1	87.99	-0.93	3.20E-11
ankyrin repeat domain 30A	26.27	-0.93	1.44E-12
solute carrier family 27 (fatty acid transporter), member 2 •	25.88	0.91	1.12E-10
ATP-binding cassette, sub-family B (MDR/TAP), member 5; small nuclear	101.42	0.88	1.18E-15
hypothetical LOC100129500; apolipoprotein E •	5185.12	0.88	5.55E-16
RAS-like, family 10, member B	254.46	0.88	5.61E-13
complement component 6	741.90	0.87	1.28E-12

The baseMean gives the mean count number between all samples. The logFC indicates the strength of expression change before and after WR (minus indicates a down-regulation). P-values are corrected for multiple testing, black circles mark transcripts involved in metabolism.

5.3.2.3 Functional annotation of differentially expressed genes in adipose tissue over weight reduction

Next we aimed to classify the highly altered genes with respect to their function. To get a first overview of gene function we searched for functional annotations like GO-terms, protein information resource or sequence feature using DAVID. Thereby we detected a predominantly alteration of genes involved in lipid pathways as indicated by Table 13 (Table 14). Further on, genes involved in insulin signalling, nutrient sensing and oxidative stress were revealed by this analysis.

Table 14: Functional annotation analysis of highly differentially expressed genes over WR phase.

Category	Term	Number of genes
GOTERM_BP_FAT	fatty acid metabolic process	12
GOTERM_BP_FAT	steroid metabolic process	12
GOTERM_BP_FAT	response to hormone stimulus	14
GOTERM_BP_FAT	lipid biosynthetic process	13
GOTERM_BP_FAT	glycerolipid metabolic process	9
KEGG_PATHWAY	biosynthesis of unsaturated fatty acids	5
GOTERM_BP_FAT	cholesterol metabolic process	7
SP_PIR_KEYWORDS	secreted	30
GOTERM_BP_FAT	response to oxygen levels	7
SP_PIR_KEYWORDS	lipid transport	5
GOTERM_BP_FAT	response to nutrient levels	8
GOTERM_BP_FAT	response to insulin stimulus	6
GOTERM_BP_FAT	lipid localization	6
GOTERM_BP_FAT	response to oxidative stress	6
SP_PIR_KEYWORDS	nadp	5

Terms were selected according to possible metabolic relevance. Number of genes indicates the number of differentially regulated genes over WR and involved in this pathway. SP_PIR = protein information resource, BP = biological process.

The SP_PIR_KEYWORD "secreted" was assumed to be of interest for endocrine function (Table 14). An in-depth analysis revealed 30 transcripts of secreted proteins highly modified by WR (Table 15). This list also includes leptin (logFC -0.7, p=6.87E-15). Other genes encode for proteins involved in cholesterol or lipid metabolism, extracellular matrix proteins and inflammatory response.

Table 15: Genes encoding for secreted proteins differentially expressed over WR.

Name	Possible function	logFC	P-value
complement component 6	-	0.87	1.28E-12
collagen, type XI, alpha 1	-	-0.93	3.20E-11
semaphorin 3C	Inflammatory response	-0.57	6.00E-17
cholesteryl ester transfer protein, plasma	Lipid metabolism	0.85	2.61E-10
matrix metallopeptidase 2	Inflammatory response, ECM	0.57	2.98E-17
carboxypeptidase X (M14 family), member 1	ECM	0.66	4.85E-08
matrix-remodelling associated 5	-	-0.64	1.10E-09
olfactomedin 2	Smooth muscle development	-0.60	1.89E-07
midkine (neurite growth-promoting factor 2)	Obesity, adipocyte	0.56	3.61E-18
glycosylphosphatidylinositol specific phospholipase D1	ECM, lipid metabolism	-0.58	7.93E-09
secreted protein, acidic, cysteine-rich (osteonectin)	-	-0.56	2.21E-20
microfibrillar-associated protein 2	-	0.55	2.49E-13
fibromodulin	ECM	-0.51	1.40E-13
casein alpha s1	-	-0.72	5.74E-07
carboxypeptidase A4	-	-0.63	3.40E-06
hepatocellular carcinoma-associated gene TD26	-	-0.61	4.26E-06
hypothetical LOC100129500; apolipoprotein E	Lipid metabolism	0.88	5.55E-16
apolipoprotein C-I	Lipid metabolism	0.61	1.63E-06
angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	CVD	-0.58	2.24E-06
growth differentiation factor 15	Inflammatory response,	0.51	4.62E-05
serum amyloid A2	Lipid metabolism,	-0.52	3.44E-04
chloride channel accessory 2	-	-0.54	3.64E-08
prokineticin 1	Obesity	0.52	1.15E-04
ADAM metallopeptidase with thrombospondin type 1 motif, 4	-	-0.51	1.21E-08
C1q and tumor necrosis factor related protein 4	-	0.52	3.60E-06
leptin	Obesity	-0.72	6.87E-15
von Willebrand factor A domain containing 3A	-	0.50	3.04E-06
hypothetical protein FLJ37543	-	-1.04	1.62E-17
silver homolog (mouse)	-	0.51	2.26E-05
glutathione peroxidase 3 (plasma)	Obesity	0.61	4.32E-17

Recent knowledge from literature pointing to a possible function. The logFC indicates the strength of expression change before and after WR (minus indicates a down-regulation). P-values are corrected for multiple testing. ECM = extra cellular matrix, CVD = cardio vascular disease.

For confirmation we used the GSVA package implanted in R to test for differentially expressed KEGG pathways. Thereby 95 KEGG pathways were identified to be modified by WR. The identified top 10% altered KEGG pathways are shown in Table 16. In accordance to the results mentioned above, TCA and biosynthesis of unsaturated FA are under the top highly altered pathways.

Table 16: Top 10 KEGG pathways altered by WR.

Name	logFC	P-value
KEGG_RIBOSOME	-0.57	4.30E-11
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	0.51	1.73E-13
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS •	0.46	1.67E-10
KEGG_ASCORBATE_AND_ALDARATE_METABOLISM	0.44	1.15E-07
KEGG_LIMONENE_AND_PINENE_DEGRADATION	0.37	2.82E-06
KEGG_CITRATE_CYCLE_TCA_CYCLE •	0.29	2.76E-04
KEGG_BUTANOATE_METABOLISM	0.28	1.29E-04
KEGG_PROPANOATE_METABOLISM	0.28	2.27E-04
KEGG_OTHER_GLYCAN_DEGRADATION	-0.28	2.13E-04

Lipid and carbohydrate metabolic pathways are marked with an black circles. P-values are adjusted for multiple testing. logFC = log fold change over WR.

Thus, genes involved in lipid and carbohydrate metabolism seemed to be highly modified by WR in adipose tissue. Therefore we further focussed on lipid and carbohydrate metabolic pathways in detail. In fact 10 of all KEGG pathways altered by WR are related to lipid and carbohydrate metabolism (Table 17).

The logFC indicates that pathways involved in lipid and carbohydrate metabolism were altered over the WR phase. However, pathway analysing algorithms do not take activating or inhibiting functions into account.

Table 17: KEGG pathways involved in lipid carbohydrate metabolism influenced by WR.

Name	logFC	P-value
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	0.46	1.67E-10
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.29	2.76E-04
KEGG_GLYCOLYSIS_GLUCONEOGENESIS	0.21	1.54E-04
KEGG_FATTY_ACID_METABOLISM	0.20	3.09E-03
KEGG_PPAR_SIGNALING_PATHWAY	0.14	1.36E-02
KEGG_INSULIN_SIGNALING_PATHWAY	0.10	3.36E-02
KEGG_STARCH_AND_SUCROSE_METABOLISM	0.26	1.51E-05
KEGG_PYRUVATE_METABOLISM	0.25	1.73E-04
KEGG_ARACHIDONIC_ACID_METABOLISM	-0.17	4.68E-03
KEGG_GLYCEROLIPID_METABOLISM	0.14	5.60E-03

P-values are adjusted for multiple testing. logFC = log fold change over WR.

Taken together, our single gene and pathway analyses showed a substantial alteration of particularly lipid metabolism in adipose tissue during WR.

5.3.3 Functional networks and anthropometric parameters

As we are interested in WR induced changes, relations between gene expression changes and phenotypic changes might be a possible indication towards regulatory processes involved in this

process. To identify correlations between changes in gene expression and phenotypic parameters we used the logFC of gene expression (change during WR) and the relative WR induced changes of the phenotypic parameters (%).

5.3.3.1 Anthropometric parameters – changes over weight reduction

Functional annotation analysis revealed that a decrease of BMI during the WR period (ΔBMI) was associated with gene expression changes (logFC genes) involved in mitochondrial processes as well as glucose and lipid metabolism in adipose tissue (Table 18).

Table 18: Summary of functional annotation analysis of identified gene expression alteration in relation to ΔBMI in adipose tissue using DAVID.

Category	Term	Number of genes
GOTERM_CC_FAT	mitochondrion	168
GOTERM_CC_FAT	mitochondrial membrane	80
GOTERM_CC_FAT	mitochondrial inner membrane	65
KEGG_PATHWAY	oxidative phosphorylation	35
GOTERM_BP_FAT	glucose metabolic process	32
GOTERM_BP_FAT	fatty acid metabolic process	33
KEGG_PATHWAY	glycolysis / gluconeogenesis	16
KEGG_PATHWAY	PPAR signalling pathway	15
GOTERM_BP_FAT	lipid biosynthetic process	37
GOTERM_BP_FAT	mitochondrion organization	18

Terms were selected according to their possible metabolic relevance. Number of genes indicates the number of genes correlating with ΔBMI and involved in this pathway. SP_PIR = protein information resource, BP = biological process, CC = cellular compartment.

Similar to our previous results on differentially expressed genes we found no metabolic cluster in skeletal muscle expression changes correlating with ΔBMI.

5.3.4 Functional networks and lipid metabolism

As shown in Table 12, RQ exhibited a significant decrease during WR, whereas FFA levels were not significantly modified. Similar to our approach at baseline we assessed gene-phenotype associations using changes in plasma FFA levels and RQ.

Investigating changes in plasma FFA (ΔFFA) and possible associations with gene expression changes we found several functional clusters in adipose tissue (Table 19). A major part of these genes could be grouped into lipid metabolic clusters. However genes of glucose metabolism as well as mitochondrial function and inflammation are also associated with ΔFFA.

Table 19: Summary of functional annotation analysis of identified genes expression alterations in relation to ΔFFA in adipose tissue using DAVID.

Category	Term	Number of genes
GOTERM_CC_FAT	mitochondrion	170
GOTERM_BP_FAT	glucose metabolic process	40
GOTERM_BP_FAT	acetyl-CoA metabolic process	16
GOTERM_BP_FAT	fatty acid metabolic process	45
GOTERM_CC_FAT	mitochondrial inner membrane	59
KEGG_PATHWAY	citrate cycle (TCA cycle)	16
GOTERM_BP_FAT	pyruvate metabolic process	17
GOTERM_BP_FAT	glycolysis	17
KEGG_PATHWAY	PPAR signalling pathway	22
GOTERM_BP_FAT	inflammatory response	52
SP_PIR_KEYWORDS	lipid synthesis	21
GOTERM_BP_FAT	fat cell differentiation	15
KEGG_PATHWAY	insulin signalling pathway	27
GOTERM_BP_FAT	glycerolipid metabolic process	26
KEGG_PATHWAY	adipocytokine signalling pathway	15

Terms were selected according to their possible metabolic relevance. Number of genes indicates the number of genes correlating with ΔFFA and involved in this pathway. SP_PIR = protein information resource, BP = biological process, CC = cellular compartment.

As skeletal muscle plays a detrimental role in FFA metabolism, we analysed the relationship between changes in muscle gene expression and ΔFFA. And again, changes of myocellular genes involved in lipid metabolism were associated with ΔFFA. According to the insulin resistance mediating effects of FFA we also found a cluster of changed myocellular genes involved in insulin signalling (Table 20).

Table 20: Summary of functional annotation analysis of identified genes expression alterations in relation to ΔFFA in skeletal muscle tissue using DAVID.

Category	Term	Number of genes
GOTERM_BP_FAT	glycerolipid metabolic process	25
GOTERM_BP_FAT	glucose metabolic process	24
KEGG_PATHWAY	insulin signalling pathway	21
GOTERM_CC_FAT	sarcomere	16
GOTERM_BP_FAT	muscle organ development	24
GOTERM_BP_FAT	regulation of lipid metabolic process	15

Terms were selected according to their possible metabolic relevance. Number of genes indicates the number of genes correlating with ΔFFA and involved in this pathway. BP = biological process, CC = cellular compartment.

Investigating associations of gene expression changes and substrate utilisation (ΔRQ) during WR only a small list of genes, which did not include metabolic clusters, were associated.

Even if several adipose transcripts of lipid metabolism were associated with RQ at baseline (5.2.5.1), no specific metabolic clusters were associated with WR induced changes of RQ in adipose tissue.

Using functional annotation we found one small cluster of myocellular transcripts involved in oxidative phosphorylation to be associated with ΔRQ (SP_PIR_KEXWORDS "oxidative phosphorylation", number of genes involved = 10). However, a large group of genes involved in transcription and transcriptional regulation was identified (e.g. GOTERM_BP_FAT " translation", number of genes involved = 81).

5.3.5 Expressional changes in cellular metabolism over 3-month weight reduction

Taken together our results of differentially regulated genes over the 3-month WR and the gene-phenotype associations, we speculated that expression of cellular metabolic genes involved in lipid metabolism, glucose metabolism and insulin signalling is related to anthropometric and metabolic improvements during WR. To confirm our hypothesis, we have chosen "key-genes" involved in all named cellular processes (Table 21).

Table 21 List of metabolic "key-genes".

Gene Symbole	Gene Symbole	Gene Symbole	Gene Symbole	Gene Symbole	Gene Symbole	Gene Symbole	Gene Symbole
ACACA	ALDOB	FBP1	IRS1	PDHB	PPARG	ADRB2	PLIN1
ACACB	ALDOC	FOXO1	IRS2	PFKM	LXRA	GIPR	PLIN2
ACADL	CPT1A	G6PC	LDHA	PGAM2	ESR1	OGDH	PPARA
ACADM	CPT1B	GAPDH	LDHB	PPARGC1A	LPL	LEP	SDHA
ACADS	CPT2	GCK	LIPE	SCD	CD36	LEPR	UCP1
ACADSB	CS	GPI	MCAT	SLC2A4	MGL	FABP4	UCP2
ACLY	EHHADH	GSK3B	MDH1	SOCS1	ATGL	FAS	UCP3
ACOX1	ELOVL2	GYS1	MDH2	SOCS2	ESR2	FGF19	VLCAD
ACOX3	ELOVL5	HADHA	PC	SOCS3	ADRA2A	FGF21	
ACSL1	ELOVL6	HADHB	PCK1	CACT	ADIPOQ	MACD	
ACSL3	FADS1	IDH1	PCK2	ACSL5	ADIPOR1	NPR1	
ACSL4	FADS2	IDH2	PDHA1	AGPAT9	ADIPOR2	NPR2	
ALDOA	FASN	INSR	PDHA2	SREBF1	ADRB1	NPR3	

We investigated the expression pattern over the WR phase. In Table 22 the significant altered genes of these key genes are presented.

Table 22 Significant regulated key cellular metabolic genes in adipose tissue.

Gene Name	Mean before WR	Mean after WR	P-value
	Lipid synthesis		
ACSL4	2101.12 ± 449.13	1784.87 ± 362.50	1.41E-04
ACSL3	1560.95 ± 281.13	1322.48 ± 188.89	7.09E-09
ACSL1	32325.83 ± 11660.74	23420.87 ± 9175.68	1.08E-07
ACSL5	1017.77 ± 131.59	1128.25 ± 162.42	3.03E-05
AGPAT9	139.33 ± 48.48	188.68 ± 148.43	9.77E-05
	Fatty acid elongation		
ELOVL5	11081.17 ± 3427.59	7628.67 ± 2326.58	7.38E-11
SCD	67488.83 ± 42122.02	18617.92 ± 21333.13	7.55E-12
ELOVL6	926.53 ± 3706.81	266.13 ± 445.43	1.81E-08
FADS2	1974.4 ± 1107.95	782.42 ± 591.83	1.49E-11
FADS1	1948.56 ± 2161.09	634.24 ± 363.15	9.99E-12
	Lipid catabolism		
EHHADH	742.63 ± 172.30	525.49 ± 131.31	1.92E-10
ACOX1	4420.17 ± 1061.33	3147.99 ± 810.38	2.79E-10
CACT	1030.56 ± 155.32	877.56 ± 139.08	8.21E-09
ACADSB	1053.49 ± 207.34	887.32 ± 153.09	1.11E-05
ACADL	728.71 ± 200.59	562.53 ± 139.88	3.13E-08
ACADM	2069.21 ± 536.35	1748.4 ± 402.91	8.39E-07
MGL	21122 ± 4380.39	17608.76 ± 3950.31	2.77E-06
	TCA cycle		
PCK2	712.59 ± 242.75	992.59 ± 446.66	4.21E-10
PDHA1	2161.09 ± 478.81	1880.24 ± 402.30	3.45E-05
PDHB	1434.49 ± 236.64	1290.43 ± 126.38	7.49E-08
MDH1	6391.99 ± 1953.78	5424.76 ± 1658.02	3.80E-05
CS	6551.17 ± 1457.72	5915.91 ± 1170.03	1.53E-03
OGDH	3806.77 ± 495.18	3470.57 ± 431.13	2.38E-04
ACLY	3807.49 ± 6081.26	2349.19 ± 540.71	1.92E-10
IDH1	5243.11 ± 1394.55	4240.16 ± 1051.29	6.96E-07
	Glycolysis/Gluconeogenesis		
ALDOC	4440.09 ± 1956.99	2052.79 ± 1269.84	1.37E-11
LDHB	12009.99 ± 2356.28	9903.47 ± 2281.29	7.09E-09
LDHA	12773.63 ± 1981.62	10888.09 ± 1781.01	4.85E-08
ALDOB	25.12 ± 13.21	18.67 ± 9.82	2.39E-05
ALDOA	14657.57 ± 2562.17	13206.73 ± 2688.63	2.44E-02
FBP1	283.89 ± 243.75	345.32 ± 213.72	4.26E-03
	Insulin Signalling		
SOCS1	58.13 ± 18.50	89.23 ± 36.33	8.93E-07
GSK3B	1166.84 ± 168.73	1015.23 ± 133.45	3.22E-06
GYS1	1777.8 ± 604.67	1431.64 ± 450.13	1.20E-04
FASN	33199.63 ± 32095.23	22470.13 ± 12157.56	4.97E-04
FOXO1	2562.99 ± 540.80	2234.15 ± 563.66	2.88E-03

Gene Name	Mean before WR	Mean after WR	P-value
	Regulatory function		
NPR3	1004.95 ± 488.41	556.68 ± 465.60	1.70E-09
ADRB2	347.11 ± 57.27	401.92 ± 95.66	1.11E-04
ADRA2A	3042.07 ± 773.05	2566.85 ± 675.20	1.41E-04
ESR2	93.28 ± 46.40	76.23 ± 31.93	1.46E-02
LXRA	1932.09 ± 369.29	2566.81 ± 566.71	1.78E-10
LPL	49438.2 ± 14593.09	36113.56 ± 11739.99	5.54E-09
CD36	92963.73 ± 18192.90	77662.51 ± 14776.52	4.89E-06
LEP	31715.07 ± 10716.88	18040.04 ± 8301.87	4.46E-11
ADIPOR2	5380.08 ± 1556.45	4101.91 ± 702.48	1.04E-10
LDLR	527.28 ± 224.09	349.31 ± 103.62	6.60E-09
GIPR	165.47 ± 64.89	213.75 ± 92.39	1.25E-05

Expression is shown before and after WR. P-values adjusted for multiple testing.

Further analysis of skeletal muscle gene expression showed only two of the genes to be slightly down-regulated (Table 23).

Table 23 Significant regulated key cellular metabolic genes in skeletal muscle tissue.

	before WR	after WR	
Gene Name	Mean ± SD	Mean ± SD	P-value
FADS1	121.85 ± 37.76	100.01 ± 25.49	1.42E-02
FADS2	304.33 ± 71.70	259.31 ± 55.42	1.96E-02

Expression is shown before and after WR. P-values adjusted for multiple testing.

According to the previous approach we investigated the associations of gene expression changes of all cellular metabolic genes (independent of a significant expressional change) and phenotypic parameters as before.

We found several positive associations between changes in cellular metabolic genes in adipose tissue and ΔBMI (Table 24). In terms of changes in metabolic parameters including ΔRQ and ΔFFA we detected associations of several metabolic transcriptional changes in both tissues. Interestingly, we observed several changes in adipose tissue gene expression negatively associated with ΔFFA (Table 24). This included enzymes involved in FA synthesis and elongation, as well as FA degradation. In turn, we found a negative correlation between ΔFFA and transcriptional changes of ESR1/ERα in skeletal muscle.

In contrast, we observed a limited number of gene changes to be linked with ΔRQ and ΔFFA in skeletal muscle (Table 25).

Table 24 Gene expression changes significant associated with phenotypic changes over WR in adipose tissue.

Gene Name	R	P-value
	ΔBMI	
SCD	0.37	9.66E-02
LDHB	0.38	5.88E-02
ALDOC	0.47	1.59E-03
LEP	0.50	9.92E-04

Gene Name	R	P-value
	ΔFFA	
LEP	-0.57	7.42E-04
FADS2	-0.48	3.14E-03
FADS1	-0.48	6.37E-03
ELOVL5	-0.46	5.27E-03
MGL	-0.45	1.91E-02
ADIPOR2	-0.44	4.18E-02
IDH1	-0.43	2.32E-02
LPL	-0.42	3.36E-02
SCD	-0.41	3.00E-02
GYS1	-0.41	4.59E-02
FASN	-0.40	5.74E-02
FABP4	0.42	9.26E-02
CPT1A	0.46	6.55E-03

Linear regression adjusted for age, gender, BMI_{T-3}. P-value adjusted for multiple testing and significance set to P-value ≤ 0.1.

Table 25 Gene expression changes significant associated with phenotypic changes over WR in skeletal muscle tissue.

Gene Name	R	P-value
	ΔRQ	
LDLR	0.48	1.79E-04
	ΔFFA	
ESR1	-0.45	1.01E-03

Linear regression adjusted for age, gender, BMI_{T-3}. P-value adjusted for multiple testing and significance set to P-value ≤ 0.1.

