Obesity due to stress

A change in glucose and lipid metabolism due to altered cortisol levels

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Content

Nederlandse samenvatting

Tijdens deze literatuurstudie is er onderzoek gedaan naar stress geïnduceerde obesitas. Allereerst zijn de gevaren en oorzaken van obesitas besproken. Vervolgens wordt het mechanisme van stress uitgelegd met onder andere de HPA as, die verantwoordelijk is voor de secretie van het glucocorticoïd cortisol. Cortisol kan binden aan twee verschillende receptoren en op die manier meerdere processen beïnvloeden. In deze scriptie wordt de invloed van cortisol op 3 systemen beschreven. Allereest het glucosemetabolisme. Daar heeft cortisol invloed op onder andere de gluconeogenese, glycogenolyse en een speciaal enzym dat de inactieve vorm van cortisol omzet naar de actieve vorm: 11β-hydroxysteriod dehydrogenase type 1. Cortisol heeft ook invloed op het vetmetabolisme. Daar stimuleert het vooral de afbraak van vetweefsel (lipolyse), wat resulteert in een grote hoeveelheid vrije vetzuren in het plasma. Dit heeft weer als gevolg dat andere processen geremd of gestimuleerd worden. Niet alleen het vet –en glucose metabolisme worden door cortisol beïnvloed, ook het centrale zenuwstelsel. Verschillende regio's van het brein reageren op cortisol door de morfologie van neuronen te veranderen, wat onder andere leidt tot "stress eten" en geprikkeld zijn. Al deze effecten van cortisol kunnen leiden tot obesitas en een gevaar voor de mens vormen. Gelukkig zijn er verschillende manieren waarop dit wellicht geremd kan worden en dat is het onderwerp van het laatste hoofdstuk.

Abstract

In this literature study different topics concerning stress-induced obesity are described. First of all the dangers and causes of obesity are discussed. Next, the mechanism of stress is explained with among others the HPA axis, which is responsible for the secretion of the glucocorticoid cortisol. Cortisol is able to bind to two different receptors and thereby influence different processes. In this thesis, the effects of cortisol on three systems are thoroughly described. One of these processes is the glucose metabolism. Cortisol influences gluconeogenesis, glycogenolysis and a special enzyme catalyzing the conversion of the inactive form of cortisol to the active form: 11β - hydroxysteroid dehydrogenase type 1. Cortisol also has an effect on the lipid metabolism. Within this metabolism it mainly stimulates the breakdown of adipose tissue (lipolysis), which results in a large amount of free fatty acids in the plasma. This in turn has an effect on other processes which will be inhibited or stimulated. Not only the lipid metabolism and glucose metabolism are affected by cortisol, also the central nervous system. Different parts of the brain respond to cortisol by changing morphology of neurons, which among others leads to "stress eating" and being agitated. All these effects of cortisol can lead to obesity and form a danger for human. Fortunately, there are many ways in which this might be treated and this is discussed in the last chapter of this study.

List of Abbreviations

11β-HSD1	11β-hydroxysteroid dehydrogenase type 1
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
ATGL	Adipose triglyceride lipase
AVP	Vasopressin
BAT	Brown adipose tissue
BMI	Body mass index
cAMP	cyclic AMP
CHD	Chronic heart disease
CREB	cAMP response element binding protein
CRH	Corticotropin-releasing hormone
CRHR1	CRH1 receptor
CRHR2	CRH2 receptor
CVD	Cardiovascular disease
DNL	*De novo* lipogenesis
ECGC	Epigallocatechin gallate
Fbpase	Fructose 1,6-bisphosphatase
FFA	Free fatty acids
FoxOs	Forkhead box class Os
G6Pase	Glucose 6 phosphatase
G/G6P	Glucose/glucose-6-phosphate
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HPA	Hypothalamic-pituitary-adrenal
HSL	Hormone-sensitive lipase
IKK	IkappaB-kinase
IRS-1	Insulin receptor substrate 1
IRSs	Insulin-receptor substrates
JNK	c-jun N-terminal kinase
LMO3	LIM domain only 3
LPL	Lipoprotein lipase
LSGRA	Liver-selective glucocorticoid receptor antagonist
MAG	Monoacylglycerol
MGL	Monoglyceride lipase
MR	Mineralocorticoid receptor
NEFAs	Nonesterified fatty acids
NPY	Neuropeptide Y
NPY2R	Neuropeptide Y receptor
p38MAPK	p38 MAP kinase
p70SK	S6 kinase p70
PC	Pyruvate carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
PKA	Protein kinase A
PKC	Protein kinase C
POMC	Pro-opiomelanocortin
PVN	Paraventricular nucleus (located in hypothalamus)
ROS	Reactive oxygen species
SAM	Sympatho-adrenomedullary
SAPK	Stress-activated protein kinase