5.4 Prediction of body weight using cellular metabolic gene expression

In summary our data indicated that predominantly lipid metabolism in adipose tissue is altered during WR and these changes were associated with improvement of several metabolic and anthropometric parameters. Therefore we asked whether the basal expression of genes involved in metabolism was predictive for subsequent WR and whether gene expression after WR predicted long-term weight maintenance.

5.4.1 Prediction of body weight after weight reduction

We further on investigated whether the transcriptome before WR can predict the body weight after WR. Therefore linear regression models adjusted for age, gender, BMI before WR were performed. In a first step we concentrated on metabolic genes. As described in section 5.3.2.3 we used the GSVA package to calculate enrichment scores.

Surprisingly we did not find any metabolic pathway to be predictive for body WR. However, the BMI is influenced by several processes and influenced by several organs. We therefore studied body compositions parameters, which might be more related to adipose tissue and the development of obesity, including waist circumference and TBF.

We found one pathway to be predictive in terms of waist circumference after WR and none for TBF (Biosynthesis of unsaturated FA, r = -0.15, p = 0.02). Nevertheless, adjusting for multiple testing did not yield any significant results.

Further investigation of metabolic key genes revealed 5 genes to be predictive for waist circumference (Table 26). In fact a positive correlation with several lipid metabolic genes including ACADL, FADS and LPL was found. In turn, the leptin receptor (LEPR) before WR showed a positive correlation with waist circumference after WR (Table 26). However, this was not further significant after adjustment for multiple testing.

Table 26 Genes significant associated with waist circumference after WR in adipose tissue.

Gene Name	R	P-value
ACADL	-0.12	4.70E-02
FADS2	-0.14	1.27E-02
IDH2	-0.17	4.50E-03
LPL	-0.14	2.96E-02
LEPR	0.13	2.64E-02

Linear regression adjusted for age, gender, BMI_{T0} and randomization.

Similar to our findings concerning the BMI after WR, we did not detect any relationship between metabolic gene expression before WR and TBF after WR. Thus neither BMI, waist circumference nor TBF were predicted by basal gene expression in adipose tissue.

In a next step associations of basal skeletal muscle pathways as well as gene expression and BMI, waist and TBF after WR were investigated.

We found only one gene to be associated with T0 BMI and waist (Table 27). In contrast, basal expression of several skeletal muscle genes seem to influence TBF after WR or vice versa. Several transcripts involved in lipid metabolism (LIPE, VLCAD, ACADL, NPR3, MGL) were identified in this analysis (Table 27). Again, after adjusting for multiple testing only phosphofructokinase (PFKM) showed a significant association to TBF after WR (adjusted p = 0.04).

Table 27 Genes significant associated with body composition after WR in skeletal muscle tissue.

Gene Name	R	P-value
BMI after WR		
ALDOC	0.06	6.76E-03
Waist circumference after WR		
MGL	0.13	2.62E-02
TBF after WR		
NPR3	-0.17	2.18E-02
LIPE	0.16	7.91E-03
VLCAD	0.14	4.66E-02
ACADL	-0.22	3.14E-03
MCAT	0.12	3.41E-02
ACSL4	-0.13	2.26E-02
MGL	0.19	1.02E-02
CACT	0.17	1.01E-02
ACOX1	0.15	2.08E-02
ALDOC	0.16	7.31E-03
PFKM	-0.22	4.93E-04

Linear regression adjusted for age, gender, BMI_{T0} and randomization.

5.4.2 Prediction of body weight after 12-month life style intervention

After the initial WR period, we observed a moderate body weight regain of 0.73 ± 2.57 kg/m² (2.41 ± 7.82%) after 12 month in the entire study cohort. Comparing the intervention and control group we found a significant difference between the two groups after the 12 month life style intervention (Figure 21).

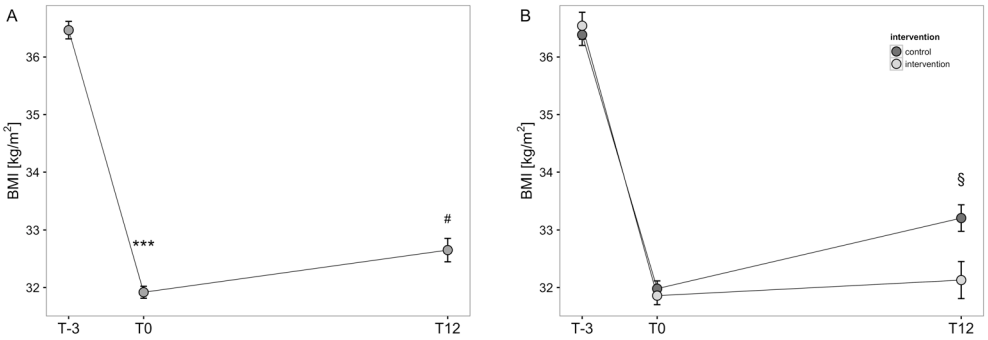

Figure 21: BMI development over 12 month. T-3 is before dietary intervention, T0 is after 3 month of dietary intervention, T12 is 12 month after initial WR. BMI development in the **A** entire cohort, **B** between control and intervention group. Error bars represent SEM. Significance is depicted by *** P < 0.001 tested between T-3 and T0, # P < 0.05 tested between T0 and T12, § P < 0.05 tested between control and intervention group.

As levels after WR might be crucial for further weight course, we investigated the potential predictive effects of metabolic gene expression pattern after WR (at T0) on long-term body weight outcome after 12 month. For the following analysis we focused only on single gene expression. To adjust for different stages of obesity we used the BMI after WR as a confounder in our analysis. As the intervention group showed a significant lower BMI_{T12} we also included the randomisation as a potential confounder in the analysis.

Table 28 Genes significant associated with BMI 12 month after WR (T12) in adipose tissue.

Gene Name	R	P-value
FABP4	-0.17	6.02E-03
PLIN2	-0.12	4.60E-02
HADHA	-0.17	7.35E-03
HADHB	-0.14	4.25E-02
FADS2	0.24	3.42E-02
SCD	0.30	2.05E-02
SREBF1	0.15	1.60E-02
NPR1	-0.14	4.67E-02
ALDOC	0.29	1.42E-02
IDH1	0.16	3.97E-02
PDHA1	0.16	3.62E-02

Linear regression adjusted for age, gender, BMI_{T0} and randomization.

After WR, genes involved in lipid oxidation (HADHA and HADHB) showed a negative association with BMI_{T12}. In contrast, lipid synthesis genes (SCD, FADS2) were positive correlated with BMI_{T12}. However, after adjustment for multiple testing all these associations were no longer significant.

Investigating a potential role of skeletal muscle gene expression regarding prediction of WR outcome we found negative correlations between the genes involved in lipid catabolism (ACADL, CPT2) and BMI_{T12} (Table 29).

Vice versa, positive associations were seen for lipid synthesis genes (ELOVL6, FADS1, FADS2, SCD) and regulatory genes (ADIPOQ, ADIPOR2, ADRA2A) (Table 29).

Again, after adjusting for multiple testing no significant results were identified. However, we observed a trend for association of FADS2 (adjusted p = 0.1).

The here presented expression data are descriptive in nature. Even if no significant correlation was found, the majority of genes are involved in lipid metabolism. We therefore assume that lipid metabolism is an important factor in body weight regulation and maintenance. To further explore whether lipid metabolic parameters can be used to predict long-term weight preservation we investigated the role of FFA and RQ.

Table 29 Genes significant associated with BMI 12 month after WR (T12) in skeletal muscle tissue.

Gene Name	R	P-value
ACADL	-0.14	7.68E-03
ELOVL6	0.17	1.47E-02
FADS1	0.21	1.34E-02
FADS2	0.25	1.18E-03
SCD	0.25	2.42E-02
ADIPOQ	0.17	1.83E-02
ADIPOR2	0.13	3.71E-02
UCP2	-0.17	2.92E-03
CPT2	-0.11	4.36E-02
ALDOA	-0.12	3.60E-02
ALDOC	0.15	3.45E-02
IDH1	0.16	4.03E-03

Linear regression adjusted for age, gender, BMI_{T0} and randomization.

In line with the gene expression data we found higher FFA levels after body WR in subjects maintaining the lost weight (maintainer 0.72 ± 0.03 vs. regainer 0.64 ± 0.03 mmol/l; $p < 0.05$) (Figure 22). Furthermore these subjects showed a tendency of a lower RQ after WR (Figure 22). Changes of RQ and FFA are not different between maintainer and regainer.

Taken together the expression data and the phenotypic parameters, our data showed that WR induced changes of lipid metabolism might play an important role in long-term body weight maintenance.

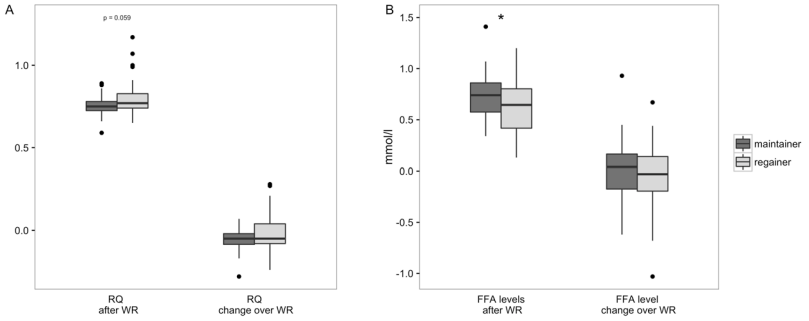

Figure 22: Metabolic parameters before and after WR in maintainer (BMI regain < 3% T0-T12) and regainer (BMI regain > 3% T0-T12). **A** RQ, **B** FFA levels (* $p < 0.05$, vs. regainer).

5.4.3 Prediction of body weight after 18 month of initial weight reduction

After the 12 month intervention phase all subjects were observed for further 6 month in a free-living phase.

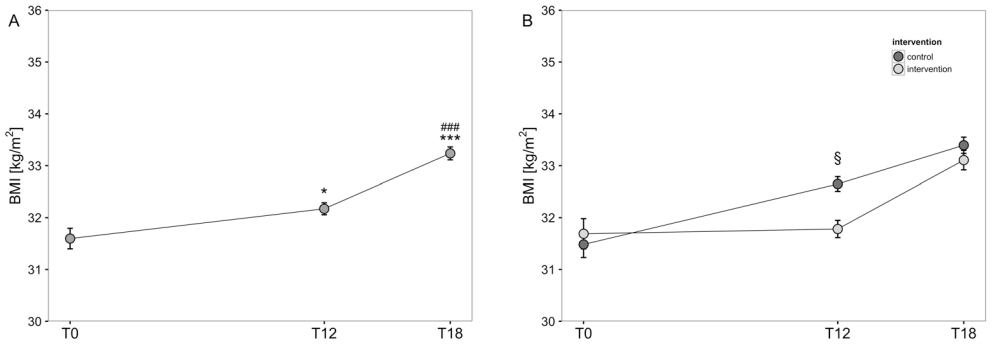

Figure 23: BMI development over 18 month. T0 is after 3 month of dietary intervention, T12 is 12 month after initial WR, T18 is 18 month after initial WR. BMI development in **A** the entire cohort, **B** between control and intervention group. Error bars show standard error of the mean. Significance is depicted by # P < 0.05 tested between T0 and T12, *** P < 0.001 tested between T0 and T18, § P < 0.05 tested between control and intervention group.

In this phase a weight regain of 1.06 ± 1.16 kg (3.35 ± 3.68%) was observed. Comparing the control and the intervention group there was no significant differences after 18 month of initial WR observable.

The expression of cellular metabolic genes after WR (T0) was used to predict the BMI at T18.

Genes involved in FA metabolism including ACADSB, ACOX1, CPT1A, ELOLV5 and FASD1 showed an association with BMI_{T18} (Table 30). Interestingly, UCP2 and UCP3 expression was negatively associated with BMI_{T18} (Table 30).

Table 30 Genes significant associated with BMI 18 month after WR (T18) in adipose tissue.

Gene Name	R	P-value
ACADSB	0.18	2.1E-02
ACOX1	0.21	1.6E-02
CPT1A	-0.23	6.3E-03
ELOVL5	0.17	2.7E-02
FADS2	0.18	3.1E-02
GSK3B	0.19	1.4E-02
IDH1	0.18	1.4E-02
UCP2	-0.30	1.4E-03
UCP3	-0.17	1.7E-02

Linear regression adjusted for age, gender, BMI_{T0}, randomization.

In skeletal muscle tissue similar results concerning UCP2 and UCP3 were found (Table 31).

Table 31 Genes significant associated with BMI 18 month after WR (T18) in skeletal muscle tissue.

Gene Names	R	P-value
SOCS3	-0.10	3.61E-02
LPL	0.19	4.56E-02
UCP2	-0.20	1.09E-02
UCP3	-0.17	1.42E-02

Linear regression adjusted for age, gender, BMI_{T0}, randomization.

Several results showed an important role of FA metabolism in body weight regulation. We found the KEGG pathway "Biosynthesis of unsaturated fatty acid" to be regulated by WR. Additionally, we revealed it to be predictive in terms of TBF after WR. We therefore used the gene expression of involved genes after WR to predict long-term body weight maintenance. Indeed, three genes (ELOVL5, ACOX1 and FADS2) showed a positive correlation with BMI_{T18} (R 0.17, P 0.03; R 0.21, P 0.02; R 0.18, P 0.03).

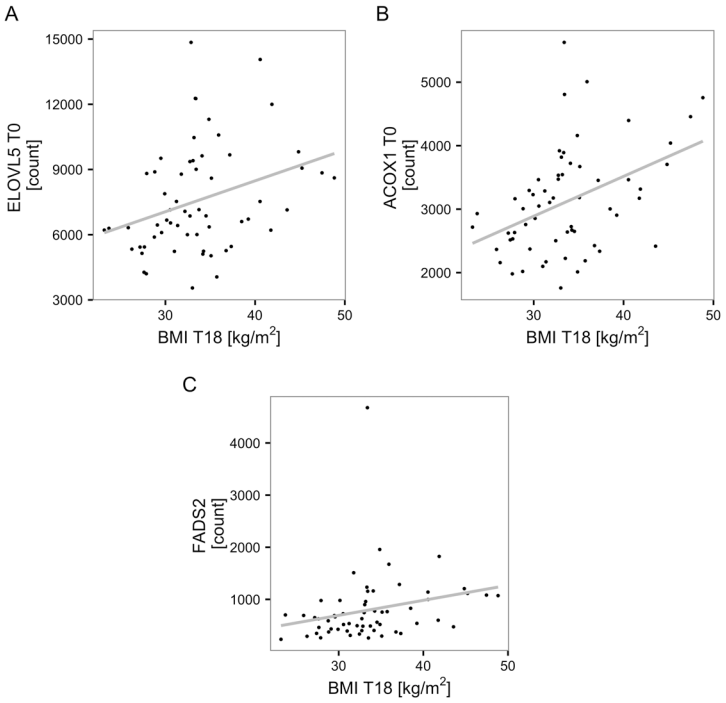

Figure 24: Gene expression after WR in relation to BMI_{T18}. **A** Expression of ELOVL5 , **B** expression of ACOX1, **C** expression of FADS2.

We further investigated physiological parameters of lipid metabolism in terms of body weight development. The RQ showed a decrease in the whole cohort after 3-month WR (0.81 ± 0.06 vs.

0.77 ± 0.07, Table 12). In line with our finding for T12, subjects maintaining their body weight showed a lower RQ after WR (maintainer 0.75 ± 0.06 vs. regainer 0.79 ± 0.084, P < 0.05) (Figure 25). In contrast to the previous described results for T12 the change of the RQ over WR in subjects maintaining the body weight after 18 month seemed to be higher in the maintainer group compared to the regainer (maintainer -0.07 ± 0.065 vs. regainer -0.027 ± 0.093, P =0.055) (Figure 25). Analysing FFA in these groups showed general higher levels in maintainer after WR (maintainer 0.74 ± 0.17 mmol/l vs. regainer 0.64 ± 0.24 mmol/l, P < 0.05). However, the reduction in plasma FFA levels was not different between both groups (Figure 25).

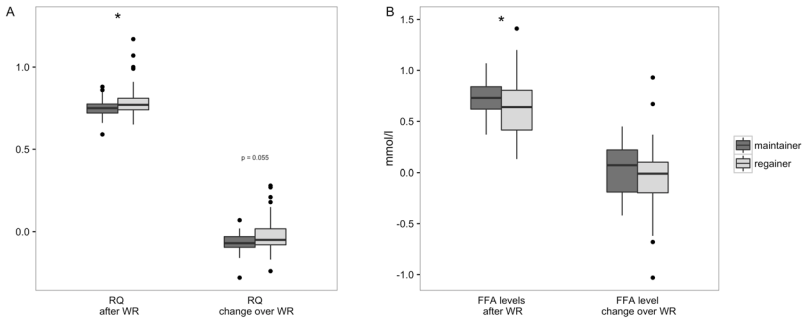

Figure 25: Metabolic parameters before and after WR (WR) in maintainer (BMI regain < 3% T0-T18) and regainer (BMI regain > 3% T0-T18). **A** RQ, **B** FFA levels (* p < 0.05, vs. regainer).

Taken together these results might show an important involvement of cellular lipid metabolism in body weight maintenance. The main aim of this work was to identify possible molecular predictors for long-term body weight maintenance. Therefore we further investigated certain lipid species, the acylcarnitines (AC).

5.4.3.1 Acylcarnitines in long-term body weight maintenance

Single AC as well as the grouped short, medium and long-chain AC levels were analysed over WR. Several plasma AC species were decreased after WR (Table 32).

Further on, we focused on a possible association of AC with the BMI_{T18}.

Table 32 Plasma concentration of AC species before and after WR

	Before WR			After WR			
AC species [nmol/l]	Mean	±	SD	Mean	±	SD	
short-chain AC	12753.63	±	2824.06	12879.20	±	3174.53	
medium-chain AC	864.19	±	528.14	764.55	±	441.64	***
long-chain AC	556.66	±	136.27	549.31	±	146.47	
C2	10405.57	±	2486.13	10812.73	±	2930.15	
C4	209.45	±	90.59	198.86	±	82.25	
C4DC	96.68	±	23.14	88.33	±	20.03	***
C6	850.57	±	580.60	658.90	±	362.81	***
C6DC	59.83	±	19.72	59.02	±	21.67	
C8	145.01	±	155.54	125.47	±	117.61	***
C8DC	27.05	±	8.28	27.39	±	8.91	
C9	68.48	±	16.54	63.45	±	22.47	***
C10	258.11	±	252.31	227.98	±	204.83	**
C10:1	115.92	±	48.35	109.26	±	60.31	**
C10:2	24.23	±	7.21	21.05	±	7.10	***
C10:3	68.01	±	28.31	55.72	±	27.11	***
C10DC	30.15	±	6.12	28.72	±	6.02	**
C12	78.69	±	43.97	65.68	±	32.02	***
C12:1	76.23	±	33.99	68.72	±	30.72	**
C12DC	24.03	±	4.40	23.06	±	3.90	*
C14	38.00	±	11.84	34.53	±	10.78	***
C14:1	76.24	±	39.31	73.89	±	35.01	
C14:2	31.13	±	13.43	29.71	±	13.36	
C14OH	11.67	±	3.23	12.68	±	3.83	**
C16	31.17	±	11.31	31.48	±	11.97	***
C16:1	121.46	±	26.06	115.76	±	28.12	
C16OH	7.67	±	1.92	7.85	±	1.97	
C16:1OH	10.19	±	2.54	10.37	±	2.84	
C16DC	47.92	±	10.04	42.85	±	10.24	***
C18	49.46	±	12.25	44.72	±	11.59	***
C18:1	114.07	±	28.09	122.49	±	35.66	**
C18:2	29.42	±	8.43	29.68	±	8.07	
C18:1OH	7.35	±	1.98	7.91	±	2.48	*
C18:2OH	17.29	±	3.02	16.98	±	2.87	
C18OH	26.80	±	8.03	28.26	±	11.75	**
C18DC	4.58	±	1.64	4.24	±	1.34	
C20	4.98	±	1.59	4.65	±	1.46	**
C20:1	6.71	±	1.90	8.10	±	2.60	***
C20:2	2.91	±	0.90	3.33	±	1.13	***
C20:3	7.41	±	1.42	6.64	±	1.29	***
C20DC	8.90	±	2.69	8.27	±	2.73	**
C22	4.59	±	1.68	4.28	±	1.74	***

Results are depicted as mean ± SD. *** P < 0.001, ** P < 0.01, * P < 0.05, vs. before WR.

We detected associations mainly between plasma levels of long-chain AC species after WR and BMI_{T18} (data not shown). Interestingly, after WR AC C18:1 levels showed a significant positive correlation with the RQ and FFA levels (R 0.1, P = 0.01; R 0.14, P < 0.001). AC C18:1 levels increased over WR thus next we investigated whether this change might be predictive in terms of long-term body weight maintenance.

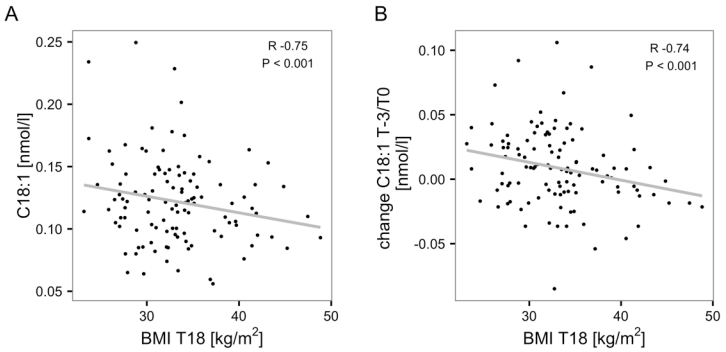

Figure 26: Plasma AC C18:1 levels in relation to the BMI_{T18}. **A** AC C18:1 levels after WR, **B** changes of AC C18:1 over WR (T-3 to T0). R and P depicte the value statistics from the linear model (confounder: age, gender, BMI_{T0} and randomization), not adjusted for multiple testing.

Indeed changes in plasma AC C18:1 showed a negative correlation with BMI_{T18} (R -0.74, P < 0.001). Taken together, these data might indicate an important role of metabolic flexibility involving lipid metabolism in long-term body weight regulation.

5.5 Single gene analysis

In addition to this unbiased approach we aimed to examined a potential role of selected signal cascades involved in substrate utilization and regulation of energy metabolism. Therefore we selected the estrogen receptor signalling in adipose tissue and the myocellular myostatin system.

5.5.1 Estrogen receptor signalling in adipose tissue of obese females

In the past a functional impact of steroid responsive elements like ERβ was already described by Lundholm and colleagues (Sethi and Vidal-Puig 2007b). Interestingly the GOTERM "response to steroid hormone stimulus", which reflects steroid responsive elements including ERβ, was one of the other pathways differently regulated between men and women (Table 3).

Thus, we investigated ERβ signalling on transcriptional level in adipose tissue in terms of lipolysis and lipogenensis.

Looking for expressional changes over WR we found significant changes of ERα (p = 0.04) and ERβ (p = 0.03) in women and no change in men. Estrogen is known to have an important role in fat mass distribution in women (Wajchenburg 2014; Barros and Gustafsson 2011). In our study cohort both genders were analysed. For further analysis we decided to focus on the female cohort only (data for men not shown).

As depicted in Figure 27 we detected a significant decrease of ERβ and an increase of ERα expression over WR. Interestingly, lipogenic enzymes including FA desaturases (FADS1, FADS2 and SCD) also showed a significant strong down-regulation, whereas several lipolytic key genes were not changed (ATGL, HSL).

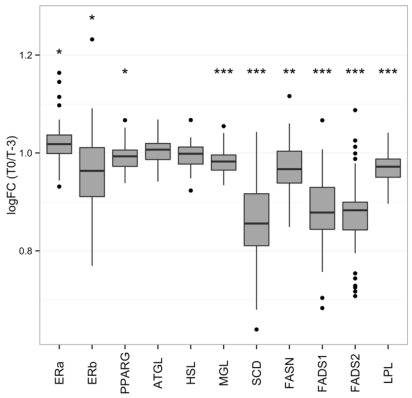

Figure 27: LogFC expression of ERs and key lipogenic and lipolytic genes in adipose tissue. LogFC is calculated between T0 and T-3. Values under 1 indicate a down-regulation and over 1 an up-regulation over WR. *** P < 0.001, * P < 0.05, vs. after WR adjusted for multiple testing.

Table 33: Correlation of lipid metabolic gene expression in adipose tissue before and over WR.

	Before WR				Over WR			
	ER-α		ER-β		ER-α		ER-β	
Gene Name	R	P-value	R	P-value	R	P-value	R	P-value
MGL	-	n.s.	0.58	5.06E-06	-	n.s.	0.53	7.18E-05
HSL	-	n.s.	0.40	4.56E-03	-	n.s.	0.31	3.58E-02
ATGL	-	n.s.	0.33	2.66E-02	-	n.s.	-	n.s.
LPL	-	n.s.	0.64	2.20E-07	-	n.s.	0.55	2.32E-05
SCD	-	n.s.	0.62	6.54E-07	-	n.s.	0.36	1.21E-02
FADS2	-	n.s.	0.55	2.60E-05	-0.37	1.01E-02	0.32	3.17E-02
FADS1	-	n.s.	0.49	2.19E-04	-0.38	8.41E-03	0.30	4.14E-02
FASN	-	n.s.	0.58	4.11E-06	-0.37	1.01E-02	0.49	3.58E-04
PPARG	-	n.s.	0.62	5.64E-07	-	n.s.	0.48	3.74E-04

Only significant results are shown. P-values adjusted for multiple testing.

Looking for gene-gene association ERβ showed positive correlation with lipolytic as well as lipogenic genes in female adipose tissue before WR. Similar results were observed over WR (change of gene expression vs. changes of phenotypic parameters) (Table 33). In contrast, ERα expression showed no links to the selected genes of lipid metabolism before WR. However, over WR a negative association between ERα and lipogenic genes (FADS1, FADS2, FASN) was found (Table 33).

Interestingly, we detected a negative correlation between ERα and body composition parameters TBF (r = -0.37, p = 3.93E-03), waist (r = -0.36, p = 4.16E-03) and BMI (r = -0.29, p = 2.11E-02) before WR. However, these parameter were not associated to ERβ.

Depicting the correlation values and creating a network shows potential changes and molecular connections. In Table 33 only significant and FDR corrected P-values are shown, whereas in Figure 28 all P-values are depicted.

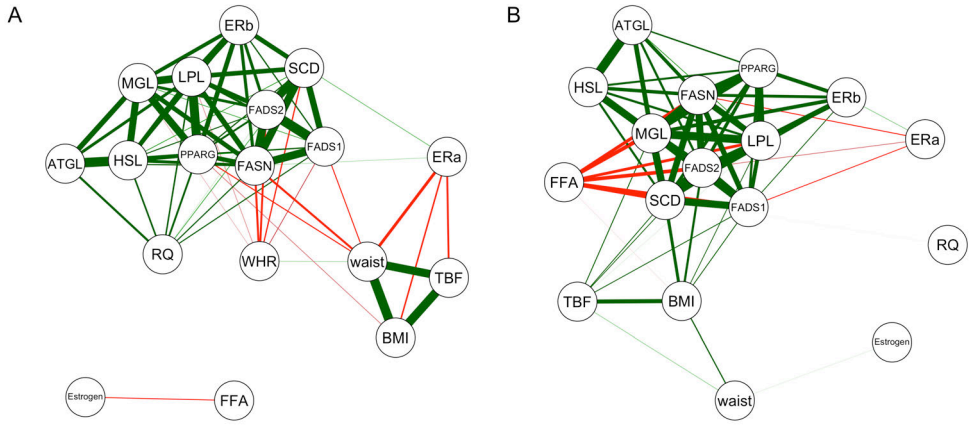

Figure 28: Correlation network of ER signalling involved genes and phenotypic associations. **A** before WR, **B** over WR. Colours indicate direction of correlation (red = negative R, green = positive R), strength of line give the strength of correlation (not corrected for multiple testing).

Figure 28 nicely showed the strong positive correlation between TBF, waist circumference and BMI as well as the inverse correlation with ERα expression levels (Figure 28 A). However, changes of ERα levels over WR did not correlated with changes in TBF, waist circumference and BMI (Figure 28 A). Our data showed strong links between lipid metabolic gene expression levels in the ER signalling pathways before and over WR. The correlation network shown in Figure 28 implied an important role of ERβ in regulating gene expression of lipid metabolic genes. ERα seemed to be less involved. However, ERα levels might have an impact on body composition in obese subjects. WR might lead to changes in this metabolic network.

5.5.2 Effects of weight reduction on myocellular myostatin expression

Given the recently assumed metabolic properties of the well characterized myokine myostatin (MSTN) (Zhao, Wall, and Yang 2005; Shan et al. 2013), we investigated a potential role of MSTN during WR. MSTN decreased in the entire cohort (203 counts before WR vs. 164 counts after WR, P < 0.01) (Figure 29).

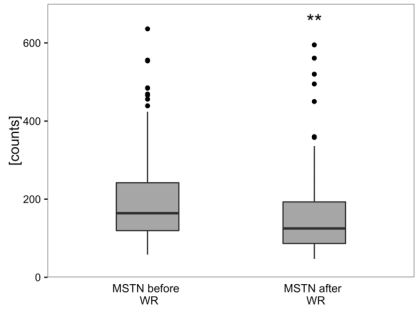

Figure 29: MSTN expression in skeletal muscle tissue of subjects underwent WR . ** P < 0.01 vs. after WR.

To confirm these data we analysed protein levels in plasma of a randomly chosen sub-cohort of 34 subjects by ELISA.

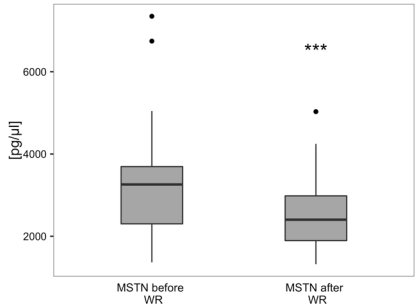

Figure 30: Plasma levels of MSTN in 34 subjects before and after WR. *** P < 0.001 vs. after WR.

As depicted in Figure 30, plasma MSTN levels showed a similar reduction as observed in the expression analysis.

We further elucidated a potential link of MSTN expression levels on gene expression of transcripts involved in lipid metabolic and insulin signalling . We therefore investigated associations of expression with phenotypic parameters before and over WR (Table 34).

Table 34: Correlation of myocellular MSTN to gene expression in skeletal muscle and phenotypic parameters before and over WR.

	Before WR		Over WR	
Gene Name	***R***	***P-value***	***R***	***P-value***
ALK4	0.24	2.35E-02	0.22	3.80E-02
MYOD1	-0.27	1.25E-02	-0.21	4.61E-02
MLXIPL	-0.32	2.95E-03	-0.36	6.78E-04
SLC2A4	-	n.s.	-0.44	5.39E-04
IRS1	0.51	1.88E-05	0.59	7.89E-08
GAPDH	0.45	3.18E-04	-	n.s.
CPT1B	-0.33	1.92E-02	-0.48	6.24E-05
CPT2	-	n.s.	-0.36	6.77E-03
CS	-	n.s.	-0.36	6.77E-03
HADHA	-	n.s.	-0.40	1.78E-03
HADHB	-0.39	3.65E-03	-0.48	6.10E-05
ISI	-0.29	6.79E-03	-	n.s.
HOMA	0.27	1.28E-02	-	n.s.
waist	0.32	2.44E-03	-	n.s.
BMI	0.36	6.62E-04	-	n.s.

Only significant results are shown. P-value adjusted for multiple testing.

At the basal state before WR MSTN showed a negative association with the estimates of insulin sensitivity (ISI and HOMA-IR index) (Table 34). Nevertheless, changes in MSTN expression were apparently not directly linked to changes of phenotypic parameters during WR (Table 34). In terms of gene-gene interaction ALK4, an interaction partner of the MSTN receptor ACVR2B showed a positive correlation with MSTN expression (Table 34). Expression levels of MSTN showed a negative correlation with the transcription factor MYOD1 before WR as well as over the WR phase. Interestingly, the glucose transporter GLUT4 (SLC2A4) was negatively associated with expression changes of MSTN but not before WR. Similar observations were found for lipid metabolic genes (CPT2, HADHA, HADHB) (Table 34).

These data indicated that MSTN was associated with insulin resistance in obese subjects and might also be linked to estimates of obesity (BMI, waist). A reduction of MSTN expression over WR might be accompanied with increases in lipid metabolic genes and GLUT4 expression. However this was not observed for functional parameters of insulin sensitivity or lipid metabolic parameters (FFA and RQ).

6 DISCUSSION

6.1 Weight reduction

WR in obese subjects leads to improvement of several metabolic parameters as well as cardiovascular risk factors (Lien et al. 2009). After 3 month of a low caloric diet a significant body WR was observed in our study. Anthropometric parameters like fat mass, waist circumference and WHR showed the expected reduction. Basal, gender-specific differences in fat mass and fat distribution were also detected. Female participants showed higher amounts of basal TBF. In line with published data, we also found a sex-specific difference in fat distribution as determined by WHR ratio (Geer and Shen 2009; Wajchenburg 2014). However, WR led to similar improvements in both genders, as we observed a comparable reduction of TBF and BMI in male and female subjects. The higher WHR reduction indicated a predominant reduction of abdominal fat in men. Together with the comparable decline in BMI and TBF this may suggest a predominant reduction of abdominal fat in men and of both, abdominal and non-abdominal fat in women. A study from Wirth and Steinmetz (Wirth and Steinmetz 1998) showed a higher reduction of abdominal fat mass in men after 3 weeks of WR. They measured specific fat layers using ultrasound and could show that women posses a thicker layer of abdominal subcutaneous fat before WR. The decrease of the abdominal fat layer was higher in the male group. Similar to our results, there was no differences in body WR between both genders. However, we found a significant gender difference between WHR changes over WR, which they did not observe. This might be due to several differences in the study outline. In our study cohort male and female participants were not matched for age and height. We did not use equal numbers of both genders as we had a higher number of female subjects. In the study from Wirth and Steinmetz (Wirth and Steinmetz 1998) the first 3 weeks of the intervention program was enrolled under controlled conditions in a hospital and also included exercise for 30 min for 5 days a week. In contrast, our WR period was performed on an ambulatory basis and participants were only advised to increase their activity levels. Therefore it is well possible, that differences in the study structure might lead to different results. Particularly differences in the study cohort, in this case a higher heterogeneity in our subjects might have a huge influence. As mentioned before, the gender-specific differences in WHR changes might also be due to higher basal levels in the male subgroup.

Metabolic parameters like TG and insulin sensitivity also showed an improvement after WR. This was accompanied by reduction of RQ during fasting conditions. As lower RQ is associated with an increased ratio of lipid oxidation compared to carbohydrate metabolism, these data indicated a switch from carbohydrate to lipid metabolism after WR. Notably FFA levels were not altered by

WR, suggesting that increased FFA oxidation may be balanced by increased synthesis (e.g lipolysis) resulting in unchanged circulating levels. This is in line with the reduction of fat mass by WR.

It is well known that the ability to switch between carbohydrate and lipid metabolism is impaired in obesity. This is usually depicted in an unchanged RQ in response to a meal or insulin infusion (Storlien, Oakes, and Kelley 2004; Galgani, Moro, and Ravussin 2008). It was also shown before that basal fasting RQ levels are different between lean and obese subjects. Glucose and FA uptake under fasting conditions were normal in obese subjects. However, a decrease in FA oxidation with a concomitant increase in FA storage was observed (Storlien, Oakes, & Kelley, 2004). Thus it is expected to observe an elevated fasting RQ in obese subjects. As we did not have a lean control group we are not able to make a statement about fasting RQ in comparison to normal subjects. However, we observed a significant decrease in RQ after WR. This shows an effect of WR on whole-body metabolism. A drop in RQ after WR indicates a higher ability to oxidate FA. This might be crucial for further weight development as obese patients who succeeded to maintain the body WR achieved initially by the very low calorie diet were characterized by a significantly lower RQ after WR (Hainer et al. 2000). Taken together, successful WR influences substrate specificity, leading to increasing amount of FA oxidation and might also be important for improvements in metabolic flexibility.

However the role of lipid metabolism regarding long-term weight maintenance as well as the underlying molecular mechanism is not well understood yet. To better understand the molecular basis of long-term body weight maintenance after the intended WR we analysed gene expression data of skeletal muscle and adipose tissue before and after WR.

6.2 Age and gender dependent effects on adipose tissue and skeletal muscle gene expression

Using PCA to assess the quality and evaluate potential experimental effects it showed a separation of gene expression data by gender. In detail, analyses revealed strong transcriptional differences between male and female subjects in adipose tissue and only minor differences in skeletal muscle.

As described before (Viguerie et al. 2012), sex differences were found in adipose tissue gene expression. We therefore had a closer look on functional clusters, including TCA cycle, glucose metabolism, FA β-oxidation, TG biosynthetic process, PPAR signalling and steroid hormone receptor signalling pathway. Interestingly the majority of involved genes were higher expressed in women. This might indicate a higher activity of lipid metabolism in female adipose tissue. However this assumption is based on expressional data only and is in some contrast to other studies reporting a lower fat oxidation in women (Nagy et al. 1996). In this study only healthy non- obese subjects were analysed. The fat oxidation rate was determined using the resting energy expenditure (REE) and not by direct measurements. They showed differences between men and

women in a healthy cohort. However, our cohort only included obese subjects, what might result in differences concerning the here described results. In a study from Schutz et al. (Schutz et al. 1992) a group of obese women were included to investigate fat oxidation. They found a clear correlation between fat oxidation and fat mass showing that gain of fat mass leads to increased FA oxidation. This was further supported by a second study investigating the effect of WR/fat mass loss on fat oxidation (Schutz et al. 1992). They could show that WR lead to decreasing levels of fat oxidation and summarized that both studies underlined the relation of fat oxidation and body fat mass. However, none of the studies measured enzyme activity or gene expression levels. Both were based on REE measurements. Schutz et al. refrained from investigating a male cohort. Comparing our data with both studies, we can only speculate that obese women show a higher lipid oxidative rate due to a higher fat mass. In general, we observed a higher body fat mass in the female group, which might further support the gene expression differences. Additional investigations concerning gender-specific differences in RQ levels might further support our assumption.

The higher levels of TG biosynthetic enzymes in our female subjects might indicate that women have a higher activity in terms of TG biosynthesis leading to increased accumulation of TG in adipose tissue and its expansion. Accordingly higher accumulation of subcutaneous adipose tissue was seen in females (Geer and Shen 2009). As our work is limited to expressional data further functional analysis will be needed.

Besides varying expression pattern between male and females we also investigated transcriptional differences between young and old subjects (old > 50 years and young < 50 years). It appeared that transcription in adipose tissue was almost not affected by age as we detected no differences between old and young subjects. In contrast, skeletal muscle transcription varied significantly between both age groups. Age related regulation of gene expression in skeletal muscle was even more pronounced than gender effects. The effect of aging on skeletal muscle is well known, among others in the context of sarcopenia (Forbes, Little, and Candow 2012). This process does not only affect muscle mass and strength but also cellular physiology and morphology. Although sarcopenia seems to be more likely in men and glycolytic muscle fibres (compared to oxidative) (Doria et al. 2012) we did not observe such a gender difference. However the small sample size of our male cohort might limit the reliability of this finding. Our data showed a lower expression of genes involved in TCA and glucose metabolism in older people which was in line with studies in mice and humans (Lee et al. 1999; Welle et al. 2003). Even if our data were only based on mRNA expression data, this might indicate impaired substrate utilisation in older subjects what is supported by previous observations by Short and colleges (Short et al. 2005). They showed reduced mitochondrial content, a decline in ATP production and a general reduced mitochondrial function in older human subjects.

Sarcopenia was also shown to have an impact on oxidative phosphorylation (Doria et al. 2012; Short et al. 2005). Similar to these reports, we found a decrease in oxidative and other

mitochondrial genes in older subjects indicating a decreased activity of respiratory chain and a decline in mitochondrial function. Further analyses in terms of functional assays will be required to better understand the functional implications of our findings.

In summary our data showed, similarly to other reports (Welle et al. 2003; Short et al. 2005; Welle et al. 2004) a decline in mitochondrial and metabolic gene expression. However, a more detailed analysis is needed for confirmation. Nevertheless, the age and gender dependent effects underline the mandatory requirement to adjust further analysis for gender and age.

6.3 Gene expression and phenotypic associations before weight reduction in obese subjects

Obesity is often linked to metabolic diseases (Flier 2004). Gene-phenotype associations in obese subjects before WR provide information of cellular processes potentially involved in obesity-associated metabolic traits. We therefore investigated the relationship between expression of cellular pathways and phenotypic parameters.

Inflammatory genes were linked to BMI before WR. Increased inflammatory response in metabolic tissue of obese subjects was already described before (Gregor and Hotamisligil 2011; Monteiro and Azevedo 2010). However, we focused only on pathways in this section and did not investigate single genes. We therefore cannot state the direction of this association. Nevertheless, identifying inflammatory pathways to be associated with BMI showed that our data is comparable to other studies and get thereby approved.

Further on, in line with the extensively described relationship between estimates of obesity and modified insulin signalling in adipose and skeletal muscle tissue (Shah, Mehta, and Reilly 2008; Zeyda and Stulnig 2009) we found a link between gene expression of insulin signalling genes and body weight or BMI. Moreover the expression data indicated an alteration of lipid metabolic genes in adipose tissue obesity. This was also supported by previous results indicating increased lipid storage and decreased lipid oxidation in obesity (Spalding et al. 2008; Kim et al. 2000). Taken together, this further supports our expression-based data set. Moreover, the above-described results showed that RNA-Seq data could be a useful tool to investigate associations between phenotypic parameters and molecular processes.

The functional relevance of our expressional data might be supported by the relationship of mitochondrial genes and lipid metabolic genes in adipose tissue to RQ as well as the link of myocellular lipid metabolic genes to FFA levels. Expression of genes involved in NADP metabolism and lipid biosynthetic processes in skeletal muscle tissue showed a relation to FFA levels. In general skeletal muscle metabolism is balanced between carbohydrates and FA. It has been shown that in obese subjects metabolism including mitochondrial metabolism is altered (Holloway, Bonen, and Spriet 2009). NADPH and its oxidized form NADP are important metabolites involved in a variety of

cellular processes. They are some enzyme relying on NADPH like glucose-6-dehydrogenase or the isocitrate dehydrogenase. Disturbances in the NADPH metabolism can lead to severe cellular dysfunctions (Mailloux and Harper 2010). Our data showed a correlation of FFA levels and NADP as well as lipid biosynthetic processes. However, the direction of this interaction was not investigated and needs to be analysed in the future. Nevertheless, these results showed a connection of plasma FFA levels and skeletal muscle metabolism in obese subjects. Similar results were seen in adipose tissue concerning the RQ. We found especially mitochondrial genes to be correlated with RQ. Energy metabolism in primary tissues like skeletal muscle and adipose tissue is highly dependent on well functioning mitochondria. As mentioned above changes in mitochondrial metabolism lead to severe metabolic dysfunction, e.g. detected in obesity (Kusminski and Scherer 2012). This correlation might indicate an important relation between adipose tissue metabolism and substrate utilisation (RQ). Again further analysis concerning single genes is needed.

Some limitations have to be mentioned. We analysed only obese subjects and a lean control group would be helpful. Thus, some of our findings may be specifically found in obese individuals, despite not being linked to body weight rather than metabolic changes in obese individuals. In general, our expression data do not provide evidence of any functional relevance. Therefore further functional analyses of candidate genes or gene patterns are urgently required. Finally, the here described results are cross-sectional in nature. Therefore we cannot determine whether gene expression causes the metabolic phenotype or vice versa. Again, functional studies will be required to address this issue.

6.4 Weight reduction-induced response of gene expression in adipose and skeletal muscle tissue

WR-induced changes in the tissue-specific gene expression may reveal possible cellular networks involved in weight regulation. Given the fact, that RNA-Seq was performed in a large number of individuals, our WR study could be regarded as rather unique. First, we confirmed previous data based on microarrays indicating that adipose tissue gene expression is highly affected by WR (Mutch et al. 2010; Viguerie et al. 2012). Differential expression analysis of our data set revealed over 8000 altered transcripts and an expected equal distribution of up- and down-regulated genes. In some contrast, skeletal muscle expression showed a rather small number of genes changed by WR. This was somewhat surprising and might be partially due to a higher intra-individual variability in skeletal muscle tissue expression. If the variability between subjects increases, gene expression differences need to be higher to become significant. This might at least in part explain the low number of differentially expressed genes in skeletal muscle tissue. In a study by Larrouy and colleagues (Larrouy et al. 2008) over 2000 genes showed a differentially expression pattern over WR in skeletal muscle. In contrast to our study Larrouy et al. only studied post-menopausal

women. This fact might have decreased the variance of gene expression changes in response to WR. As we included women and men at a broad range of ages the higher variability in skeletal muscle tissue expression changes observed in our cohort might be induced by the heterogeneity of the study cohort. This holds also true for the adipose tissue samples. However, as we observed several significant gene expression alterations, this might show that these changes are either stronger or more robust regarding the variability.