SCN	Suprachiasmatic nucleus
sER	smooth endoplasmatic reticulum
SF	Skinfold measurements
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol
WAT	White adipose tissue
WC	Waist circumference
WHR	Waist-hip ratio
WHO	World Health Organization

Introduction

Obesity

A spectrum of diseases is known to occur more frequently in people with obesity. Obesity is a serious worldwide problem, nowadays affecting approximately 10% of the world's population (World Health Organization, www.who.int/gho/ncd/risk_factors/obesity_text/en/).

Definition & Measurements

Obesity is defined as an accumulation of adipose tissue that is of sufficient magnitude to impair life (Robbins & Cotran, 8th edition). One method to measure obesity is the use of the body mass index (BMI). The BMI of a person can be calculated by dividing the weight (kg) with the height in square (m^2) (Sweeting, 2007; Roche *et al.*, 1981). The World Health Organisation (WHO) stated that a median BMI for an adult population should be in the range of 21 - 23kg/m^2 to achieve optimum health. A BMI of 25 – 29,9kg/m^2 increases risk for co-morbidities, and moderate to severe risk of co-morbidities occur at a BMI of 30kg/m2 and higher (World Health Organization). An overview of BMI classification is shown in **table 1.**

Table 1. Classification of BMI. Adapted from World Health Organization

BMI	Classification
< 18.5	Underweight
18.5 – 24.9	Normal weight
25 – 29.9	Pre- obese
30 – 34.9	Obese class I
35 – 39.9	Obese class II
≥ 40	Obese class III

Multiple authors have described disadvantages of the BMI method (Garn *et al*. 1986; Daniels *et al*., 1997; Prentice and Jebb, 2001; Wells *et al*., 2002). One of the biggest disadvantages is that BMI reflects both fat and fat-free components of weight and thereby does not directly measure adipose tissue (Wells *et al.*, 2002). Therefore other measurements are necessary.

These other measurements include complex methods and most of them are limited to research settings (Sweeting, 2007). Some easier, anthropometric methods are skinfold measurements (SF), waist circumference (WC) and waist-hip ratio (WHR) (Sweeting, 2007). These methods together with BMI can diagnose a person to suffer from obesity or not.

Prevalence

In 2008, 35% of adults aged 20+ (worldwide) were overweight (BMI ≥ 25 kg/m^2) (34% men and 35% women). The worldwide prevalence of obesity has nearly doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 kg/m^2), compared with 5% for men and 8% for women in 1980 (World Health Organization). In the Netherlands in 2011, 41% of the population suffers from obesity, 10% of this percentage suffers from severe obesity (CBS, www.cbs.nl/nl-NL/menu/themas/gezondheid-welzijn/publicaties/artikelen/archief/2012/2012-3651-wm.htm).

The prevalence of overweight and obesity is highest in America (62% & 26%) and lowest in South East Asia (14% & 3%). In all WHO regions women were more likely to be obese than men. The

prevalence of raised body mass index increases with income level of countries (World Health Organization) suggesting that being wealthy results more likely in obesity compared to being poor.

Health risks

Some of the diseases thought to occur more frequently in people suffering from obesity are among others metabolic syndrome, diabetes, cardiovascular disease and respiratory effects (Bray, 2004; Kopelman, 2007).