Further on, there are differences concerning the WR program. In our study subjects underwent a very low caloric diet for 12 weeks and lost about 12% of their body weight. In contrast Larrouy et al. performed a WR phase of 7-10 weeks which was followed by a weight stabilising phase of 4 weeks, resulting in an overall WR of 10%. The effect of modified body weight and composition might be the predominant driver of expressional changes. In contrast, a partially existing negative energy balance at the end of an acute WR may additionally influence expression levels. Therefore further studies are clearly warranted to better understand the here observed differences.

6.4.1 Adipokine expression changes over weight reduction

We aimed to confirm known data regarding WR to validate our data. Therefore we selected a panel of known adipokine transcripts. As reported before (Viguerie et al. 2012), we detected a significant reduction of leptin expression over WR. Other known adipokines like apelin and retinol binding protein 4 (RBP4) also showed a decreased expression after WR, which was again in line with previous data (Ouchi et al. 2011). Interestingly, we did not observe an increase in adiponectin expression. Several other studies showed that plasma adiponectin level increase by WR (Bobbert et al. 2005). It was also shown that obese subjects compared to lean individual have lower mRNA levels of adiponectin (Gabriella Milan et al. 2002; Degawa-Yamauchi et al. 2005). This also leads to the assumption that WR would results in increasing mRNA levels of adiponecting in adipose tissue. However, similar to our results Campbell and colleague described no changes in adiponectin expression after 6 month WR (Campbell et al. 2013). In general, WR induced effects on adiponectin secretion and expression are controversial discussed in the literature (Klempel and Varady 2011). It was also described before that adiponectin levels are affected by gender differences. In general, women have higher adiponectin levels (Iglesias et al. 2006). Our study included both gender and might therefore lead to different results. In a study using isolated adipocytes from healthy overweight patients, Skurk and colleagues (Skurk et al. 2007) found adiponectin secretion associated with adipocyte cell size while adiponectin expression in adipose tissue did not show any correlations to cell size. Even if we did not observe increased adiponectin expression in adipose tissue after WR, we also did not assess changes in circulating adiponectin levels. This hinderes the interpretation somehow. Furthermore adipocyte size was not analysed in our cohort to further elucidate this topic.

6.4.2 Differentially regulated genes in adipose tissue

Focusing further on differentially regulated genes our data indicated that predominantly metabolic genes involved in lipid metabolism were modified by WR in adipose tissue. This could be shown by several analytical approaches. In fact top differentially expressed genes in adipose tissue with a logFC over +/- 0.5 during 3 month WR included genes of unsaturated FA biosynthesis (SCD, FADS1, FADS2, ELOVL6). It seems that WR due to caloric restriction resulted in a down-regulation of expression of genes mediating unsaturated FA synthesis. This was in line with previous results observed e.g in the DIOGenes study (Viguerie et al. 2012; Mutch et al. 2010). Interestingly, we found additional differences in certain intracellular signalling transcripts involved in lipid metabolism, insulin signalling and differentiation. This was confirmed by gene set enrichment analysis showing insulin signalling to be altered by WR.

As several enzymes are primarily regulated by post-translational modifications our transcriptional data do not necessarily provide evidence for functional changes, although substantial changes might indicate a potential regulatory mechanisms in response to WR. Indeed, WR-induced changes at functional level (reduction of RQ without concomitant changes of FFA levels) (Toledo, Watkins, and Kelley 2006), support this assumption. Nevertheless, further functional validation of our transcriptional data is urgently needed.

In obese subjects high levels of basal lipolysis caused ectopic fat accumulation (Mcquaid et al. 2011). However, WR reduces ectopic fat accumulation due to increased FFA oxidation (Gray et al. 2003). In turn decreased FFA levels might lead to improved whole-body insulin sensitivity as well as adipose tissue-specific insulin sensitivity, as FFA are known to induce insulin resistance (K. Mai et al. 2010). Accordingly an increase in an estimate of whole-body (ISI_{clamp}) as well as an estimate of hepatic insulin resistance (HOMA-IR) was found in our cohort. Insulin acts on adipose tissue by inhibiting lipolysis (Arner 2005). Thus increased insulin sensitivity in adipose tissue might counteract stimulated lipolysis and lipid utilisation in adipose tissue during WR. Even if myocellular and whole-body insulin sensitivity is increasing by WR (Goodpaster et al. 1999; Wing et al. 1994), effects on adipose tissue-specific insulin efficacy are only minor topics in research. Data more than 45 years ago indicated an increased insulin sensitivity after WR in adipose tissue (Salans, Knittle, and Hirsch 1968). However, these data only included 5 obese individuals. Further data on adipose tissue insulin sensitivity showed that in T2DM patient not only liver and muscle tissue are insulin resistant but also adipose tissue was in a state of insulin resistance (Kotronen et al. 2008). Insulin resistance in adipose tissue leads to increased lipolytic activity and a higher efflux of FFA. This was further supported by findings showing a positive correlation between adipose tissue insulin resistance and hepatic lipid content (Korenblat et al. 2008; Kotronen et al. 2008; Lomonaco et al. 2012). This again indicates a higher FFA release from adipose tissue leading to ectopic lipid accumulation Our data not directly showed a decrease in lipolytic genes, however involved genes are strongly regulated by post-translational modification (Arner 2005) and

therefore we might not be able to detect changes on gene expression level. Concerning adipose tissue insulin sensitivity we also detected some genes involved in insulin response to be regulated by WR. In fact our data indicated a down-regulation of selected insulin target genes like GSK3B and FASN. Even if only based on transcriptional data, the apparently lower insulin response seems to be consistent with higher lipolytic activity of adipose tissue, indicated by decreased transcription of several lipogenic genes as well as circulating parameters after WR. This is very surprising as our data indicated an improvement in insulin sensitivity. However, as it was shown before by other, excessive FFA can lead to insulin resistance (Galgani, Moro, and Ravussin 2008). Based on only transcriptional data it is speculative to assume that a decreasing lipogenic potential together with a decrease in downstream genes of the insulin signalling could lead to higher FFA efflux. This higher FFA levels might lead in turn to a local adipose tissue insulin resistance. As we observed an improved liver and muscle tissue insulin sensitivity, this might on the other hand imply an improved FA oxidation in those tissues.

A decrease in lipogenic genes might lead to lower lipid storage and therefore to higher loss in fat mass over WR. It was shown before that subjects with transcriptional reduction of acyl-CoA synthase, a key enzyme of *de novo* lipid synthesis, after bariatric surgery were characterized by a higher loss in fat mass (Garrido-Sánchez et al. 2012). However, these authors focused only on a selected number of genes. In contrast, we used an unbiased approach but still found similar modifications. Interestingly, FFA levels in our cohort did not decrease during WR. This might reflect a higher capacity of skeletal muscle tissue to oxidise FFA and was supported by the decrease in RQ during WR.

Nevertheless, as this data is based on changes of mRNA expression, determination of protein levels and functional analysis of enzymatic activity would be desirable to confirm our results at gene expression level.

6.5 Gene expression changes are linked to body composition and lipid metabolism

Adipose and skeletal muscle tissues are metabolically active and play an important regulatory role in body homeostasis. Changes of gene expression due to WR have been shown before (Marquez-Quinones et al. 2010; Mutch et al. 2010). However, it is still not fully understood how these changes are linked to certain parameters of body composition and substrate homeostasis.

We analysed the impact on WR induced changes of estimates of obesity (BMI).

Concentrating on lipid metabolism pathways, especially expressional changes in SCD (stearoyl-CoA desaturase), LDHB (lactate dehydrogenase B) and ALDOC (aldolase C) showed a positive association with ΔBMI. SCD is involved in FA synthesis and our data indicated that higher WR is associated with stronger reduction of SCD mRNA expression. This supports our hypothesis of reduced lipogenic potential in adipose tissue after WR (see 6.4.2).

Our data might therefore indicate an effect of WR on lipid metabolic gene expression, which might reflect improved adipose tissue function after WR.

Our results did not reveal an association of changes at mRNA levels of skeletal muscle and WR-induced alterations of BMI.

We also investigated potential associations of gene expression and lipid metabolic parameters. FFA are the end product of TG breakdown in adipocytes, released into circulation. FFA are either shuttled into the liver or muscle to be metabolised. They can also be taken up by adipocytes and used for re-esterification into TG (Gaidhu et al. 2010; Zechner et al. 2012). Alterations of genes involved in lipid and carbohydrate metabolism are both connected to plasma FFA level alterations. In detail, genes involved in acetyl-CoA metabolism, FA metabolism and lipid synthesis were correlated. Acetyl-CoA is an important metabolite, connecting carbohydrate and lipid metabolism and playing a role in several regulatory loops (Dharuri et al. 2014). On single gene level WR-induced changes of adipose tissue key genes embedded in lipid synthesis were negatively related to ΔFFA. This implies that WR leads to reduction in expression of lipid synthetic genes (e.g SCD, FADS1, FADS2) with a concomitant increase in FFA levels. One might speculate that this reduction in lipid synthesis is accompanied by increased FFA release from adipose tissue. As previously discussed the lower RQ after WR indicated at least an increased oxidation of these released FFA. Surprisingly genes encoding lipid catabolism were also decreased by WR, which apparently contradicts such an assumption. However reduction of lipid synthesis and elongation genes was much higher compared to the observed decline in catabolic genes. Even if these are only transcriptional data, this points towards the shift of the anabolic/catabolic balance to a relative increased FA catabolism, which is likely to be the basis of our clinical finding, the reduction of body fat mass and body weight. Similar findings were shown before, indicating a catabolic phase during very low caloric diets (Bryson et al. 1996).

The positive association of WR-induced changes of adipose CPT1A (carnintie palmitoyltransferase 1A) expression and ΔFFA further supported this assumption. CPT1A is important to shuttle lipid moieties from the cytosol into the mitochondria for β-oxidation (Houten and Wanders 2010). Given the positive association between changes of FFA and CPT1, it is tempting to speculate that increased FFA levels due to higher release will be accompanied by a compensatory up-regulation of FFA oxidation.

Although we identified several functional links concerning gene expression changes and the phenotypic outcome of WR, further analysis are needed to elucidate these results.

6.6 Prediction of body weight after weight reduction and maintenance

In the previous section the association between gene expression and phenotypic characteristics was discussed. We could show that genes involved in lipid and carbohydrate metabolism were

affected by WR and associated to body composition and metabolic parameters. In a next approach we investigated the predictive impact of basal gene expression on future WR by analysing the effect on BMI after WR, adjusted also for basal BMI. Interestingly, none of the genes showed a correlation to the degree of WR. Thus basal gene expression was not relevant for short-term success of a diet-based WR intervention. As regulation of BMI underlies a complex network of inter-organ crosstalk including several tissues like liver, brain and muscle and adipose tissue we further focused on estimates of fat mass reduction. Improvement of waist circumference as a surrogate parameter of abdominal adiposity was predicted by a subset of basal genes involved in lipid metabolism. For instance, our data suggested that high levels of adipose ACADL (long-chain acyl-CoA dehydrogenase) before WR might result in higher reduction of waist circumference. ACADL is involved in the first step of β-oxidation by dehydrating the fatty acyl-CoA (Kurtz et al. 1998; Lea et al. 2000). A higher expression of ACADL should lead to higher flux through the β-oxidation and may result in higher mitochondrial activity including the TCA cycle. Next we found IHD2 (isocitrate dehydrogenase 2), a key regulator of the TCA cycle (Reitman and Yan 2010) positively linked to waist circumference after WR. Together this might imply that a higher basal mitochondrial activity by β-oxidation and TCA is beneficial in terms of improvement in abdominal adiposity. It was shown before that obesity and T2DM is accompanied by mitochondrial dysfunction in white adipose tissue resulting in a lower oxidative capacity (Patti and Corvera 2010; Kusminski and Scherer 2012). Several mouse and *in vitro* studies also revealed that an increase in oxidative phosphorylation is possible in adipose tissue and will be beneficial in terms of body weight regulation (Keuper et al. 2014; Vernochet et al. 2012; Kusminski et al. 2012). Our data also indicates an central role of mitochondrial oxidation in long-term regulation of metabolic health. However, further analysis concentrating on oxidative phosphorylation is needed.

This all may point towards an important influence of lipid metabolism in body weight regulation. However, we are not able to state how enzyme activity is related to mRNA levels and we therefore can only speculate about a functional relevance of our findings. The fact, that none of these predictions persisted after adjusting for multiple testing, may indicate, that the impact of a single gene is rather low. However 4 of 5 genes predictive for improvement of abdominal obesity represented genes of lipid metabolism. This may support the mentioned assumptions that basal state of lipid metabolism is important for subsequent degree of body WR.

Our results of gene expression of skeletal muscle tissue are less clear. Only single genes showed a correlation with BMI and waist circumference. However, we found that lower expression levels of the mitochondrial gene PFKM (phosphofructokinase) might be beneficial over WR. PFKM is an essential enzyme involved in glycolysis. It is important for the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate (Tirone and Brunicardi 2001). Lower expression levels of PFKM might indicate a lower flux through glycolysis and therefore increase lipid oxidation for energy generation. This is further supported by a decrease in RQ after WR indicating a higher usage of

lipids. Additionally higher expression of the β-oxidative gene VLCAD (acyl-CoA dehydrogenase, very long-chain) was fount to be beneficial in terms of body weight regulation. This again points towards a beneficial effect of FA oxidation in body weight regulation and metabolic health.

Expression of several lipid metabolic genes showed an association with TBF after WR. Lipolytic genes including MGL (monoglyceride lipase) and LIPE (HSL, hormone sensitive lipase) showed a positive association. One hallmark of obesity is the accumulation of ectopic lipids, like in skeletal muscle (intramyocellular lipids). This can effect the muscular metabolism and lead to insulin resistance (Toledo and Goodpaster 2013). Higher expression of lipolytic genes in skeletal muscle might lead to reduction of these intramyocellular lipids. As we did not measure intramyocellular lipids further confirmative data is needed.

The majority of subjects is not able to maintain the reduced body weight after intended WR (Maclean et al. 2011). Nevertheless a high variability of regain is known (Hensrud et al. 1994). We aimed to analyse, whether this variability could be explained by tissue-specific gene expression.

Further analysis was focused on BMI after the 12-month life-style intervention as well as after 18 month of initial WR. We found expression of several lipid metabolic genes after WR to be predictive for the development of the body weight. Our data indicated that subjects with higher expression of lipid storage (FABP4 and PLIN2), β-oxidative (HADHA and HADHB) and lipolysis genes (NPR1) are able to maintain their weight after 12-month lifestyle intervention. This may indicate an important role of β-oxidation on long-term body weight maintenance. In line with the results after 12 month, data from the literature could show that obese subjects possess an increased lipolytic rate and a decrease in TG storage. This could result in higher efflux of FFA from the adipocytes to other tissue like liver or muscle (Attie and Scherer 2008). High expression of lipid synthetic (FADS1, FADS2, ELOVL5 and SCD) and glucose metabolic genes (ALDOC, IDH1 and PDHA1) are linked to weight regain after 12 month. We showed before that lipogenesis in adipose tissue was down-regulated over WR and associated with BMI. Similarly we found the tendency of lipid oxidative genes to be beneficial in terms of WR/fat mass loss. After 12-month lifestyle intervention the participant still showed a lower body weight as the control group. However, we included the randomization state in our analysis.

After 18 month expression of genes involved in β-oxidation (ACADS and ACOX1) were positively associated with the BMI indicating a different regulation as seen 12 month after WR. We additionally detected the lipid synthetic gene FASD2 (FA desaturase 2) and the glucose metabolic gene IDH1 (isocitrate dehydrogenase 1) to be linked to weight gain after 18 month. This is in line with the results after 12 month.

However, the here described differences between BMI_{T12} and BMI_{T18} might be due to the intervention. After 12 month all participants enter a free-living phase and did not receive any counselling. Comparing the intervention and the control group after 18 month there were no significant differences in body weight. This further showed the effect of the lifestyle intervention.

It might additionally indicate that metabolic changes or metabolic flexibility in adipose tissue are important in long-term body weight maintenance. As it was shown recently, adipocytes are able to switch between glucose and lipids oxidation depending on substrate availability to maintain intracellular ATP concentration (Keuper et al. 2014). However, it is not clear how this might affect body weight or body weight maintenance.

Comparing the data observed in adipose tissue with skeletal muscle gene expression, we found similar associations regarding lipid catabolism. Interestingly, UCP2 (uncoupling protein 2) and CPT2 (carnitine palmitoyltransferase 2) expression seem to be beneficial for body weight maintenance. UCP2, similar to UCP1 is involved in uncoupling of proton transport in mitochondria. It was also shown that UCP2 participates in the regulation of energy metabolism and in some cases is linked to obesity (Patrick Schrauwen and Hesselink 2002). Higher levels of UCP2 mRNA in adipose and skeletal muscle were found in obese subjects, which showed a reduction over WR (P Schrauwen et al. 2000). However, the influence of UCP2 is still discussed controversial (Stone and Yang 2006). Our data suggest a beneficial role of UCP2 in long-term body weight maintenance, indicating that subjects with a higher level of UCP2 after WR will be able to maintain their reduced body weight. Higher levels of CPT2 expression also seem to be assigned to maintaining a lower body weight. Together with higher level of UPC2 this might indicate an advantageous effect of increased mitochondrial metabolism in skeletal muscle.

We further looked for physiological and circulating parameters reflecting lipid metabolism to confirm the assumption indicated by transcriptional data. As described before in this work, we used plasma FFA levels and RQ. In line with the above described link concerning lipid catabolic gene expression, we observed the tendency of a lower RQ in subjects maintaining the lost body weight. This indicated a switch to lipid oxidation. Interestingly, the maintainer cohort was also characterised by higher FFA levels. This data was additionally supported by the investigation of plasma AC levels. We identified AC C18:1 to be predictive in terms of long-term body weight maintenance. It was shown before that AC might be an indicator for incomplete β-oxidation (Adams et al. 2009; M. Mai et al. 2013; Mihalik et al. 2010). However, our data showed a negative correlation of AC C18:1 levels and RQ after WR and a positive association to FFA levels. We did not observe any correlation with medium- or short-chain AC. This further supports our hypothesis that body weight maintenance is associated with myocellular lipid oxidation.

Taken together the expressional and the phenotypic data, we hypothesize that a decreased lipogenic capacity as well as a higher metabolic flexibility in adipocytes together with higher mitochondrial activity (lipid oxidation) in skeletal muscle tissue might be beneficial in terms of long-term body weight maintenance. Similar observation were shown in a study analysing metabolic factors in weight regain in obese subjects (Valtueña, Salas-Salvadó, and Lorda 1997). This group showed that differences in RQ could explain differences in body weight maintenance. Similar to our results, individuals with a lower RQ were able to maintain the WR indicating that a

higher metabolic flexibility might be beneficial in terms of long-term body weight maintenance. Further investigation of adipocyte metabolism as well as lipid metabolites like AC in terms of body weight maintenance will be necessary to address the remaining questions.

6.7 Alterations in estrogen receptor signalling in female obese subjects

Several studies have shown that the female sex hormone estrogen is involved in metabolic functions like fat mass distribution and might be protective in terms of T2DM and cardio-metabolic diseases (Cignarella, Kratz, and Bolego 2010; Szmuilowicz, Stuenkel, and Seely 2009; Pedersen et al. 2004).

Investigating single genes of interest, we focused our analysis on the estrogen receptor (ER) network. The steroid hormone receptor signalling pathway was identified in this work to be different expressed between male and female participants. Further on, it was already described that ERα levels are decreased in obese subjects (Gao and Dahlman-Wright 2013).

We detected a significant up-regulation of ERα expression and a significant down-regulation of ERβ over WR in females. This was not visible in male subjects and was independent of age and thereby menopause. Estrogen can affect lipolysis as well as lipogenesis and has an important role in the regulation of fat distribution (Pedersen et al. 2004). We found positive correlations of ERβ with several lipogenic enzymes including SCD, LPL, FADS1, FASN and PPARγ before WR. According to the literature ERβ was positively involved in the regulation of PPARγ transcription and its target genes (Gao and Dahlman-Wright 2013; Barros and Gustafsson 2011; Foryst-Ludwig et al. 2008). ERβ expression changes showed a positive correlation with changes in transcripts of lipogenic enzyme. In contrast, WR-induced alteration of ERα expression correlated negatively with expressional changes of lipogenic enzymes. This was not observed before WR.

Lipolytic genes showed a positive correlation with ERβ before WR. This indicates a positive regulation of lipolysis by ERβ according to the literature (Foryst-Ludwig & Kintscher, 2010). However, this was only found in terms of ERβ expression.

We further investigated potential associations of involved genes and phenotypic parameters. We found a negative correlation of ERα and waist circumference as well as TBF before WR indicating that females with lower expression of ERα have a higher amount of fat tissue. This is in line with data from mice showing increased fat mass in ERα knockout animals (Faulds et al. 2012) and human data showing decreased ERα levels in obese subjects (Gao and Dahlman-Wright 2013).

Both ER isoforms are known to lead to a suppression of lipogenesis. However, in our data we have seen a positive association of ERβ and lipogenic genes, leading to an induction of lipogenesis. This might indicate a dysregulation of ERβ in adipose tissue of obese women. The outcome could be an accumulation of subcutaneous abdominal adipose tissue leading to higher waist circumference. During WR ERβ levels showed a decrease. This might further support the hypothesis of an

unfavourable effect of high levels of ERβ activity in obese subjects. In contrast, the observed increase in ERα expression might be beneficial. Our data indicate that increased expression of ERα during WR led to decreased expression of lipogenic genes and an indirect decreased in fat mass accumulation. This is in line with a study in ERα-knock out mice, which showed an obese phenotype (Naaz et al. 2002). If mice were ovariectomised (leading to removal of ERβ signalling), these mice showed decreased body weight, adipocyte size and adipose tissue weight. The authors therefore speculated that the two ER mediate opposite metabolic effects (Naaz et al. 2002). Nevertheless, more functional data regarding regulation of the ER network in humans and the involvement in body weight and composition is required.

6.8 Regulation of myostatin expression over weight reduction

MSTN is a negative regulatory of muscle growth and the decrease in expression and plasma levels might play an important role in preventing higher skeletal muscle loss due to WR.