The metabolic syndrome is a combination of metabolic risk factors that consist the following: atherogenic dyslipidemia (elevated triglycerides, ≥150 mg/dl), elevated blood pressure (≥130/85 mm Hg), elevated blood glucose (≥100 mg/dl), prothrombotic state and proinflammatory state (Grundy, 2004). It was found that many factors are increased in obese people plasma: Nonesterified fatty acids (NEFAs), inflammatory cytokines, adiponectin, leptin and resistin. Al these factors are thought to contribute to the development of the metabolic syndrome (Guerre-Millo, 2002) and explains why obesity results in an increased risk for this disease.

Another health risk developing with obesity is diabetes type 2 (T2DM). About 90-95% of people with diabetes have type 2 and in 80% of all people with diabetes type 2, obesity is the cause. When diabetes type 2 is diagnosed, the pancreas is producing enough insulin, but the body does not respond to it properly, also called insulin resistance (NIDDK, The National Institute of Diabetes and Digestive and Kidney Diseases, http://diabetes.niddk.nih.gov/dm/pubs/overview/). Insulin resistance leads to a decreased uptake of glucose in the muscle, reduced glycolysis and fatty acid oxidation in the liver and an impairment to suppress hepatic gluconeogenesis. This in total results in an accumulation of glucose in the blood, called hyperglycemia (Robbins & Cotran, 8th edition). Insulin resistance can be reversed, for example by bilio-pancreatic diversion, causing lipid malabsorption (Mingrone *et al*., 1997). Because of the insulin resistance the pancreas first tends to secrete even more insulin to compensate. However, the body will still not react properly to it and this will finally lead to β-cell dysfunction (production site of insulin) (Robbins & Cotran, 8th edition). This dysfunction causes irreversibility of the insulin resistance and T2DM has occurred.

Obese persons have a higher prevalence of hypertension compared to lean persons. This is a strong risk factor for cardiovascular disease (CVD) (Grundy, 2004; Chobanioan *et al*.,2003). Well-known complications of hypertension are chronic heart disease (CHD), stroke, left ventricular hypertrophy, heart failure, and chronic renal failure (Grundy, 2004; Ejerblad *et al*., 2006). Some of these complications can also be caused by inflammation, which in turn also is a risk of obesity (Grundy, 2004).

Accumulation of fat tissue impairs ventilatory function by reducing volume and total lung capacity (Chin *et al*., 1996; Poulain *et al*., 2006). Impaired ventilatory function is caused by the mechanical effects of fat on the diaphragm and the chest wall (Ray *et al*., 1983; Poulain *et al*., 2006). Another consequence of obesity is obstructive sleep apnea. Increased fat tissue deposition in the pharyngeal region obstructs the upper airway and increases its collapsibility. This results in repetitive closures of the airway during sleep (Resta *et al*., 2001). Obstructive sleep apnea is also associated with increased mortality due to the high incidence of cardiovascular disorders reported in this condition (Wolk *et al*., 2003).

Obesity and Stress

Obesity can be caused by an inactive lifestyle, environmental factors (work schedules, oversized food portions and lack of access to healthy food), genetic factors and family history (NIH, National Institutes of Health, http://www.nhlbi.nih.gov/health/health-topics/topics/obe/causes). Many of these factors can be counteracted by changing lifestyle. However, besides all listed causes, there may be additional factors associated with the development of obesity, metabolic syndrome and disease. One of these factors is stress and will be the main subject of this thesis.

All living organisms strive towards a dynamic equilibrium, called homeostasis. This involves chemical and other processes to maintain the most optimal conditions for life. Homeostasis is continually disrupted by environmental factors, external and internal stimuli. Physical and physiological events causing the loss of optimal conditions are called stressors and can be experienced as having stress. Stressors also trigger physiological and behavioural responses that are aimed to maintain homeostasis. In response to stress, the brain can activate different systems (De Kloet *et al*., 2005).

This literature study is about those different systems activated by the brain and how this can lead to obesity. Therefore the responses to stress will be further described. Next, this will be related to changes in both glucose metabolism and lipid metabolism. Besides these peripheral effects of stress, there also are some relevant effects within the central nervous system. For both aspects of metabolism (glucose and lipid), mechanisms to prevent obesity due to stress, will be discussed. Finally a summary of the literature will be given.

Mechanisms of stress

When a situation is perceived as stressful, mainly two pathways are activated in order to maintain homeostasis. The effects of both pathways will be explained with use of **figure 2**.

Autonomic nervous system responses

The responses of the autonomic nervous system (ANS) are indicated in blue in **figure 2**. Exposure to stressors results in activation of sympathetic neurons in the thoracolumbar (T1-L2) spinal cord. These neurons in turn project to prevertebral or paravertebral ganglia which project to organs and cells of the adrenal medulla. This sympathetic activation, also called the sympatho-adrenomedullary (SAM) axis, represents the classic 'fight or flight' response which generally increases circulating levels of adrenaline (from the adrenal medulla) and noradrenaline (from sympathetic nerves). Heart rate and force of contraction, peripheral vasoconstriction, and energy mobilization are also increased by this activation. Besides the sympathetic activation, the parasympathetic system can also be modulated during stress. This is indicated in **figure 2** by red dots. In the parasympathetic system, activation of cranial nuclei results in changes in the cardiovascular system and activation of sacral nuclei modulates changes in the abdominal viscera. Parasympathetic actions are generally opposite to those of the sympathetic system (Ulrich-Lai & Herman, 2009).

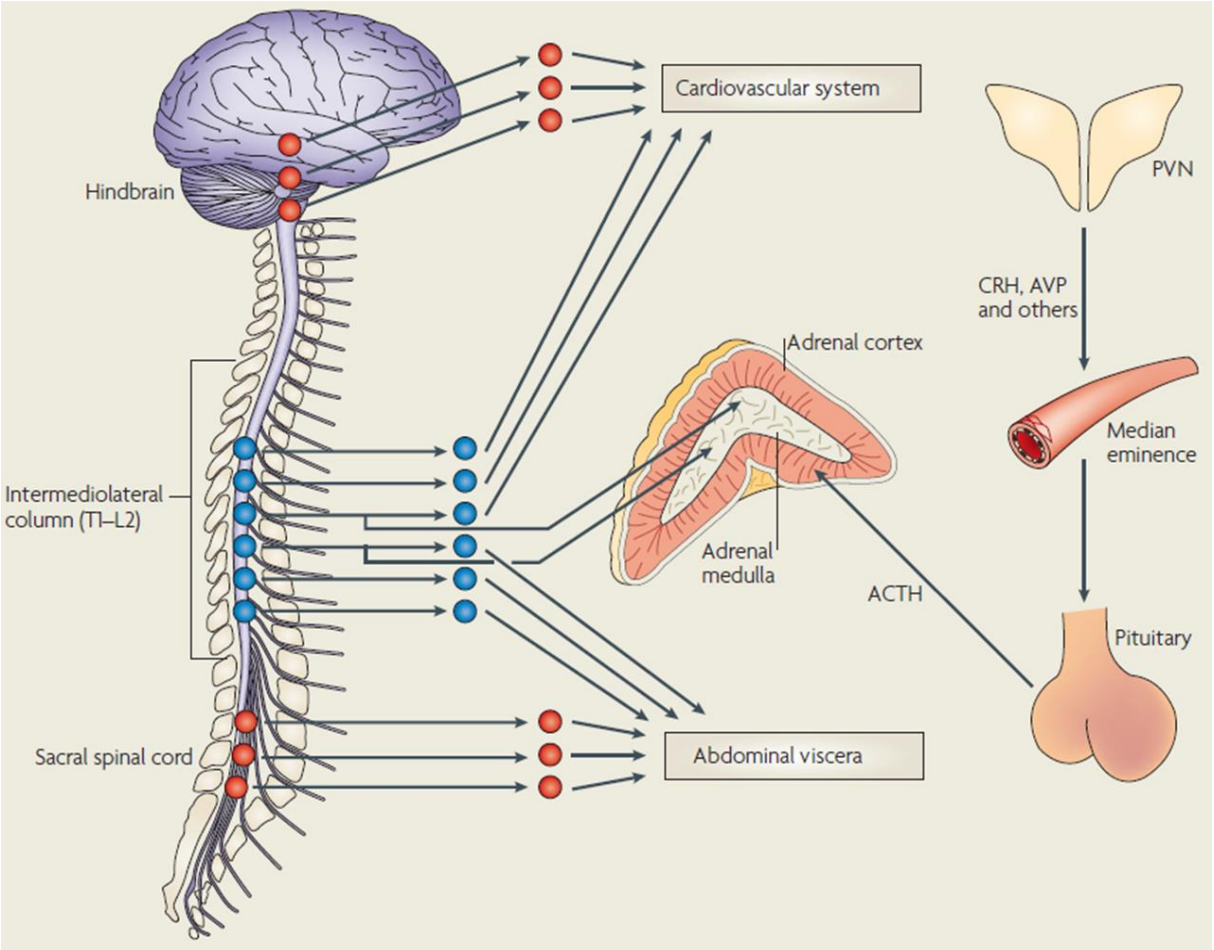

Figure 2. The sympatho-adrenomedullary axis (left side) and the hypothalamic-pituitary-adrenal axis (right side). The two main pathways activated by stress exposure to maintain homeostasis. Adapted from Ulrich-Lai & Herman, 2009

The hypothalamic-pituitary-adrenal (HPA) axis

The other pathway responding to stress is the hypothalamic-pituitary-adrenal (HPA) axis. This pathway will be the main focus of this thesis and is depicted on the right side of **figure 2**. Exposure to stressors activate the production of corticotropin-releasing hormone (CRH) and vasopressin (AVP) by the parvocellular neurons located in the hypothalamic paraventricular nucleus (PVN). These neurons secrete the peptides into the portal vessel system where they can bind the CRH1 receptor (CRHR1) and activate the synthesis of pro-opiomelanocortin (POMC) in the anterior pituitary. POMC in turn gets processed into among others adrenocorticotropic hormone (ACTH), opioid and melanocortin peptides. CRH-induced release of ACTH stimulates the adrenal cortex to synthesize and secrete the glucocorticoid cortisol. Cortisol acts as a negative feedback signal, inhibiting ACTH and CRH secretion (Herman *et al.*, 2003; De Kloet *et al.*, 2005; Silverthorn, 5th edition).

The hypothalamic-pituitary-adrenal (HPA) axis & circadian rhythm