Interestingly, MSTN expression levels as well as protein levels measured in a sub-cohort during WR showed a significant reduction of MSTN. Reduced levels of MSTN during WR were shown before (G. Milan et al. 2004; Park et al. 2006). MSTN knockout mice not only showed an increased muscle mass but also a decrease in fat mass and an abnormal glucose metabolism (McPherron and Lee 2002). We further investigated possible associations of MSTN expression and phenotypic parameters as well as genes involved in glucose homeostasis. As observed before (Zhao, Wall, and Yang 2005; T. Guo et al. 2009) our data also showed that high levels of MSTN are associated with insulin resistance. We also found a positive correlation between MSTN mRNA and BMI as well as abdominal fat mass (waist circumference) before WR.

On gene expression level we found a negative correlation between MSTN and MyoD (myogenic determination factor 1). Langley and colleagues showed before that MSTN inhibits muscle differentiation by down-regulating MyoD (Langley 2002). This data might indicate that reduced mRNA levels over WR might lead to higher levels of MyoD, leading to a higher potential of skeletal muscle differentiation. It might therefore be a protective effect against muscle mass loss due to WR.

Regarding metabolic gene expression we found several negative correlations to changes in MSTN levels. MSTN was shown before to have an important role in metabolic control (T. Guo et al. 2009). Interestingly, we saw a negative effect of MSTN expression on the glucose transporter GLUT4 (SLC2A4) as well as lipid catabolic genes involved in FA transport and β-oxidation (CPT1B, CPT2, HADHA, HADHB). This might further indicate that decreased levels of MSTN over WR might be protective against higher muscle mass loss. A reduction of MSTN over WR might lead to higher lipid oxidative capacity and insulin sensitivity. As mentioned before this data is only based on transcriptional data and further studies will be necessary to support our theory.

In summary, using our RNA-Seq data we showed that the expression landscape of adipose tissue is altered by WR and might be predictive in terms of long-term body weight maintenance. This data can be useful for further investigation of metabolic dysregulation in obese subjects.

7 MATERIAL AND METHODS

7.1 Study design and phenotypic assessment of the MAINTAIN cohort

7.1.1 Setting and Participants

156 overweight or obese subjects (120 female and 36 male) (BMI ≥ 27 kg/m^2) participated in a structured WR program for 12 weeks. Participants were initially screened for any systemic disease or biochemical evidence of severe hepatic or renal dysfunction, for serious health problems and the intake of medication. A recent weight change above 5 kg during the last 2 months, changes of smoking or diet behavior during the last 3 months were considered as exclusion criterion. Furthermore subjects with severe chronic diseases like instable coronary heart disease, severe renal insufficiency (eGFR< 30 ml/min), liver diseases, severe psychological diseases, severe endocrine disorders, cancer, chronic infections or comparable chronic disorders were excluded. Except of thyroxin, all drugs modifying energy homeostasis and body weight were not allowed during this trial. The study protocols were approved by the Institutional Review Board of the Charité Medical School, Campus Benjamin Franklin and all subjects gave written informed consent.

7.1.2 Study design

WR phase: The protocol of the standardized 12 weeks WR program consisted of three parts: caloric restriction, nutritional counseling and physical exercises. The caloric restriction was based on a replacement of all 3 meals by a very-low energy formula diet (800 kcal, Optifast 2, Nestlé HealthCare Nutrition GmbH, Frankfurt am Main, Germany) for 8 weeks, provided by the trial team. An advise was given to the participants not to consume any additional food., An additional consumption of 2 cups of low-starch vegetables was allowed during this phase, if subjects were not able to strictly keep on caloric restriction. The amout of additional food had to be documented. After the 8 weeks of caloric restricotion, the diet was switched to an energy-reduced healthy diet (distribution of macronutrients: carbohydrates 35-45%, fat 25-35%, and protein 25-30%) to facilitate further WR. A daily calorie intake of approximately 1500 kcal was recommended.

12 months randomized weight maintenance phase: Subjects with a loss of a minimum 8 % of body weight during the WR period were randomized (n=143). Subjects were distributed into an intervention and a control group, using a stratified randomization. Stratification considered gender and body weight at baseline (3 BMI strata). Subjects of the control group did not receive any form of counseling. In contrast, subjects in the intervention group continued to obtain

advice and counseling for the next 12 months. 36 meetings were offered every participants of the intervention group during the weight maintenance period. In detail, weekly group sessions were performed for the first 16 weeks, comparable to the WR phase. Afterwards meetings took place on a fortnightly basis over a period of two months. In the final period meetings occurred once a month until the end of the maintenance phase.

The dietary advices given to the intervention group were similar to the final phase of the WR intervention. Further WR was allowed. In case of body weight gain within this intervention period, a lower energy intake (500 kcal below the calculated energy demand) was recommended.

A supervised physical activity regime was provided for the first 12 weeks of the weight maintenance period. Afterwards, participants were encouraged to participate in sports at least twice a week.

6 month follow-up period: After twelve months all subjects underwent a free living period of six months without any further active intervention.

7.1.3 Phenotyping

All participants underwent a comprehensive phenotyping before (T-3), after (T0) WR, after 12 months (T12) and after 18 month (T18). Each subject was studied using a five-day protocol. This included anthropometric, hormonal and metabolic assessment using an oral glucose tolerance test, indirect calorimetry and bioimpedance analysis, hyperinsulinemic euglycemic clamp as well as biopsies from skeletal muscle and adipose tissue before and WR. In total biopsies were taken from 119 subjects.

In detail, following a 10-hour overnight fast all subjects were investigated in the clinical research center at the Charité Medical School, Campus Benjamin Franklin at 8.00 a.m. The respiratory quotient was estimated after a 20 minute resting period by indirect calorimetry (Vmax ENCORE, CareFusion Germany 234 GmbH, Germany). Bioelectric impedance analysis was used to measured body composition on resting participants using AKERN BIA 101 (SMT medical GmbH & Co. KG, Würzburg, Germany). Total body fat (TBF) was additionally measured estimating the skinfold thickness (Durnin and Womersley 1974). The amount of TBF in % was calculated by

$$\left(\frac{4.95}{\frac{a-(b*\log_{10}(d_{biceps}+d_{triceps}+d_{suprillica}+d_{subscapular})}{1000}}-4.5\right)*100$$

d = skinfold thickness in mm; $a_{♂}$ = 1176.5; $b_{♂}$ = 74.4; $a_{♀}$ = 1156.7 ; $b_{♀}$ =71.7

At 9.00 a.m. an oral glucose tolerance test with 75 g glucose was performed as described previously (K. Mai et al. 2007). Blood samples were taken at 0, 30, 60, 90, 120 and 180 min.

A hyperinsulinemic-euglycemic clamp was performend on a different day as described previously (K. Mai et al. 2007). In brief, 40 mIU · m^{-2} · min^{-1} human insulin (Actrapid®, Novo Nordisk, Bagsvaard, Denmark) and a variable infusion of 10 % glucose (Serag Wiessner, Naila, Germany) was used. Capillary glucose concentration was monitored every 5 minutes and kept stable between 4.0 and 4.9 mmol/l by variation of the glucose infusion rate. Blood samples were collected before the clamp and at least two hours after starting during steady-state conditions. All blood samples were centrifuged, and plasma and serum samples were frozen immediately at -80°C.

Biopsies were taken at T-3 and T0 from altered body sides. Abdominal subcutaneous adipose tissue samples were gained (0.5 to 1.0 g) by repeated needle biopsies from the periumbilical region using a 12 G biopsy-cut needle (CR Bard GmbH, Karlsruhe, Germany). Muscle biopsies were taken from the gastrocnemius muscle using the same approach. The skin was anesthetized with 1% lidocaine without epinephrine, a skin incision (3-4 mm) was made and the biopsies were obtained as discribed above. Samples were snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

7.1.4 Laboratory tests

Capillary blood glucose was measured using the glucose oxidase method (Dr. Müller Super GL, Freital, Germany). TG were measured by standard laboratory methods using Cobas ISE direct and c111 Analyzer (Roche Diagnostics, Mannheim, Germany) and Sysmex XE 5000 (Sysmex Deutschland GmbH, Norderstedt, Germany). Serum insulin was measured using fluoroimmunometric assay (AutoDelfia; Perkin Elmer, Rodgau, Germany) (inter-assay CV 2.3 - 3.5%, intra-assay CV 1.7 - 2.4%). FFA were quantified in serum using a commercially available colorimetric assay (NEFA HR2, Wako, Neuss, Germany) performed on ABX Pentra 400 (HORIBA ABX, Montpellier, France) (inter-assay CV < 5%, intra-assay CV < 1%). Leptin was analyzed using commercial ELISA (R&D Systems, Abingdon, UK) (inter-assay CV 3.5 - 5.4%, intra-assay CV 3.0 - 3.3%). Acylcarnitine (AC) species were assessed in plasma samples by LC-MS/MS using commercially available MS kit for newborn screening (MassChrom® Amino Acids and Acylcarnitines from Dried Blood; Chromsystems, Munich/Germany) as previously described (Mihalik et al. 2010; Metz et al. 2012).

7.2 RNA Sequencing

In total, 178 adipose tissue biopsy samples and 216 skeletal muscle biopsy samples were used for RNA extraction and sequencing.

7.2.1 RNA isolation from human tissue

The RNA isolation was performed with TRIZOL® reagent (life technologies, Darmstadt, Germany) following the manufactures instruction. In short, biopsy samples were carefully thawed on ice in 500 µl TRIZOL® reagent. Homogenization of tissue was performed with a rotor-stator homogenizer (IKA Labortechnik, Staufen, Germany, Ultra Turrax T25 basic, disperging tool S25N-8G (for adipose tissue) and disperging tool S10N-5G (for muscle tissue). In general 5 cycles for about 10 seconds were performed or until completely disruption of tissue samples. After each cycle, the homogenzier was washed with 500 µl TRIZOL® reagent, which was added to the tissue sample after complete homogenization. In total 1 ml TRIZOL® reagent was used for tissue homogenization.

For adipose tissue samples, we performed two centrifugation steps at 4°C, 13.000 rm for 10 min to clear samples from lipids. 200 µl chloroform (Roth, Karlsruhe, Germany) was added to each sample, mixed by inverting and incubated for 5 min at room temperature. Phase separation was achieved by centrifugation at 4°C, 12.000 rpm for 15 min. The upper, aqueous phase was transferred into a new tube and 500 µl isopropernol (Sigma-Aldrich, Steinheim, Germany) was added. The mix was incubated for 20 min at room temperature and centrifuged at 4°C, 14.000 rpm for 30 min. The supernatant was discarded and the RNA pellet was washed twice with 1 ml 75 % ethanol in RNase-free water and centrifugation at 4°C, 14.000 rpm for 5 min. The dried RNA pellet was re-suspended in 33 µl RNase-free water. RNA concentration was measured with the NanoDrop 2000/2000c Spectrophotometers (Thermo scientific, Schwerte, Germany).

7.2.2 Library preparation

1 µg of total RNA was used for library preparation (TruSeq RNA sample Preparation Kit A and B, Illumina, San Diego, USA). First, poly-T beads were used to purify mRNA containing ploy-A tail. The mRNA was fragmented and primed. In a second step double stranded cDNA was synthesized. During fragmentation, overhangs are created which are converted into blunts end to avoid self-ligation. To avoid ligation of blunt ends, a single adenine is added to the cDNAs. This also simplifies the adapter ligation, since they possess of a thymidine. Adapter 1-12 were used and ligated to the cDNA.. To enrich cDNA fragments containing adapter sequences on both ends, a short 15 cylce polymerase chain reaction (PCR) using adapter specific primers was done. Library concentration was measured with the Quibit® dsDNA HS Assay kit (life Technologies,

Darmstadt, Germany) and quality was assessed with the Bioanalyzer (Agilent DNA 1000 kit, Agilent, Waldbronn). 6 samples were pooled and loaded onto the flow cell.

7.2.3 Sequencing

Sequencing was performend by 2 time 100 cycles of paired end reads on a HiSeq2000 platform (Kits: TruSeq PE Cluster Kit v3 - cBOT – HS and TruSeq SBS Kit v3- HS 200 cycles (illumina) (in cooperation with Prof. N. Hübner and Dr. W. Chen, Max-Delbrück-Centre for molecular medicine, Berlin).

7.2.4 Raw image data processing

Further data analysis was done using CASAVA (v1.8) to gain FASTQ files. In short, CASVA was used for image analysis, base calling and de-multiplexing of multiplexed samples.
FASTQ files were aligned and mapped to the human genome (Homo_sapiens.GRCh37.66) by TopHat. BAM files were either used directly for quality assessment or for raw count extraction via HTSeq (in cooperation with Prof. Dr. N. Hübner, Max-Delbrück-Centre for molecular medicine, Berlin).
Sequencing quality and depth was checked looking at aligned and perfect mapped reads.

7.3 RNA sequencing data analysis

All analysing steps were performed in R (version 3.1.1) (R Core Team, 2014), if not indicated otherwise.

7.3.1 Quality control

The TopHat output was analysed concerning mapped (reads could be mapped to potential genomic side), proper-paired (pairs of read 1 and read 2 which could be mapped correctly together), read 1/read 2 (both reads of a paired-end sequencing analysis) and singletons (reads without a mate-pair).
Samples with a high number of singletons (> 500000 reads) and a low number in proper paired (< 15000000 reads) were excluded. After quality control the final data set included 150 adipose samples (75 subjects before after WR) and 174 skeletal muscle samples (87 subjects before and after WR).

7.3.2 Normalization of count data

For further analysis of the count data, normalization was done using the R package DESeq2 (Love, Anders, and Huber 2014).The count data was normalized for different library size and within-sample variability (for further explanations see 7.3.4).

7.3.3 Cluster analysis of count data

As an unbiased approach to investigate data structure principle component analysis (PCA) was used. The used function is based on calculating single value decomposition. Log2-transformed of normalized top 1000 genes selected by highest inter-sample variance were used for data analysis. The first two principal components were plotted.

7.3.4 Differentially expressed genes

Differential expression was calculated using raw counts per gene calculated by HTSeq and the R package DESeq2 (Love, Anders, and Huber 2014). Modelling in the DESeq2 package is based on negative binominal distribution. It estimates the mean and the dispersion of all samples and computed the log fold change expression between different conditions per gene. The dispersion is in this case equal to the biological coefficient of variation and represents the squared coefficient of variation of the expression levels between each sample under the same condition (here: before and after WR) (Anders et al. 2013). Analysing different expressed genes between old and young subjects, age groups were set to old > 50 years and young < 50 years.
For differential expression estimation the Wald test with a paired design matrix (~*patient+time*) was used and p-values were adjusted for multiple testing using the Bejamini & Hochberg method. Genes were assumed to be differentially regulated with a false discovery rate (FDR) < 5% (p-value < 0.05).

7.3.5 Estimation of gene-phenotype associations

Linear regression modelling was used to determine gene-phenotype associations. Either the normalized expression before or the log fold-change of gene expression changes over WR was used. Phenotypic parameters were similarly added using either before WR or the change during WR (T0 to T-3). The model was adjusted for confounders. Anthropometric parameters including BMI, waist and total body fat were adjusted for sex and age. Metabolic parameters including free-FA and RQ were adjusted for sex, age and BMI before WR. For association of expression levels before WR and anthropometric parameters (BMI) the linear model was adjusted for sex, age and BMI before WR. In case of associations between gene expression after WR and parameters after 12 and 18-month maintenance confounders included sex, age, intervention and

BMI after WR. AC species were grouped in short- (C2- and C4-AC), medium- (C6- to C14-AC) and long-chain AC (C16- to C22-AC).

7.3.6 Gene-Phenotype Correlation network

For graphical visualization the correlation data was plotted using the R package qgraph (Epskamp et al. 2011). The cut off was set to a rho > 0.2. The line strength and colour intensity gives the strength of the correlation (higher intensity and stronger line = rho > 0.4).

7.3.7 Pathway analysis

7.3.7.1 Differentially altered pathways

To estimate differentially altered pathways the R package GSVA (Hänzelmann, Castelo, and Guinney 2013) was used. This methods estimates gene sets using the entire expression set and not only a sub-list of specific genes of interest. In contrast to other Gene Set Enrichment Analysis (GSEA) the here used method is an unsupervised approach, as the sample matrix is not based on the phenotypic parameter. In this work, the GSVA package was used to estimate relative differences in pathways over the three-month WR. Therefore, normalized count data was used to determine alternated pathways. First, the expression data is used to calculate a sample-wise enrichment score (ES) by determining highly and lowly expressed genes in the sample taken the distribution into account. The ES were calculated as the maximum distances of the random walk using a bimodal distribution. Genes were rank to their sign and the minimum sizes of each set were set to 5. A linear regression model implemented in the limma package (Ritchie et al. 2015) was used to determine the differentially regulated pathways over WR.

7.3.7.2 Determining pathways from single gene lists

To group identified genes into pathways, we used the Internet database DAVID (the database for annotation, visualization and integrated discovery) Bioinformatics Resources 6.7 (Huang et al. 2009; Huang, Sherman, and Lempicki 2009).

DAVID is an online-based integrated biological knowledgebase where gene lists can be analysed concerning their biological function. We used the function “Functional annotation chart” to group a gene list of interest into biological functions. This included several different databases like GO terms, KEGG pathways, sequence features and others. For this analysis the EASE score threshold was set to 0.05, giving only significant enriched genes. Stringency was further increased by only including terms with a number of involved genes higher 5 (count threshold).

7.3.7.3 Graphical interphase for DAVID gene clusters

Gene lists of differentially expressed genes were used for functional annotation clustering by DAVID. This estimated the relationship among the annotations terms and is useful to further interpret biological modules. Clusters therefore do not show single functional categories (as in the functional annotation chart) but clusters of these categories. The "classification stringency" was set to "high" to get a lower number of more specific clusters. For visualization the R package FGNet (Aibar et al. 2014) was used.

7.4 Quantitative real-time PCR (qPCR)

DNase digestion (DNase 1, Thermo scientific, Schwerte, Germany) and reverse transcription was performed according to the manufactures protocol (Revert-Aid M Mul V Reverse Transcriptase, Thermo scientific).

10 ng cDNA was used per qPCR reaction (Applied Biosystems, life technologie, Darmstad, Germany). The total reaction volume was 10 µl and Maxima SYBR Green/Rox qPCR Master Mix (2x) (Thermo scientific) was used. Primers are listed in Table 35. Data was evaluated using the $2^{-\Delta\Delta ct}$ method with cyclophilin, actin and GUSB as internal control.

Table 35: Primer sequences used for the qPCR.

Gene name	Sequence forward	Sequence reverse
cyclophillin	TGTGAAGTCACCACCCTGACACAT	AGACAAGGTCCCAAAGACAGCAGA
actin	ACAGAGCCTCGCCTTTGC	CACGATGGAGGGGAAGAC
GUSB	AAGTGGTGCGTAGGGACAAG	TTGCTCACAAAGGTCACAGG
SHIP2	AGCTTCCTGGTCCGAGACA	GGTCTGCACAGCCAAGAAAT
PGC1a	GGGAAAGTGAGCGATTAGTTGAG	CATGTAGAATTGGCAGGTGGAA
INSR	GGCAACATCACCCACTACCT	GGCCGAATCCTCATACTCAC
IRS1	TCACAGCAGAATGAAGACCTAAATG	TGAGTTAGAAGAGGATTTGCTGAGG
AMPHa1	AAACATATGCTGCAGGTGGA	TGATGGATCCTCAGGAAAGA
p110a	CGTGCATGTGGGATGTATTT	TGGTCGCCTCATTTGCTC
PPARg	GAGCCCAAGTTTGAGTTTGC	TGTCTTCAATGGGCTTCACA
SCD	ACACTTGGGAGCCCTGTATG	GACGATGAGCTCCTGCTGTT
NPR3	GTGGCCTAGAAGAATCGGCA	GGGTTCGCCTCTCAATGGTT

7.5 Enzyme linked immuosorbent assay (ELISA)

Plasma myostatin was measured using the ELISA kit (R&D Systems, Abingdon, UK). The assay was performed according to the manufactures protocol.

7.6 Statistics

All statics were performed using R (version 3.1.1). The here presented data is expressed as mean value plus/minus standard error of the mean (SEM). Comparison of subjects before and after

WR or between male and females was done using a pairwise *t* test or (in case of non-normal distributed data) a Wilcoxen signed-rank Test. Normal distribution of data was tested by Shapiro-Wilk test. If indicated, P-values were adjusted to multiple testing using the Benjamini and Hochberg method. Pearson correlation coefficients were used to investigate associations of single gene-phenotype associations. Changes of phenotypic parameters over the three-month WR were calculated (after WR – before WR) and expressed in %. If not indicated otherwise significance was assumed as $p \leq 0.05$. A clinically significant weight regain was defined as an increase of BMI between T0 and T12/T18 of at least 3 % of the BMI at T0 (weight-regain group (regainer)). Subjects who gained less than 3% or lost body weight during that period were classified as weight-maintenance group (maintainer).

8 APPENDIX

8.1 Literature

Adams, Sean H, Charles L Hoppel, Kerry H Lok, Ling Zhao, Scott W Wong, Paul E Minkler, Daniel H Hwang, John W Newman, and W Timothy Garvey. 2009. 'Plasma Acylcarnitine Profiles Suggest Incomplete Long-Chain Fatty Acid B -Oxidation and Altered Tricarboxylic Acid Cycle Activity in Type 2 Diabetic African-American Women 1 – 3.' *The Journal of Nutrition* 139: 1073–81. doi:10.3945/jn.108.103754.1073.

Aibar, Sara, Celia Fontanillo, Conrad Droste, and Javier De Las Rivas. 2014. 'FGNet Functional Gene Networks Derived from Biological Enrichment Analyses', 1–34.

Alemany, Marià. 2011. 'Utilization of Dietary Glucose in the Metabolic Syndrome.' *Nutrition & Metabolism* 8 (1): 74. doi:10.1186/1743-7075-8-74. http://www.nutritionandmetabolism.com/content/8/1/74.

Anders, Simon, Davis J McCarthy, Yunshun Chen, Michal Okoniewski, Gordon K Smyth, Wolfgang Huber, and Mark D Robinson. 2013. 'Count-Based Differential Expression Analysis of RNA Sequencing Data Using R and Bioconductor.' *Nature Protocols* 8 (9): 1765–86. doi:10.1038/nprot.2013.099. http://www.nature.com/doifinder/10.1038/nprot.2013.099.

Apostolopoulou, Martha, Christos Savopoulos, Konstantinos Michalakis, Simon Coppack, Theodoros Dardavessis, and Apostolos Hatzitolios. 2012. 'Age, Weight and Obesity.' *Maturitas* 71 (2). Elsevier Ireland Ltd: 115–19. doi:10.1016/j.maturitas.2011.11.015. http://dx.doi.org/10.1016/j.maturitas.2011.11.015.