The HPA axis is normally continuously active under the influence of the ANS. A basic characteristic of the HPA axis is that unstressed animals have a circadian rhythm of ACTH and glucocorticoid secretion. This rhythm is coordinated by outputs from the suprachiasmatic nucleus (SCN) of the hypothalamus (Lightman & Conway-Campbell, 2010). The circadian rhythm appears to have an underlying ultradian (hourly) activity of the HPA axis. The origin of ultradian rhythmicity in the HPA axis is not known. Although there has been a general assumption that there must be a hypothalamic pulse generator, there is little evidence to support this (Lightman & Conway-Campbell, 2010). However, it is thought that simple feedforward and feedback interactions between the pituitary and adrenal cortex can account for the glucocorticoid rhythms that have been observed experimentally (Lightman & Conway-Campbell, 2010). Finally researchers conclude that both the circadian and ultradian rhythms are crucial for optimal responsiveness of glucocorticoid-sensitive neural processes (Lightman & Conway-Campbell, 2010). Stress is one of those neural processes where responsiveness should be optimal.

Cortisol and its effects

Cortisol belongs to a family called steroid hormones. This family can be further subdivided into three classes: sex hormones, mineralocorticoids (named because of its effect on the minerals sodium and potassium) and glucocorticoids (named because of its effect on plasma glucose concentrations). Cortisol belongs to the glucocorticoids and is synthesized from cholesterol in the zona fasciculata of the adrenal cortex which is the only zone containing the correct enzymes (21-hydroxylase and 17-α-hydroxylase) for the synthesis (Silverthorn, 5th edition).

Approximately 75% of the cortisol in the circulation is bound to a plasma protein named transcortin or corticosteroid binding globulin (CBG). Another 15% is bound to albumin, and the remaining 10% is unbound or free. Free cortisol is biologically active. It is also the concentration of free cortisol that is regulated. The half-life of cortisol in the circulation is 60-90 min and it is metabolized in the liver. Most of the cortisol is reduced to dihydrocortisol and next to tetrahydrocortisol which is conjugated to glucuronic acid. Some cortisol is converted to cortisone, which is an inactive glucocorticoid. Cortisone is also reduced and conjugated to form tetrahydrocortisone glucuronide. The tetrahydroglucuronide derivatives (like glucuronic acid) of cortisol and cortisone are water soluble and are excreted in the urine (Boron & Boulpaep, 1st edition).

Cortisol can bind to both the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). However, the receptors have a different affinity for the hormone. The MR has a 10-fold higher affinity for cortisol compared to the GR. Therefore the MR is active at both low and high concentrations of the hormone and the GR becomes activated at only high concentrations of cortisol (Funder, 1997; De Kloet *et al*., 2005). When cortisol binds the receptors (depending on the concentration) they form dimers (either homo –or heterodimers) and migrate to the nucleus. There they will interact with specific DNA sequences called glucocorticoid response elements (GRE), which changes the transcription of multiple target genes. GR monomers can also interact with stress-induced transcription factors (TFs) or other proteins to dampen their transcriptional activity (Stokes *et al*., 2000; De Kloet *et al*., 2005). The described pathways are depicted in **figure 3** below.

By changing the transcription of multiple target genes, cortisol has many metabolic effects, mainly preventing hypoglycemia via stimulation of catabolic processes. Some of the processes cortisol stimulates are: suppression of the immune system, creating a negative calcium balance (by adjusting calcium absorption and secretion and promoting breakdown of calcified bone matrix), promoting gluconeogenesis and lipolysis and the breakdown of skeletal muscle proteins. (Silverthorn, 5th edition). The last two processes (gluconeogenesis and lipolysis) are part of the glucose and lipid metabolism and will be extensively explained in the next chapters. The breakdown of skeletal muscle proteins fits with lipolysis and gluconeogenesis because it serves the function of releasing energy substrate for other processes.

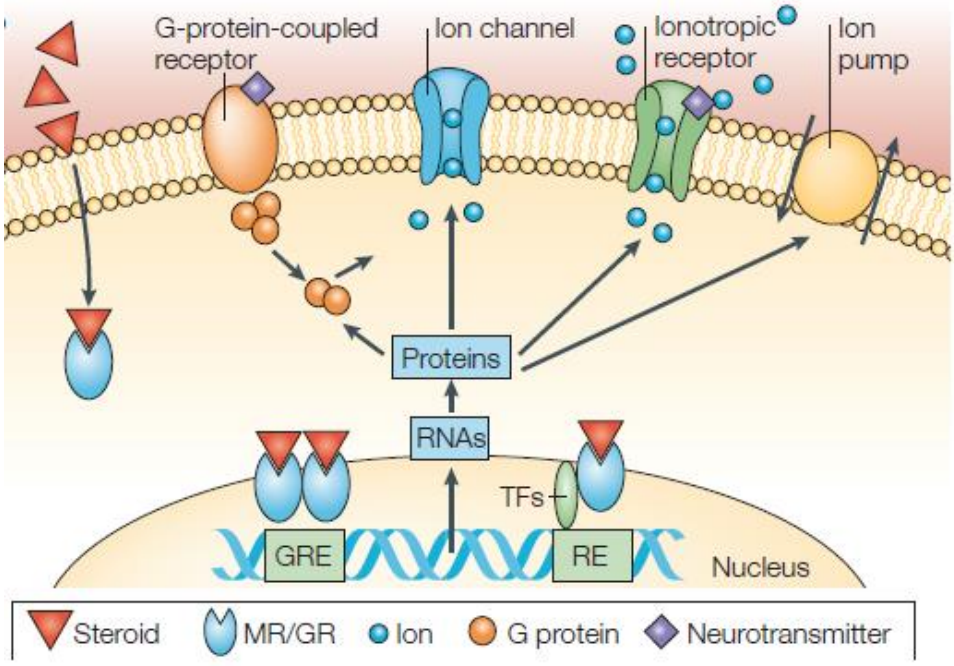

Figure 3. Overview of the pathway after binding of cortisol to either the mineralocorticoid receptor or glucocorticoid receptor. Adapted from De Kloet *et al*., 2005

The effects of cortisol & stress on the glucose metabolism

In this chapter the influence of cortisol on the glucose metabolism will be described. Cortisol has multiple effects on the glucose metabolism overall resulting in hyperglycemia.