Arner, Peter. 2005. 'Human Fat Cell Lipolysis: Biochemistry, Regulation and Clinical Role.' *Best Practice & Research. Clinical Endocrinology & Metabolism* 19 (4): 471–82. doi:10.1016/j.beem.2005.07.004. http://www.ncbi.nlm.nih.gov/pubmed/16311212.

Attie, A. D., and P. E. Scherer. 2008. 'Adipocyte Metabolism and Obesity.' *The Journal of Lipid Research* 50 (Supplement): S395–99. doi:10.1194/jlr.R800057-JLR200. http://www.jlr.org/cgi/doi/10.1194/jlr.R800057-JLR200.

Barros, R P, and J A Gustafsson. 2011. 'Estrogen Receptors and the Metabolic Network.' *Cell Metab* 14: 289–99. doi:S1550-4131(11)00312-3 [pii]\r10.1016/j.cmet.2011.08.005. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21907136.

Blaak, E. E. 2004. 'Basic Disturbances in Skeletal Muscle Fatty Acid Metabolism in Obesity and Type 2 Diabetes Mellitus.' *Proceedings of the Nutrition Society* 63 (02): 323–30. doi:10.1079/PNS2004361. http://www.journals.cambridge.org/abstract_S0029665104000436.

Bobbert, Thomas, Helmut Rochlitz, Uta Wegewitz, Suzan Akpulat, Knut Mai, Martin O Weickert, Matthias Möhlig, Andreas F H Pfeiffer, and Joachim Spranger. 2005. 'Changes of Adiponectin Oligomer Composition by Moderate Weight Reduction.' *Diabetes* 54 (9): 2712–19. doi:54/9/2712 [pii]. http://www.ncbi.nlm.nih.gov/pubmed/16123361.

Bruun, J. M. 2006a. 'Diet and Exercise Reduce Low-Grade Inflammation and Macrophage Infiltration in

Adipose Tissue but Not in Skeletal Muscle in Severely Obese Subjects.' *AJP: Endocrinology and Metabolism* 290 (5): E961–67. doi:10.1152/ajpendo.00506.2005. http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.00506.2005.

Bruun. 2006b. 'Diet and Exercise Reduce Low-Grade Inflammation and Macrophage Infiltration in Adipose Tissue but Not in Skeletal Muscle in Severely Obese Subjects.' *AJP: Endocrinology and Metabolism* 290 (5): E961–67. doi:10.1152/ajpendo.00506.2005. http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.00506.2005.

Bryson, J M, S E King, C M Burns, L A Baur, S Swaraj, and I D Caterson. 1996. 'Changes in Glucose and Lipid Metabolism Following Weight Loss Produced by a Very Low Calorie Diet in Obese Subjects.' *International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 20 (4): 338–45.

Buchakjian, Marisa R., and Sally Kornbluth. 2010. 'The Engine Driving the Ship: Metabolic Steering of Cell Proliferation and Death.' *Nature Reviews Molecular Cell Biology* 11 (10). Nature Publishing Group: 715–27. doi:10.1038/nrm2972. http://www.nature.com/doifinder/10.1038/nrm2972.

Campbell, K. L., K. E. Foster-Schubert, K. W. Makar, M. Kratz, D. Hagman, E. A. Schur, N. Habermann, et al. 2013. *Gene Expression Changes in Adipose Tissue with Diet- And/or Exercise-Induced Weight Loss*. *Cancer Prevention Research*. Vol. 6. doi:10.1158/1940-6207.CAPR-12-0212. http://cancerpreventionresearch.aacrjournals.org/cgi/doi/10.1158/1940-6207.CAPR-12-0212.

Cheng, Zhiyong, Yolanda Tseng, and Morris F. White. 2010. 'Insulin Signaling Meets Mitochondria in Metabolism.' *Trends in Endocrinology & Metabolism* 21 (10). Elsevier Ltd: 589–98. doi:10.1016/j.tem.2010.06.005. http://www.sciencedirect.com/science/article/pii/S1043276010000998\nhttp://pdn.sciencedirect.com/science?_ob=MiamiImageURL&_cid=271121&_user=1072900&_pii=S1043276010000998&_check=y&_origin=article&_zone=toolbar&_coverDate=31-Oct-2010&view=c&originContentFa.

Cignarella, Andrea, Mario Kratz, and Chiara Bolego. 2010. 'Emerging Role of Estrogen in the Control of Cardiometabolic Disease.' *Trends in Pharmacological Sciences* 31 (4). Elsevier Ltd: 183–89. doi:10.1016/j.tips.2010.01.001. http://www.ncbi.nlm.nih.gov/pubmed/20138672.

Cloonan, Nicole, Alistair R R Forrest, Gabriel Kolle, Brooke B A Gardiner, Geoffrey J Faulkner, Mellissa K Brown, Darrin F Taylor, et al. 2008. 'Stem Cell Transcriptome Profiling via Massive-Scale mRNA Sequencing.' *Nature Methods* 5 (7): 613–19. doi:10.1038/nmeth.1223. http://www.nature.com/doifinder/10.1038/nmeth.1223.

Cloonan, Nicole, Qinying Xu, Geoffrey J. Faulkner, Darrin F. Taylor, Dave T P Tang, Gabriel Kolle, and Sean M. Grimmond. 2009. 'RNA-MATE: A Recursive Mapping Strategy for High-Throughput RNA-Sequencing Data.' *Bioinformatics* 25 (19): 2615–16. doi:10.1093/bioinformatics/btp459. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2752615&tool=pmcentrez&rendertype=abstract.

Coll, Anthony P., I. Sadaf Farooqi, and Stephen O'Rahilly. 2007. 'The Hormonal Control of Food Intake.' *Cell* 129 (2): 251–62. doi:10.1016/j.cell.2007.04.001. http://linkinghub.elsevier.com/retrieve/pii/S0092867407004473.

Costa, Valerio, Claudia Angelini, Italia De Feis, and Alfredo Ciccodicola. 2010. 'Uncovering the Complexity

of Transcriptomes with RNA-Seq.' *Journal of Biomedicine and Biotechnology* 2010 (January): 1–19. doi:10.1155/2010/853916. http://www.hindawi.com/journals/bmri/2010/853916/.

De Ferranti, Sarah, and Dariush Mozaffarian. 2008. 'The Perfect Storm: Obesity, Adipocyte Dysfunction, and Metabolic Consequences.' *Clinical Chemistry* 54 (6): 945–55. doi:10.1373/clinchem.2007.100156. http://www.ncbi.nlm.nih.gov/pubmed/18436717.

Degawa-Yamauchi, Mikako, Katherine a Moss, Jason E Bovenkerk, Sudha S Shankar, Charles L Morrison, Christopher J Lelliott, Antonio Vidal-Puig, RoseMarie Jones, and Robert V Considine. 2005. 'Regulation of Adiponectin Expression in Human Adipocytes: Effects of Adiposity, Glucocorticoids, and Tumor Necrosis Factor Alpha.' *Obesity Research* 13 (4): 662–69. doi:10.1038/oby.2005.74.

Després, Jean-Pierre, and Isabelle Lemieux. 2006. 'Abdominal Obesity and Metabolic Syndrome.' *Nature* 444 (7121): 881–87. doi:10.1038/nature05488. http://www.nature.com/doifinder/10.1038/nature05488.

Després, Jean-Pierre, Isabelle Lemieux, Jean Bergeron, Philippe Pibarot, Patrick Mathieu, Eric Larose, Josep Rodés-Cabau, Olivier F Bertrand, and Paul Poirier. 2008. 'Abdominal Obesity and the Metabolic Syndrome: Contribution to Global Cardiometabolic Risk.' *Arteriosclerosis, Thrombosis, and Vascular Biology* 28 (6): 1039–49. doi:10.1161/ATVBAHA.107.159228.

Dharuri, Harish, Peter a C 't Hoen, Jan B van Klinken, Peter Henneman, Jeroen F J Laros, Mirjam a Lips, Fatiha El Bouazzaoui, et al. 2014. 'Downregulation of the Acetyl-CoA Metabolic Network in Adipose Tissue of Obese Diabetic Individuals and Recovery after Weight Loss.' *Diabetologia* 57 (11): 2384–92. doi:10.1007/s00125-014-3347-0. http://www.ncbi.nlm.nih.gov/pubmed/25099943\nhttp://link.springer.com.ep.fjernadgang.kb.dk/article/10.1007/s00125-014-3347-0.

Dina, Christian. 2008. 'New Insights into the Genetics of Body Weight.' *Current Opinion in Clinical Nutrition and Metabolic Care* 11 (4): 378–84. doi:10.1097/MCO.0b013e328303e5e6. http://www.ncbi.nlm.nih.gov/pubmed/18541995.

Doria, Enrico, Daniela Buonocore, Angela Focarelli, and Fulvio Marzatico. 2012. 'Relationship between Human Aging Muscle and Oxidative System Pathway.' *Oxidative Medicine and Cellular Longevity*. doi:10.1155/2012/830257. http://www.hindawi.com/journals/omcl/2012/830257/.

Durnin, J. V. G. A., and J. Womersley. 1974. 'Body Fat Assessed from Total Body Density and Its Estimation from Skinfold Thickness: Measurements on 481 Men and Women Aged from 16 to 72 Years.' *British Journal of Nutrition* 32 (01): 77–97. doi:10.1079/BJN19740060. http://www.journals.cambridge.org/abstract_S0007114574000614.

Eckardt, Kristin, Henrike Sell, and Juergen Eckel. 2008. 'Novel Aspects of Adipocyte-Induced Skeletal Muscle Insulin Resistance.' *Archives of Physiology and Biochemistry* 114 (April): 287–98. doi:10.1080/13813450802404761. http://www.ncbi.nlm.nih.gov/pubmed/18946789.

Epskamp, S, A O J Cramer, L J Waldorp, V D Schmittmann, and D Borsboom. 2011. 'Qgraph: Network Representations of Relationships in Data.' *R Package Version 0.4* 10 (4): 1–18.

Eswaran, Jeyanthy, Dinesh Cyanam, Prakriti Mudvari, Sirigiri Divijendra Natha Reddy, Suresh B Pakala, Sujit S Nair, Liliana Florea, Suzanne a W Fuqua, Sucheta Godbole, and Rakesh Kumar. 2012. 'Transcriptomic Landscape of Breast Cancers through mRNA Sequencing.' *Scientific Reports* 2

(January): 264. doi:10.1038/srep00264. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3278922&tool=pmcentrez&rendertype=abstract.

Farooqi, I Sadaf, Giuseppe Matarese, Graham M Lord, Julia M Keogh, Elizabeth Lawrence, Chizo Agwu, Veronica Sanna, et al. 2002. 'Beneficial Effects of Leptin on Obesity, T Cell Hyporesponsiveness, and Neuroendocrine/metabolic Dysfunction of Human Congenital Leptin Deficiency.' *The Journal of Clinical Investigation* 110 (8): 1093–1103. doi:10.1172/JCI15693. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=150795&tool=pmcentrez&rendertype=abstract.

Farooqi, IS, SA Jebb, G Langmack, E Lawrence, CH Cheetham, AM Prentice, IA Hughes, MA McCamish, and S O'Rahilly. 1999. 'Effects of Recombinant Leptin Therapy in a Child with Congenital Leptin Deficiency.' *New England Journal of Medicine* 341: 879–84. doi:10.1056/NEJM199909163411204. http://www.nejm.org.ezproxy.uws.edu/doi/pdf/10.1056/NEJM199909163411204.

Faulds, Malin Hedengran, Chunyan Zhao, Karin Dahlman-Wright, and Jan Åke Gustafsson. 2012. 'The Diversity of Sex Steroid Action: Regulation of Metabolism by Estrogen Signaling.' *Journal of Endocrinology*. doi:10.1530/JOE-11-0044.

Flier, Jeffrey S. 2004. 'Obesity Wars: Molecular Progress Confronts an Expanding Epidemic.' *Cell* 116 (2): 337–50. doi:10.1016/S0092-8674(03)01081-X. http://www.ncbi.nlm.nih.gov/pubmed/14744442.

Forbes, Scott C, Jonathan P Little, and Darren G Candow. 2012. 'Exercise and Nutritional Interventions for Improving Aging Muscle Health.' *Endocrine*. doi:10.1007/s12020-012-9676-1.

Foryst-Ludwig, Anna, Markus Clemenz, Stephan Hohmann, Martin Hartge, Christiane Sprang, Nikolaj Frost, Maxim Krikov, et al. 2008. 'Metabolic Actions of Estrogen Receptor Beta (ERbeta) Are Mediated by a Negative Cross-Talk with PPARgamma.' *PLoS Genetics* 4 (6): e1000108. doi:10.1371/journal.pgen.1000108. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2432036&tool=pmcentrez&rendertype=abstract.

Foryst-Ludwig, Anna, and Ulrich Kintscher. 2010. 'Metabolic Impact of Estrogen Signalling through ERalpha and ERbeta.' *The Journal of Steroid Biochemistry and Molecular Biology* 122 (1-3). Elsevier Ltd: 74–81. doi:10.1016/j.jsbmb.2010.06.012. http://linkinghub.elsevier.com/retrieve/pii/S0960076010002852.

Franz, Marion J., Jeffrey J. VanWormer, a. Lauren Crain, Jackie L. Boucher, Trina Histon, William Caplan, Jill D. Bowman, and Nicolas P. Pronk. 2007. 'Weight-Loss Outcomes: A Systematic Review and Meta-Analysis of Weight-Loss Clinical Trials with a Minimum 1-Year Follow-Up.' *Journal of the American Dietetic Association* 107 (10): 1755–67. doi:10.1016/j.jada.2007.07.017. http://www.ncbi.nlm.nih.gov/pubmed/17904936.

Gaidhu, Mandeep P, Nicole M Anthony, Prital Patel, Thomas J Hawke, and Rolando B Ceddia. 2010. 'Dysregulation of Lipolysis and Lipid Metabolism in Visceral and Subcutaneous Adipocytes by High-Fat Diet: Role of ATGL, HSL, and AMPK.' *American Journal of Physiology. Cell Physiology* 298 (4): C961–71. doi:10.1152/ajpcell.00547.2009. http://www.ncbi.nlm.nih.gov/pubmed/20107043.

Galgani, J. E., C. Moro, and E. Ravussin. 2008. 'Metabolic Flexibility and Insulin Resistance.' *AJP:*

Endocrinology and Metabolism 295 (5): E1009–17. doi:10.1152/ajpendo.90558.2008. http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.90558.2008.

Gao, Hui, and Karin Dahlman-Wright. 2013. 'Implications of Estrogen Receptor Alpha and Estrogen Receptor Beta for Adipose Tissue Functions and Cardiometabolic Complications.' *Hormone Molecular Biology and Clinical Investigation* 15 (3): 81–90. doi:10.1515/hmbci-2013-0021.

Garber, Manuel, Manfred G Grabherr, Mitchell Guttman, and Cole Trapnell. 2011. 'Computational Methods for Transcriptome Annotation and Quantification Using RNA-Seq.' *Nature Methods* 8 (6). Nature Publishing Group: 469–77. doi:10.1038/nmeth.1613. http://dx.doi.org/10.1038/nmeth.1613.

Garrido-Sánchez, Lourdes, Joan Vendrell, Diego Fernández-García, Victoria Ceperuelo-Mallafré, Matilde R Chacón, Luis Ocaña-Wilhelmi, Juan Alcaide, Francisco J Tinahones, and Eduardo García-Fuentes. 2012. 'De Novo Lipogenesis in Adipose Tissue Is Associated with Course of Morbid Obesity after Bariatric Surgery.' *PloS One* 7 (2): e31280. doi:10.1371/journal.pone.0031280. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3285616&tool=pmcentrez&rendertype=abstract.

Geer, Eliza B., and Wei Shen. 2009. 'Gender Differences in Insulin Resistance, Body Composition, and Energy Balance.' *Gender Medicine* 6. Excerpta Medica Inc.: 60–75. doi:10.1016/j.genm.2009.02.002. http://linkinghub.elsevier.com/retrieve/pii/S1550857909000072.

Gonzalez-Cadavid, Nestor F, and Shalender Bhasin. 2004. 'Role of Myostatin in Metabolism.' *Current Opinion in Clinical Nutrition and Metabolic Care* 7 (4): 451–57. doi:10.1097/01.mco.0000134365.99523.7f. http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00075197-200407000-00015.

Goodpaster, Bret H, David E Kelley, Rena R Wing, Amy Meier, and F Leland Thaete. 1999. 'Effects of Weight Loss on Regional Fat Distribution and Insulin Sensitivity in Obesity.' *Diabetes* 48 (4): 839–47. doi:10.2337/diabetes.48.4.839. http://www.ncbi.nlm.nih.gov/pubmed/10102702.

Grant, R W, B M Vester Boler, T K Ridge, T K Graves, and K S Swanson. 2013. 'Skeletal Muscle Tissue Transcriptome Differences in Lean and Obese Female Beagle Dogs.' *Animal Genetics* 44 (5): 560–68. doi:10.1111/age.12035. http://www.ncbi.nlm.nih.gov/pubmed/23488938.

Gray, Robert E, Charles J Tanner, Walter J Pories, Kenneth G MacDonald, and Joseph a Houmard. 2003. 'Effect of Weight Loss on Muscle Lipid Content in Morbidly Obese Subjects.' *American Journal of Physiology. Endocrinology and Metabolism* 284: E726–32. doi:10.1152/ajpendo.00371.2002.

Gregor, Margaret F., and Gökhan S. Hotamisligil. 2011. 'Inflammatory Mechanisms in Obesity.' *Annual Review of Immunology* 29 (1): 415–45. doi:10.1146/annurev-immunol-031210-101322. http://www.annualreviews.org/doi/abs/10.1146/annurev-immunol-031210-101322.

Guo, Tingqing, William Jou, Tatyana Chanturiya, Jennifer Portas, Oksana Gavrilova, and Alexandra C. McPherron. 2009. 'Myostatin Inhibition in Muscle, but Not Adipose Tissue, Decreases Fat Mass and Improves Insulin Sensitivity.' *PLoS ONE* 4 (3): 1–11. doi:10.1371/journal.pone.0004937. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2654157&tool=pmcentrez&rendertype=abstract.

Guo, Zengkui, Donald D. Hensrud, C. Michael Johnson, and Michael D. Jensen. 1999. 'Regional Postprandial

Fatty Acid Metabolism in Different Obesity Phenotypes.' *Diabetes* 48 (August): 1586–92. doi:10.2337/diabetes.48.8.1586.

Hainer, V, M Kunesová, J Parízková, V Stich, R Mikulová, and S Slabá. 2000. 'Respiratory Quotient in Obesity: Its Association with an Ability to Retain Weight Loss and with Parental Obesity.' *Sbornik Lekarsky* 101 (1): 99–104. http://www.scopus.com/inward/record.url?eid=2-s2.0-0033642911&partnerID=tZOtx3y1.

Hänzelmann, Sonja, Robert Castelo, and Justin Guinney. 2013. 'GSVA: Gene Set Variation Analysis for Microarray and RNA-Seq Data.' *BMC Bioinformatics* 14: 7. doi:10.1186/1471-2105-14-7. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3618321&tool=pmcentrez&rendertype=abstract.

Hardie, D. Grahame, Fiona a. Ross, and Simon a. Hawley. 2012. 'AMPK: A Nutrient and Energy Sensor That Maintains Energy Homeostasis.' *Nature Reviews Molecular Cell Biology*. Nature Publishing Group. doi:10.1038/nrm3311. http://dx.doi.org/10.1038/nrm3311.

Harris, R B S. 2014. 'Direct and Indirect Effects of Leptin on Adipocyte Metabolism.' *Biochimica et Biophysica Acta* 1842 (3). Elsevier B.V.: 414–23. doi:10.1016/j.bbadis.2013.05.009. http://www.ncbi.nlm.nih.gov/pubmed/23685313.

Havekes, Bas, and Hans P Sauerwein. 2010. 'Adipocyte-Myocyte Crosstalk in Skeletal Muscle Insulin Resistance; Is There a Role for Thyroid Hormone?' *Current Opinion in Clinical Nutrition and Metabolic Care* 13 (6): 641–46. doi:10.1097/MCO.0b013e32833e341d. http://www.ncbi.nlm.nih.gov/pubmed/20689414.

Hensrud, Donald D., Roland L. Weinsier, Betty E. Darnell, and Gary R. Hunter. 1994. 'A Prospective Study of Weight Maintenance in Obese Subjects Reduced to Normal Body Weight without Weight-Loss Training.' *American Journal of Clinical Nutrition* 60 (5): 688–94. http://www.ncbi.nlm.nih.gov/pubmed/7942574.

Holloway, Graham P, Arend Bonen, and Lawrence L Spriet. 2009. 'Regulation of Skeletal Muscle Mitochondrial Fatty Acid Metabolism in Lean and Obese Individuals 1 – 4.' *American Journal of Clinical Nutrition* 89: 455–62. doi:10.3945/ajcn.2008.26717B.INTRODUCTION.

Houten, Sander Michel, and Ronald J. A. Wanders. 2010. 'A General Introduction to the Biochemistry of Mitochondrial Fatty Acid β-Oxidation.' *Journal of Inherited Metabolic Disease* 33 (5): 469–77. doi:10.1007/s10545-010-9061-2. http://link.springer.com/10.1007/s10545-010-9061-2.

Huang, Da Wei, Richard A Lempicki, and Brad T Sherman. 2009. 'Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources.' *Nature Protocols* 4 (1): 44–57. doi:10.1038/nprot.2008.211. http://www.ncbi.nlm.nih.gov/pubmed/19131956.

Huang, Da Wei, Brad T. Sherman, and Richard a. Lempicki. 2009. 'Bioinformatics Enrichment Tools: Paths toward the Comprehensive Functional Analysis of Large Gene Lists.' *Nucleic Acids Research* 37 (1): 1–13. doi:10.1093/nar/gkn923. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2615629&tool=pmcentrez&rendertype=abstract.

Huang, Da Wei, Brad T. Sherman, Xin Zheng, Jun Yang, Tomozumi Imamichi, Robert Stephens, and Richard A. Lempicki. 2009. 'Extracting Biological Meaning from Large Gene Lists with DAVID.' *Current*

Protocols in Bioinformatics Chapter 13 (September): Unit 13.11. doi:10.1002/0471250953.bi1311s27. http://doi.wiley.com/10.1002/0471250953.bi1311s27.

Iglesias, María J, Sonia Eiras, Roberto Piñeiro, Diego López-otero, and Rosalía Gallego. 2006. 'Gender Differences in Adiponectin and Leptin Expression in Epicardial and Subcutaneous Adipose Tissue .' *Revista Espanola de Cardiologia* 59 (12): 1252–60.

Jéquier, E, and L Tappy. 1999. 'Regulation of Body Weight in Humans.' *Physiological Reviews* 79 (2): 451–80. http://www.ncbi.nlm.nih.gov/pubmed/22948429.

Jeukendrup, Asker E. 2002. 'Regulation of Fat Metabolism in Skeletal Muscle.' *Annals of the New York Academy of Sciences* 967: 217–35. doi:10.1111/j.1749-6632.2002.tb04278.x.

Karastergiou, Kalypso, Steven R Smith, Andrew S Greenberg, and Susan K Fried. 2012. 'Sex Differences in Human Adipose Tissues – the Biology of Pear Shape.' *Biology of Sex Differences*. BioMed Central Ltd. doi:10.1186/2042-6410-3-13. http://www.bsd-journal.com/content/3/1/13.

Kelley, David E, and Lawrence J Mandarin. 2000. 'Fuel Selection in Human Skeletal Muscle in Insulin Resistance. A Reexamination.' *Diabetes* 49: 677–83.

Keuper, Michaela, Martin Jastroch, Chun Xia Yi, Pamela Fischer-Posovszky, Martin Wabitsch, Matthias H. Tschöp, and Susanna M. Hofmann. 2014. 'Spare Mitochondrial Respiratory Capacity Permits Human Adipocytes to Maintain ATP Homeostasis under Hypoglycemic Conditions.' *FASEB Journal* 28 (2): 761–70. doi:10.1096/fj.13-238725.

Kim, J Y, R C Hickner, R L Cortright, G L Dohm, and J a Houmard. 2000. 'Lipid Oxidation Is Reduced in Obese Human Skeletal Muscle.' *American Journal of Physiology. Endocrinology and Metabolism* 279 (5): E1039–44.

Klempel, Monica C, and Krista A Varady. 2011. 'Reliability of Leptin, but Not Adiponectin, as a Biomarker for Diet-Induced Weight Loss in Humans.' *Nutrition Reviews* 69 (3): 145–54. doi:10.1111/j.1753-4887.2011.00373.x. http://www.ncbi.nlm.nih.gov/pubmed/21348878.

Könner, A. Christine, and Jens C. Brüning. 2012. 'Selective Insulin and Leptin Resistance in Metabolic Disorders.' *Cell Metabolism* 16 (2): 144–52. doi:10.1016/j.cmet.2012.07.004. http://linkinghub.elsevier.com/retrieve/pii/S1550413112002811.

Korenblat, Kevin M., Elisa Fabbrini, B. Selma Mohammed, and Samuel Klein. 2008. 'Liver, Muscle, and Adipose Tissue Insulin Action Is Directly Related to Intrahepatic Triglyceride Content in Obese Subjects.' *Gastroenterology* 134 (5): 1369–75. doi:10.1053/j.gastro.2008.01.075.

Kotronen, Anna, Leena Juurinen, Mirja Tiikkainen, Satu Vehkavaara, and Hannele Yki-Järvinen. 2008. 'Increased Liver Fat, Impaired Insulin Clearance, and Hepatic and Adipose Tissue Insulin Resistance in Type 2 Diabetes.' *Gastroenterology* 135 (1): 122–30. doi:10.1053/j.gastro.2008.03.021. http://www.ncbi.nlm.nih.gov/pubmed/18474251.

Kovalik, Jean Paul, Dorothy Slentz, Robert D. Stevens, William E. Kraus, Joseph A. Houmard, James B. Nicoll, Y. Renee Lea-Currie, et al. 2011. 'Metabolic Remodeling of Human Skeletal Myocytes by Cocultured Adipocytes Depends on the Lipolytic State of the System.' *Diabetes* 60 (7): 1882–93. doi:10.2337/db10-0427. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3121442&tool=pmcentrez&rendertype=abstract.

Koves, Timothy R., John R. Ussher, Robert C. Noland, Dorothy Slentz, Merrie Mosedale, Olga Ilkayeva, James Bain, et al. 2008. 'Mitochondrial Overload and Incomplete Fatty Acid Oxidation Contribute to Skeletal Muscle Insulin Resistance.' *Cell Metabolism* 7 (1): 45–56. doi:10.1016/j.cmet.2007.10.013. http://linkinghub.elsevier.com/retrieve/pii/S1550413107003063.

Kurtz, D M, P Rinaldo, W J Rhead, L Tian, D S Millington, J Vockley, D a Hamm, et al. 1998. 'Targeted Disruption of Mouse Long-Chain Acyl-CoA Dehydrogenase Gene Reveals Crucial Roles for Fatty Acid Oxidation.' *Proceedings of the National Academy of Sciences of the United States of America* 95 (December): 15592–97. doi:10.1073/pnas.95.26.15592.

Kusminski, Christine M, William L Holland, Kai Sun, Jiyoung Park, Stephen B Spurgin, Ying Lin, G Roger Askew, et al. 2012. 'MitoNEET-Driven Alterations in Adipocyte Mitochondrial Activity Reveal a Crucial Adaptive Process That Preserves Insulin Sensitivity in Obesity.' *Nature Medicine* 18 (10). Nature Publishing Group: 1539–49. doi:10.1038/nm.2899. http://www.nature.com/doifinder/10.1038/nm.2899.

Kusminski, Christine M., and Philipp E. Scherer. 2012. 'Mitochondrial Dysfunction in White Adipose Tissue.' *Trends in Endocrinology & Metabolism* 23 (9). Elsevier Ltd: 435–43. doi:10.1016/j.tem.2012.06.004. http://linkinghub.elsevier.com/retrieve/pii/S1043276012000963.

Lafontan, Max, and Dominique Langin. 2009. 'Lipolysis and Lipid Mobilization in Human Adipose Tissue.' *Progress in Lipid Research* 48 (5). Elsevier Ltd: 275–97. doi:10.1016/j.plipres.2009.05.001. http://linkinghub.elsevier.com/retrieve/pii/S0163782709000204.

Langin, Dominique. 2006. 'Adipose Tissue Lipolysis as a Metabolic Pathway to Define Pharmacological Strategies against Obesity and the Metabolic Syndrome.' *Pharmacological Research* 53 (6): 482–91. doi:10.1016/j.phrs.2006.03.009. http://linkinghub.elsevier.com/retrieve/pii/S1043661806000478.

Langley, B. 2002. 'Myostatin Inhibits Myoblast Differentiation by Down-Regulating MyoD Expression.' *Journal of Biological Chemistry* 277 (51): 49831–40. doi:10.1074/jbc.M204291200. http://www.jbc.org/cgi/doi/10.1074/jbc.M204291200.

Larrouy, Dominique, Pierre Barbe, Carine Valle, Sébastien Déjean, Véronique Pelloux, Claire Thalamas, Jean-Philippe Bastard, et al. 2008. 'Gene Expression Profiling of Human Skeletal Muscle in Response to Stabilized Weight Loss.' *American Journal of Clinical Nutrion*.

Lea, W, a S Abbas, H Sprecher, J Vockley, and H Schulz. 2000. 'Long-Chain Acyl-CoA Dehydrogenase Is a Key Enzyme in the Mitochondrial Beta-Oxidation of Unsaturated Fatty Acids.' *Biochimica et Biophysica Acta* 1485 (2-3): 121–28. http://www.ncbi.nlm.nih.gov/pubmed/10832093.

Lee, C K, R G Klopp, R Weindruch, and T a Prolla. 1999. 'Gene Expression Profile of Aging and Its Retardation by Caloric Restriction.' *Science (New York, N.Y.)* 285 (5432): 1390–93. doi:10.1126/science.285.5432.1390.

Lien, Lillian F, Andrea M Haqq, Michelle Arlotto, Cris A Slentz, Michael J Muehlbauer, Ross L McMahon, James Rochon, et al. 2009. 'The STEDMAN Project: Biophysical, Biochemical and Metabolic Effects of a Behavioral Weight Loss Intervention during Weight Loss, Maintenance, and Regain.' *Omics : A Journal of Integrative Biology* 13 (1): 21–35. doi:10.1089/omi.2008.0035. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2678563&tool=pmcentrez&rendertype=abstract.

Lin, Ji, Heather B Arnold, Mary Anne Della-Fera, Michael J Azain, Diane L Hartzell, and Clifton a Baile. 2002. 'Myostatin Knockout in Mice Increases Myogenesis and Decreases Adipogenesis.' *Biochemical and Biophysical Research Communications* 291 (3): 701–6. doi:10.1006/bbrc.2002.6500. http://www.ncbi.nlm.nih.gov/pubmed/11855847.

Lissner, Lauren PhD, Patricia M PhD Odell, Ralph B PhD D'Agostino, Joseph I I I M D Stokes, Bernard E M D Kreger, Albert J M A Belanger, and Kelly D PhD Brownell. 1991. 'Variability of Body Weight and Health Outcomes in the Framingham Population.' *The New England Journal of Medicine* 324 (26): 1839–44. doi:10.1056/NEJM199106273242602. http://search.proquest.com/docview/223952159?accountid=12860\nhttp://jr3tv3gd5w.search.serialssolutions.com/?ctx_ver=Z39.88-2004&ctx_enc=info:ofi/enc:UTF-8&rfr_id=info:sid/ProQ:psychologyshell&rft_val_fmt=info:ofi/fmt:kev:mtx:journal&rft.genre=unknown&rft.jtitle=The+New+England+Journal+of+Medicine&rft.atitle=Variability+of+Body+Weight+and+Health+Outcomes+in+the+Framingham+Population&rft.au=Lissner,+Lauren,+PhD;Odell,+Patricia+M,+PhD;D'Agostino,+Ralph+B,+PhD;Stokes,+Joseph,+III,+MD;Kreger,+Bernard.

Liu, Sihao, Ryan K. Alexander, and Chih Hao Lee. 2014. 'Lipid Metabolites as Metabolic Messengers in Inter-Organ Communication.' *Trends in Endocrinology and Metabolism* 25 (7). Elsevier Ltd: 356–63. doi:10.1016/j.tem.2014.05.002. http://dx.doi.org/10.1016/j.tem.2014.05.002.

Lomonaco, Romina, Carolina Ortiz-Lopez, Beverly Orsak, Amy Webb, Jean Hardies, Celia Darland, Joan Finch, et al. 2012. 'Effect of Adipose Tissue Insulin Resistance on Metabolic Parameters and Liver Histology in Obese Patients with Nonalcoholic Fatty Liver Disease.' *Hepatology* 55 (5): 1389–97. doi:10.1002/hep.25539.

Love, M. I., Simon Anders, and Wolfgang Huber. 2014. *Differential Analysis of Count Data - the DESeq2 Package*. doi:10.1101/002832. http://biorxiv.org/lookup/doi/10.1101/002832.

Lustig, Robert H. 2006. 'Childhood Obesity: Behavioral Aberration or Biochemical Drive? Reinterpreting the First Law of Thermodynamics.' *Nature Clinical Practice Endocrinology & Metabolism* 2 (8): 447–58. doi:10.1038/ncpendmet0220. http://www.nature.com/doifinder/10.1038/ncpendmet0220.

Maclean, Paul S, Audrey Bergouignan, Marc-Andre Cornier, and Matthew R Jackman. 2011. 'Biology's Response to Dieting: The Impetus for Weight Regain.' *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 301 (3): R581–600. doi:10.1152/ajpregu.00755.2010.

Mai, Knut, Janin Andres, Thomas Bobbert, Christiane Maser-Gluth, Matthias Möhlig, Volker Bähr, Andreas F H Pfeiffer, Joachim Spranger, and Sven Diederich. 2007. 'Rosiglitazone Decreases 11beta-Hydroxysteroid Dehydrogenase Type 1 in Subcutaneous Adipose Tissue.' *Clinical Endocrinology* 67: 419–25. doi:10.1111/j.1365-2265.2007.02903.x.

Mai, Knut, Thomas Bobbert, Christian Groth, Anke Assmann, Sabine Meinus, Jessica Kraatz, Janin Andres, et al. 2010. 'Physiological Modulation of Circulating FGF21: Relevance of Free Fatty Acids and Insulin.' *American Journal of Physiology. Endocrinology and Metabolism* 299 (1): E126–30. doi:10.1152/ajpendo.00020.2010. http://www.ncbi.nlm.nih.gov/pubmed/20424140.

Mai, Manuel, Anke Tönjes, Peter Kovacs, Michael Stumvoll, Georg Martin Fiedler, and Alexander Benedikt Leichtle. 2013. 'Serum Levels of Acylcarnitines Are Altered in Prediabetic Conditions.' *PloS One* 8

(12): e82459. doi:10.1371/journal.pone.0082459. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3865089&tool=pmcentrez&rendertype=abstract.

Mailloux, R. J., and M.-E. Harper. 2010. 'Glucose Regulates Enzymatic Sources of Mitochondrial NADPH in Skeletal Muscle Cells; a Novel Role for Glucose-6-Phosphate Dehydrogenase.' *The FASEB Journal* 24 (7): 2495–2506. doi:10.1096/fj.09-151803. http://www.fasebj.org/cgi/doi/10.1096/fj.09-151803.

Marguerat, Samuel, and Jürg Bähler. 2010. 'RNA-Seq: From Technology to Biology.' *Cellular and Molecular Life Sciences* 67 (4): 569–79. doi:10.1007/s00018-009-0180-6. http://link.springer.com/10.1007/s00018-009-0180-6.

Marquez-Quinones, A., D. M. Mutch, C. Debard, P. Wang, M. Combes, B. Roussel, C. Holst, et al. 2010. 'Adipose Tissue Transcriptome Reflects Variations between Subjects with Continued Weight Loss and Subjects Regaining Weight 6 Mo after Caloric Restriction Independent of Energy Intake.' *American Journal of Clinical Nutrition* 92 (4): 975–84. doi:10.3945/ajcn.2010.29808. http://www.ajcn.org/cgi/doi/10.3945/ajcn.2010.29808.

McPherron, Alexandra C., and Se Jin Lee. 2002. 'Suppression of Body Fat Accumulation in Myostatin-Deficient Mice.' *Journal of Clinical Investigation* 109 (5): 595–601. doi:10.1172/JCI200213562.

Mcquaid, E, Leanne Hodson, Matthew J Neville, A Louise Dennis, Jane Cheeseman, Sandy M Humphreys, Toralph Ruge, Marjorie Gilbert, Barbara A Fielding, and Keith N Frayn. 2011. 'Downregulation of Adipose Tissue Fatty Acid Trafficking.' *Diabetes* 60 (January): 47–55. doi:10.2337/db10-0867.

Meshkani, Reza, and Khosrow Adeli. 2009. 'Hepatic Insulin Resistance, Metabolic Syndrome and Cardiovascular Disease.' *Clinical Biochemistry* 42 (13-14). The Canadian Society of Clinical Chemists: 1331–46. doi:10.1016/j.clinbiochem.2009.05.018. http://linkinghub.elsevier.com/retrieve/pii/S0009912009002513.

Metz, Thomas F, Thomas P Mechtler, Michael Merk, Anne Gottschalk, Richard Lukačin, Kurt R Herkner, and David C Kasper. 2012. 'Evaluation of a Novel, Commercially Available Mass Spectrometry Kit for Newborn Screening Including Succinylacetone without Hydrazine.' *Clinica Chimica Acta; International Journal of Clinical Chemistry* 413 (15-16): 1259–64. doi:10.1016/j.cca.2012.04.007. http://www.ncbi.nlm.nih.gov/pubmed/22521492.

Mihalik, Stephanie J., Bret H. Goodpaster, David E. Kelley, Donald H. Chace, Jerry Vockley, Frederico G.S. Toledo, and James P. DeLany. 2010. 'Increased Levels of Plasma Acylcarnitines in Obesity and Type 2 Diabetes and Identification of a Marker of Glucolipotoxicity.' *Obesity* 18 (9). Nature Publishing Group: 1695–1700. doi:10.1038/oby.2009.510. http://doi.wiley.com/10.1038/oby.2009.510.

Milan, G., E. Dalla Nora, C. Pilon, C. Pagano, M. Granzotto, M. Manco, G. Mingrone, and R. Vettor. 2004. 'Changes in Muscle Myostatin Expression in Obese Subjects after Weight Loss.' *Journal of Clinical Endocrinology and Metabolism* 89 (6): 2724–27. doi:10.1210/jc.2003-032047. http://www.ncbi.nlm.nih.gov/pubmed/15181048.

Milan, Gabriella, Marnie Granzotto, Alessandro Scarda, Alessandra Calcagno, Claudio Pagano, Giovanni Federspil, and Roberto Vettor. 2002. 'Resistin and Adiponectin Expression in Visceral Fat of Obese Rats: Effect of Weight Loss.' *Obesity Research* 10 (11): 1095–1103. doi:10.1038/oby.2002.149.

Monteiro, Rosário, and Isabel Azevedo. 2010. 'Chronic Inflammation in Obesity and the Metabolic

Syndrome.' *Mediators of Inflammation*. doi:10.1155/2010/289645. http://www.hindawi.com/journals/mi/2010/289645/.

Mortazavi, Ali, Brian a Williams, Kenneth McCue, Lorian Schaeffer, and Barbara Wold. 2008. 'Mapping and Quantifying Mammalian Transcriptomes by RNA-Seq.' *Nature Methods* 5 (7): 621–28. doi:10.1038/nmeth.1226.

Mueckler, Mike, and Bernard Thorens. 2013. 'The SLC2 (GLUT) Family of Membrane Transporters.' *Molecular Aspects of Medicine*. doi:10.1016/j.mam.2012.07.001. http://linkinghub.elsevier.com/retrieve/pii/S0098299712000842.

Muller, G. 2011. 'Take-over: Multiple Mechanisms of Inter-Adipocyte Communication.' *Journal of Molecular Cell Biology* 3 (6): 382–382. doi:10.1093/jmcb/mjr037. http://jmcb.oxfordjournals.org/cgi/doi/10.1093/jmcb/mjr037.

Mutch, David M, Tune H Pers, M Ramzi Temanni, Veronique Pelloux, Adriana Marquez-Quinl̀ones, J Alfredo Martinez Claus Holst, Dimitris Babalis, et al. 2010. 'A Distinct Adipose Tissue Gene Expression Response to Caloric Restriction Foreshadows Successful Weight Maintenance in Obese Subjects.' *The American Journal of Clinical Nutrition* 8: 65–81. doi:10.3945/ajcn.110.006858.

Mutch, David M, M Ramzi Temanni, Corneliu Henegar, Florence Combes, Véronique Pelloux, Claus Holst, Thorkild I a Sørensen, et al. 2007. 'Adipose Gene Expression prior to Weight Loss Can Differentiate and Weakly Predict Dietary Responders.' *PloS One* 2 (12): e1344. doi:10.1371/journal.pone.0001344. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2147074&tool=pmcentrez&rendertype=abstract.

Naaz, a, M Zakroczymski, P Heine, J Taylor, P Saunders, D Lubahn, and P S Cooke. 2002. 'Effect of Ovariectomy on Adipose Tissue of Mice in the Absence of Estrogen Receptor Alpha (ERalpha): A Potential Role for Estrogen Receptor Beta (ERbeta).' *Hormone and Metabolic Research = Hormon-Und Stoffwechselforschung = Hormones et Metabolisme* 34 (11-12): 758–63. doi:10.1055/s-2002-38259.

Nagy, T R, M I Goran, R L Weinsier, M J Toth, Y Schutz, and E T Poehlman. 1996. 'Determinants of Basal Fat Oxidation in Healthy Caucasians.' *Journal of Applied Physiology* 80 (5): 1743–48.

Neiberg, Rebecca H, Rena R Wing, George A Bray, David M Reboussin, Amy D Rickman, Karen C Johnson, Abbas E Kitabchi, Lucy F Faulconbridge, Dalane W Kitzman, and Mark A Espeland. 2012. 'Patterns of Weight Change Associated with Long-Term Weight Change and Cardiovascular Disease Risk Factors in the Look AHEAD Study.' *Obesity (Silver Spring, Md.)* 20 (10): 2048–56. doi:10.1038/oby.2012.33. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3632374&tool=pmcentrez&rendertype=abstract.

Ouchi, Noriyuki, Jennifer L. Parker, Jesse J. Lugus, and Kenneth Walsh. 2011. 'Adipokines in Inflammation and Metabolic Disease.' *Nature Reviews Immunology* 11 (2). Nature Publishing Group: 85–97. doi:10.1038/nri2921. http://www.nature.com/doifinder/10.1038/nri2921.

Park, Jung-Jun, Jason R Berggren, Matthew W Hulver, Joseph a Houmard, and Eric P Hoffman. 2006. 'GRB14, GPD1, and GDF8 as Potential Network Collaborators in Weight Loss-Induced Improvements in Insulin Action in Human Skeletal Muscle.' *Physiological Genomics* 27 (2): 114–21.

doi:10.1152/physiolgenomics.00045.2006.

Patti, Mary-Elizabeth, and Silvia Corvera. 2010. 'The Role of Mitochondria in the Pathogenesis of Type 2 Diabetes.' *Endocrine Reviews* 31 (3): 364–95. doi:10.1210/er.2009-0027. http://press.endocrine.org/doi/abs/10.1210/er.2009-0027.

Pedersen, Steen B, Kurt Kristensen, Pernille A Hermann, John A Katzenellenbogen, and Bjørn Richelsen. 2004. 'Estrogen Controls Lipolysis by up-Regulating α2A-Adrenergic Receptors Directly in Human Adipose Tissue through the Estrogen Receptor α. Implications for the Female Fat Distribution.' *Journal of Clinical Endocrinology and Metabolism* 89 (4): 1869–78. doi:10.1210/jc.2003-031327.

R Team, development core. 2012. 'R: A Language and Environment for Statistical Computing.' doi:10.1007/978-3-540-74686-7. http://www.r-project.org/.

Rask-Madsen, C., and C. R. Kahn. 2012. 'Tissue-Specific Insulin Signaling, Metabolic Syndrome, and Cardiovascular Disease.' *Arteriosclerosis, Thrombosis, and Vascular Biology* 32 (9): 2052–59. doi:10.1161/ATVBAHA.111.241919. http://atvb.ahajournals.org/cgi/doi/10.1161/ATVBAHA.111.241919.

Rasouli, Neda, and Philip A. Kern. 2008. 'Adipocytokines and the Metabolic Complications of Obesity.' *The Journal of Clinical Endocrinology & Metabolism* 93 (11_supplement_1): s64–73. doi:10.1210/jc.2008-1613. http://press.endocrine.org/doi/abs/10.1210/jc.2008-1613.

Raucci, R., F. Rusolo, A. Sharma, G. Colonna, G. Castello, and S. Costantini. 2013. 'Functional and Structural Features of Adipokine Family.' *Cytokine* 61 (1). Elsevier Ltd: 1–14. doi:10.1016/j.cyto.2012.08.036. http://linkinghub.elsevier.com/retrieve/pii/S1043466612006795.

Reitman, Z. J., and H. Yan. 2010. 'Isocitrate Dehydrogenase 1 and 2 Mutations in Cancer: Alterations at a Crossroads of Cellular Metabolism.' *JNCI Journal of the National Cancer Institute* 102 (13): 932–41. doi:10.1093/jnci/djq187. http://jnci.oxfordjournals.org/cgi/doi/10.1093/jnci/djq187.

Riedmaier, Irmgard, and Michael W Pfaffl. 2013. 'Transcriptional Biomarkers--High Throughput Screening, Quantitative Verification, and Bioinformatical Validation Methods.' *Methods (San Diego, Calif.)* 59 (1). Elsevier Inc.: 3–9. doi:10.1016/j.ymeth.2012.08.012. http://www.ncbi.nlm.nih.gov/pubmed/22967906.

Ritchie, M. E., B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, and G. K. Smyth. 2015. 'Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies.' *Nucleic Acids Research* 43 (7): e47–e47. doi:10.1093/nar/gkv007. http://nar.oxfordjournals.org/lookup/doi/10.1093/nar/gkv007.

Salans, L B, J L Knittle, and J Hirsch. 1968. 'The Role of Adipose Cell Size and Adipose Tissue Insulin Sensitivity in the Carbohydrate Intolerance of Human Obesity.' *The Journal of Clinical Investigation* 47 (1): 153–65. doi:10.1172/JCI105705.

Saltiel, Alan R. 2012. 'Insulin Resistance in the Defense against Obesity.' *Cell Metabolism* 15 (6). Elsevier Inc.: 798–804. doi:10.1016/j.cmet.2012.03.001. http://dx.doi.org/10.1016/j.cmet.2012.03.001.

Saltiel, Alan R., and C. Ronald Kahn. 2001. 'Insulin Signalling and the Regulation of Glucose and Lipid Metabolism.' *Nature* 414 (6865): 799–806. doi:10.1038/414799a.

Samaras, Katherine, Natalia K. Botelho, Donald J. Chisholm, and Reginald V. Lord. 2010. 'Subcutaneous and Visceral Adipose Tissue Gene Expression of Serum Adipokines That Predict Type 2 Diabetes.' *Obesity*

18 (5). Nature Publishing Group: 884–89. doi:10.1038/oby.2009.443. http://doi.wiley.com/10.1038/oby.2009.443.

Schiaffino, S., and C. Reggiani. 2011. 'Fiber Types in Mammalian Skeletal Muscles.' *Physiological Reviews* 91 (4): 1447–1531. doi:10.1152/physrev.00031.2010. http://physrev.physiology.org/cgi/doi/10.1152/physrev.00031.2010.

Schooneman, M. G., F. M. Vaz, S. M. Houten, and M. R. Soeters. 2013. 'Acylcarnitines: Reflecting or Inflicting Insulin Resistance?' *Diabetes* 62 (1): 1–8. doi:10.2337/db12-0466. http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db12-0466.

Schrauwen, P, G Schaart, W H Saris, L J Slieker, J F Glatz, H Vidal, and E E Blaak. 2000. 'The Effect of Weight Reduction on Skeletal Muscle UCP2 and UCP3 mRNA Expression and UCP3 Protein Content in Type II Diabetic Subjects.' *Diabetologia* 43 (11): 1408–16. doi:10.1007/s001250051547. http://www.ncbi.nlm.nih.gov/pubmed/11126411.

Schrauwen, Patrick, and Matthijs Hesselink. 2002. 'UCP2 and UCP3 in Muscle Controlling Body Metabolism.' *The Journal of Experimental Biology* 205 (Pt 15): 2275–85.

Schutz, Y, A Tremblay, R L Weinsier, and K M Nelson. 1992. 'Role of Fat Oxidation in the Long-Term Stabilization of Body Weight in Obese Women.' *Am J Clin Nutr* 55 (3): 670–74. http://www.ncbi.nlm.nih.gov/pubmed/1550042\nhttp://ajcn.nutrition.org/content/55/3/670.full.pdf\nhttp://ajcn.nutrition.org/content/55/3/670.long.

Schwartz, M. W., D. G. Baskin, T. R: Bukowski, J. L. Kuijper, D. Foster, G. Lasser, D. E. Prunkard, et al. 1996. 'Specificty of Leptin Action on Elevated Blood Glucose Levels and Hyppthalamic Neuropeptide Y Gene Expression in Ob/ob Mice.' *Diabetes* 45: 531–35.

Sell, Henrike, Jürgen Eckel, and Daniela Dietze-Schroeder. 2006. 'Pathways Leading to Muscle Insulin Resistance--the Muscle--Fat Connection.' *Archives of Physiology and Biochemistry* 112 (2): 105–13. doi:10.1080/13813450600711540. http://www.ncbi.nlm.nih.gov/pubmed/16931452.

Sethi, Jaswinder K, and Antonio J Vidal-Puig. 2007a. 'Adipose Tissue Function and Plasticity Orchestrate Nutritional Adaptation.' *Journal of Lipid Research* 48: 1253–62.

Sethi. 2007b. 'Adipose Tissue Function and Plasticity Orchestrate Nutritional Adaptation.' *Journal of Lipid Research* 48: 1253–62.

Shah, A., N. Mehta, and M. P. Reilly. 2008. 'Adipose Inflammation, Insulin Resistance, and Cardiovascular Disease.' *Journal of Parenteral and Enteral Nutrition* 32 (6): 638–44. doi:10.1177/0148607108325251. http://pen.sagepub.com/cgi/doi/10.1177/0148607108325251.

Shan, T., X. Liang, P. Bi, and S. Kuang. 2013. 'Myostatin Knockout Drives Browning of White Adipose Tissue through Activating the AMPK-PGC1 -Fndc5 Pathway in Muscle.' *The FASEB Journal* 27 (5): 1981–89. doi:10.1096/fj.12-225755. http://www.fasebj.org/cgi/doi/10.1096/fj.12-225755.

Short, Kevin R, Maureen L Bigelow, Jane Kahl, Ravinder Singh, Jill Coenen-Schimke, Sreekumar Raghavakaimal, and K Sreekumaran Nair. 2005. 'Decline in Skeletal Muscle Mitochondrial Function with Aging in Humans.' *Proceedings of the National Academy of Sciences of the United States of America* 102 (15): 5618–23. doi:10.1073/pnas.0501559102.

Skurk, Thomas, Catherine Alberti-Huber, Christian Herder, and Hans Hauner. 2007. 'Relationship between Adipocyte Size and Adipokine Expression and Secretion.' *The Journal of Clinical Endocrinology &*

Metabolism 92 (3): 1023–33. doi:10.1210/jc.2006-1055. http://press.endocrine.org/doi/abs/10.1210/jc.2006-1055.

Spalding, Kirsty L., Erik Arner, Pål O. Westermark, Samuel Bernard, Bruce A. Buchholz, Olaf Bergmann, Lennart Blomqvist, et al. 2008. 'Dynamics of Fat Cell Turnover in Humans.' *Nature* 453 (7196): 783–87. doi:10.1038/nature06902. http://www.nature.com/doifinder/10.1038/nature06902.

Stone, James R, and Suping Yang. 2006. 'Hydrogen Peroxide: A Signaling Messenger.' *Antioxidants & Redox Signaling* 8 (3-4): 243–70. doi:10.1089/ars.2006.8.243. http://www.liebertonline.com/doi/abs/10.1089/ars.2006.8.1391\nhttp://www.liebertonline.com/doi/abs/10.1089/ars.2006.8.243.

Storlien, Len, Nick D Oakes, and David E Kelley. 2004. 'Metabolic Flexibility.' *The Proceedings of the Nutrition Society* 63 (2): 363–68. doi:10.1079/PNS2004349. http://journals.cambridge.org/abstract_S0029665104000497.

Sultan, M., M. H. Schulz, H. Richard, A. Magen, A. Klingenhoff, M. Scherf, M. Seifert, et al. 2008. 'A Global View of Gene Activity and Alternative Splicing by Deep Sequencing of the Human Transcriptome.' *Science* 321 (5891): 956–60. doi:10.1126/science.1160342. http://www.sciencemag.org/cgi/doi/10.1126/science.1160342.

Swinburn, Boyd a., Gary Sacks, Kevin D. Hall, Klim McPherson, Diane T. Finegood, Marjory L. Moodie, and Steven L. Gortmaker. 2011. 'The Global Obesity Pandemic: Shaped by Global Drivers and Local Environments.' *The Lancet* 378 (9793). Elsevier Ltd: 804–14. doi:10.1016/S0140-6736(11)60813-1. http://dx.doi.org/10.1016/S0140-6736(11)60813-1.

Szmuilowicz, Emily D, Cynthia a Stuenkel, and Ellen W Seely. 2009. 'Influence of Menopause on Diabetes and Diabetes Risk.' *Nature Reviews. Endocrinology* 5 (10). Nature Publishing Group: 553–58. doi:10.1038/nrendo.2009.166. http://dx.doi.org/10.1038/nrendo.2009.166.

Taniguchi, Cullen M., Brice Emanuelli, and C. Ronald Kahn. 2006. 'Critical Nodes in Signalling Pathways: Insights into Insulin Action.' *Nature Reviews Molecular Cell Biology* 7 (2): 85–96. doi:10.1038/nrm1837. http://www.nature.com/doifinder/10.1038/nrm1837.

Tchoukalova, Yourka D, Christina Koutsari, Maksym V Karpyak, Susanne B Votruba, Eliana Wendland, and Michael D Jensen. 2008. 'Subcutaneous Adipocyte Size and Body Fat Distribution.' *The American Journal of Clinical Nutrition* 87 (1): 56–63. http://www.ncbi.nlm.nih.gov/pubmed/18175737.

Tirone, Thomas A., and F. Charles Brunicardi. 2001. 'Overview of Glucose Regulation.' *World Journal of Surgery* 25 (4): 461–67. doi:10.1007/s002680020338. http://link.springer.com/10.1007/s002680020338.

Toledo, Frederico G. S., Simon Watkins, and David E. Kelley. 2006. 'Changes Induced by Physical Activity and Weight Loss in the Morphology of Intermyofibrillar Mitochondria in Obese Men and Women.' *The Journal of Clinical Endocrinology & Metabolism* 91 (8): 3224–27. doi:10.1210/jc.2006-0002. http://press.endocrine.org/doi/abs/10.1210/jc.2006-0002.

Toledo, Frederico G.S., and Bret H. Goodpaster. 2013. 'The Role of Weight Loss and Exercise in Correcting Skeletal Muscle Mitochondrial Abnormalities in Obesity, Diabetes and Aging.' *Molecular and Cellular Endocrinology* 379 (1-2). Elsevier Ireland Ltd: 30–34. doi:10.1016/j.mce.2013.06.018. http://linkinghub.elsevier.com/retrieve/pii/S0303720713002566.

Trayhurn, Paul, Christian a Drevon, and Jürgen Eckel. 2011. 'Secreted Proteins from Adipose Tissue and Skeletal Muscle - Adipokines, Myokines and Adipose/muscle Cross-Talk.' *Archives of Physiology and Biochemistry* 117 (2): 47–56. doi:10.3109/13813455.2010.535835. http://www.ncbi.nlm.nih.gov/pubmed/21158485.

Turner, N., G. J. Cooney, E. W. Kraegen, and C. R. Bruce. 2014. 'Fatty Acid Metabolism, Energy Expenditure and Insulin Resistance in Muscle.' *Journal of Endocrinology* 220 (2): T61–79. doi:10.1530/JOE-13-0397. http://joe.endocrinology-journals.org/cgi/doi/10.1530/JOE-13-0397.

Valtueña, S, J Salas-Salvadó, and P G Lorda. 1997. 'The Respiratory Quotient as a Prognostic Factor in Weight-Loss Rebound.' *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity* 21 (9): 811–17. doi:10.1038/sj.ijo.0800480.

Vernochet, Cecile, Arnaud Mourier, Olivier Bezy, Yazmin Macotela, Jeremie Boucher, Matthew J. Rardin, Ding An, et al. 2012. 'Adipose-Specific Deletion of TFAM Increases Mitochondrial Oxidation and Protects Mice against Obesity and Insulin Resistance.' *Cell Metabolism* 16 (6): 765–76. doi:10.1016/j.cmet.2012.10.016. http://linkinghub.elsevier.com/retrieve/pii/S1550413112004160.

Viguerie, Nathalie, Emilie Montastier, Jean-José Maoret, Balbine Roussel, Marion Combes, Carine Valle, Nathalie Villa-Vialaneix, et al. 2012. 'Determinants of Human Adipose Tissue Gene Expression: Impact of Diet, Sex, Metabolic Status, and Cis Genetic Regulation.' *PLoS Genetics* 8 (9): e1002959. doi:10.1371/journal.pgen.1002959. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3459935&tool=pmcentrez&rendertype=abstract.

Wajchenburg, Bernardo Leo. 2014. 'Subcutaneous and Visceral Adipose Tissue : Their Relation to the Metabolic Syndrome.' *Endocrine Reviews* 21 (July): 697–738. doi:10.1210/edrv.21.6.0415.

Wang, Y. Claire, Klim McPherson, Tim Marsh, Steven L. Gortmaker, and Martin Brown. 2011. 'Health and Economic Burden of the Projected Obesity Trends in the USA and the UK.' *The Lancet* 378 (9793). Elsevier Ltd: 815–25. doi:10.1016/S0140-6736(11)60814-3. http://dx.doi.org/10.1016/S0140-6736(11)60814-3.

Welle, Stephen, Andrew I Brooks, Joseph M Delehanty, Nancy Needler, Kirti Bhatt, Bharati Shah, and Charles A Thornton. 2004. 'Skeletal Muscle Gene Expression Profiles in 20-29 Year Old and 65-71 Year Old Women.' *Experimental Gerontology* 39 (3): 369–77. doi:10.1016/j.exger.2003.11.011. http://www.ncbi.nlm.nih.gov/pubmed/15036396.

Welle, Stephen, Andrew I Brooks, Joseph M Delehanty, Nancy Needler, and Charles A Thornton. 2003. 'Gene Expression Profile of Aging in Human Muscle.' *Physiological Genomics* 14 (2): 149–59. doi:10.1152/physiolgenomics.00049.2003. http://www.ncbi.nlm.nih.gov/pubmed/12783983.

Wells, J C K, and M Siervo. 2011. 'Obesity and Energy Balance: Is the Tail Wagging the Dog?' *European Journal of Clinical Nutrition* 65 (11). Nature Publishing Group: 1173–89. doi:10.1038/ejcn.2011.132. http://www.nature.com/doifinder/10.1038/ejcn.2011.132.

Wing, Rena R, Elaine H Blair, Patricia Bononi, Marsha D Marcus, Richard Watanabe, and Richard N Bergman. 1994. 'Caloric Restriction Per Se Is a Significant Factor in Improvements in Glycemic During Weight Loss in Obese.' *Diabetes Care* 17 (1): 30–36.

Wirth, a, and B Steinmetz. 1998. 'Gender Differences in Changes in Subcutaneous and Intra-Abdominal Fat during Weight Reduction: An Ultrasound Study.' *Obesity Research* 6 (6): 393–99. doi:10.1002/j.1550-8528.1998.tb00370.x.

Wisse, B. E. 2004. 'The Inflammatory Syndrome: The Role of Adipose Tissue Cytokines in Metabolic Disorders Linked to Obesity.' *Journal of the American Society of Nephrology* 15 (11): 2792–2800. doi:10.1097/01.ASN.0000141966.69934.21. http://www.jasn.org/cgi/doi/10.1097/01.ASN.0000141966.69934.21.

Yaspelkis, Ben B, Maziyar Saberi, Mohenish K Singh, Beatriz Trevino, and Toby L Smith. 2002. 'Chronic Leptin Treatment Normalizes Basal Glucose Transport in a Fiber Type-Specific Manner in High-Fat–Fed Rats.' *Metabolism* 7: 859–63. doi:10.1053/meta.2002.33338.

Yaspelkis, Ben B, Mohenish K Singh, Adam D Krisan, Dale E Collins, Connie C Kwong, Jeffrey R Bernard, and Andrew M Crain. 2004. 'Chronic Leptin Treatment Enhances Insulin-Stimulated Glucose Disposal in Skeletal Muscle of High-Fat Fed Rodents.' *Life Sciences* 74: 1801–16. doi:10.1016/j.lfs.2003.08.037.

You, Tongjian, Xuewen Wang, Karin M Murphy, Mary F Lyles, Jamehl L Demons, Rongze Yang, Da Wei Gong, and Barbara J Nicklas. 2014. 'Regional Adipose Tissue Hormone/cytokine Production before and after Weight Loss in Abdominally Obese Women.' *Obesity* 22 (7): 1679–84. doi:10.1002/oby.20743.

Zechner, Rudolf, Robert Zimmermann, Thomas O. Eichmann, Sepp D. Kohlwein, Guenter Haemmerle, Achim Lass, and Frank Madeo. 2012. 'FAT SIGNALS - Lipases and Lipolysis in Lipid Metabolism and Signaling.' *Cell Metabolism* 15 (3). Elsevier Inc.: 279–91. doi:10.1016/j.cmet.2011.12.018. http://linkinghub.elsevier.com/retrieve/pii/S1550413112000186.

Zeyda, Maximilian, and Thomas M. Stulnig. 2009. 'Obesity, Inflammation, and Insulin Resistance.' *Gerontology* 55 (4): 379–86. doi:10.1159/000212758. http://www.karger.com/doi/10.1159/000212758.

Zhang, C., C. McFarlane, S. Lokireddy, S. Bonala, X. Ge, S. Masuda, P. D. Gluckman, M. Sharma, and R. Kambadur. 2011. 'Myostatin-Deficient Mice Exhibit Reduced Insulin Resistance through Activating the AMP-Activated Protein Kinase Signalling Pathway.' *Diabetologia* 54 (6): 1491–1501. doi:10.1007/s00125-011-2079-7. http://link.springer.com/10.1007/s00125-011-2079-7.

Zhang, Y, R Proenca, M Maffei, M Barone, L Leopold, and J M Friedman. 1994. 'Positional Cloning of the Mouse Obese Gene and Its Human Homologue.' *Nature* 372 (6505): 425–32. doi:10.1038/372425a0.

Zhao, Baoping, Robert J. Wall, and Jinzeng Yang. 2005. 'Transgenic Expression of Myostatin Propeptide Prevents Diet-Induced Obesity and Insulin Resistance.' *Biochemical and Biophysical Research Communications* 337 (1): 248–55. doi:10.1016/j.bbrc.2005.09.044. http://linkinghub.elsevier.com/retrieve/pii/S0006291X05020371.

Zhou, Yingjiang, and Liangyou Rui. 2014. 'Leptin Signaling and Leptin Resistance.' *Front Med*. doi:10.1007/s11684-013-0263-5.Leptin.

8.2 Figures

8.3 Tables

8.4 Abbreviations

AC	Acylcarnitines
AMPK	AMP-activated protein kinase
ANP	Natriuretic peptides
ATGL	Adipose triglyceride lipase
BMI	Body mass index
CACT	Carnitine acylcarnitine translocase
CPT	Carnitine palmitoyl transferase
CVD	Cardio vascular disease
ECM	Extracellular matrix
ES	Enrichment score
FA	Fatty acids
FC	Fold change
FDR	False discovery rate
FFA	Free fatty acids
GDF	Growth differentiation factor
GLP-1	Glucagon-like peptide 1
GLUT	Glucose transporter
GSEA	Gene set enrichment analysis
GWAS	Gene wide association studies
HSL	Hormone sensitive lipase
IRS	Insulin receptor substrate
ISI_{clamp}	Insulin sensitivity index
MCH	Melanin-concentrating hormone
MGL	Monacylglycerol lipase
MYH	Myosin heavy chain
NPY	Neuropeptide Y
PCA	Principal component analysis
PDK	Pyruvate dehydrogenase kinase
PGC1α	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PI3K	Class 1A phosphaditylinositide 3-kinase
PK	Protein kinase
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
PPARα	Proliferator-activated receptor alpha
PYY	Peptide YY
RNA-Seq	RNA-Sequencing
RQ	Respiratory quotient
SNP	Single nucleotide polymorphisem
SVC	Stroma vascular cells
T2DM	Type 2 diabetes mellitus
TBF	Total body fat
TCA	Tricarboxylic acid cycle
TG	Triglycerides
VLCAD	Very long-chain acyl-CoA dehydrogenase
WR	Weight reduction

8.5 Acknowledgments

I first of all like to thank my doctoral supervisor Prof. Dr. med. Joachim Spranger to given me the great opportunity to work on this interesting and challenging project. I learned a lot in the last years about scientific progress, stagnation and nevertheless to go on.

I also like to thank my supervisor PD. Dr. med. Knut Mai for all the help and spending time discussing this data. Even if we not always agreed.

I also very much acknowledge Prof. Dr. med. Matthias Blüher and Prof. Dr. med. Andreas Pfeiffer for reviewing my work. Speical thanks to Prof. Dr. Matthias Schulze, Prof. Dr. rer. Nat. Burkhard Kleuser and Prof. Dr. med. vet. Jens Raila to be part of my examination comitee.

A big thank you to all the members of my lab for giving me such a nice and friendly environment. I do not want to miss the last years in this lab. Especially not the time spending in the „doctoral office“ having great discussions, many hours of laughing and so many times just to be remembered that I am not alone with my work.

Last, I am very glad that I found a strong and constant support in my partner, friends and family.

8.6 Declaration

I confirm that the PhD thesis entitled "Genome wide expression analysis and metabolic mechanisms predicting body weight maintanence" is the result of my own work. I did not receive any aids or support other than stated. All sources and materials applied are listed and specified in the thesis. Furthermore I confirm that this thesis has not yet been submitted as part of another examination process, neither in identical nor in similar form.

Ich versichere hiermit, dass ich die Doktorarbeit mit dem Titel „Genome wide expression analysis and metabolic mechanisms predicting body weight maintanence" selbstständig und ohne unzulässige fremde Hilfe erbracht habe. Ich habe keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie wörtliche und sinngemäße Zitate kenntlich gemacht. Die Arbeit hat in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegen.

Berlin, 22.11.15 Maria Schlöcker