Gluconeogenesis

One of the processes cortisol has an effect on is gluconeogenesis. This is the opposite of glycolysis and involves the synthesis of glucose from non-carbohydrate precursors. This process mainly takes place in the liver (Nordlie and Foster, 1999). A first sign of cortisol having a counterregulatory effect on hypoglycemia was discovered in 1989. During this experiment normal subjects got infused with insulin. During the administration of several other compounds the glucose fluxes were continuously monitored. One of the experimental groups received, despite the insulin, a blocker preventing the levels of cortisol to increase. This group appeared to have a 22% decrease of hepatic glucose production (gluconeogenesis) and a 15% increase in glucose utilization. From this could be concluded that cortisol normally plays an important counterregulatory role during hypoglycemia by stimulating glucose production and decreasing glucose utilization (De Feo *et al*., 1989). This outcome got confirmed by other research groups (Rooney *et al*., 1993; Goldstein *et al*., 1993). Other research groups were able to put these effects of cortisol in context with metabolic syndrome (Chrousos, 2000; Khani & Tayek, 2001; Anagnostis *et al*., 2009).

Cortisol is thought to stimulate gluconeogenesis by activation of key regulatory enzymes involved in this process. These enzymes are: glucose 6 phosphatase (G6Pase), fructose 1,6-bisphosphatase (Fbpase), pyruvate carboxylase (PC), and phosphoenolpyruvate carboxykinase (PEPCK). These enzymes are activated by stimulation of intracellular pathways leading to activation of the key transcription factors cAMP response element binding protein (CREB) and forkhead box class Os (FoxOs) (Oh *et al*., 2013).

Due to reciprocal regulation of glycolysis and gluconeogenesis (Weber *et al*., 1967; Berg, 6th edition) cortisol indirectly inhibits glycolysis and thereby also promotes hyperglycemia.

Glycogenolysis

If cortisol has the function to counteract hypoglycaemia, not only a stimulation of gluconeogenesis would be expected, but also an increase in glycogenolysis. This process includes the breakdown of glycogen, mostly stored in the liver, to provide glucose 6-phosphate for further metabolism. The hormones glucagon and epinephrine can stimulate glycogenolysis by binding to a G-protein coupled receptor. This finally results in activation of the enzyme glycogen phosphorylase and stimulation of glycogenolysis (Berg, 6th edition). In 1981 the effect of cortisol on glycogenolysis was discovered in chicks. After injections of cortisol, glycogen content within the liver was decreased, suggesting an increase in glycogenolysis (Eqana *et al*. 1981). This finding got confirmed by another group in the salmon (Vijayan & Leatherland, 1989).

Nowadays it is thought that cortisol stimulates glycogenolysis by facilitating the epinephrine induced activation of the enzyme glycogen phosphorylase (Kuo *et al*., 2013).

Insulin resistance

Normally, the hormone insulin counteracts the hyperglycemic effects of cortisol. However, cortisol is known to counteract insulin action and thereby creates an insulin resistance. Insulin resistance can be quantified by numerous methods, usually involving measurement of the plasma insulin concentration relative to plasma glucose concentration, or the amount of glucose infused to maintain euglycaemia at a fixed insulin concentration (called glucose tolerance test)(Ferrannini and Mari, 1998).

Normally insulin binds to the cell-surface insulin receptor (IR) which is a tyrosine kinase that autophosphorylates and next phosphorylates the insulin receptor substrate (IRS). Tyrosine-phosphorylated IRS associates with IR and activates downstream signaling pathways (Kuo *et al*., 2013). Mice treated with cortisol show to have reduced levels of tyrosine-phosphorylated IR and total IRS-1 proteins. The activity of two downstream signaling molecules: phosphoinositide-3-kinase (PI3K) and Akt are also decreased. Moreover, the phosphorylation of serine 307 of IRS-1 is increased after cortisol administration. This phosphorylation disrupts the association between IR and IRS-1 and thereby reduces the insulin response (Giorgino *et al*., 1993; Kuo *et al*., 2013).

Insulin resistance may also reflect impaired insulin-dependent down-regulation of hepatic glucose release and/or impaired insulin-mediated increase in peripheral glucose uptake (Andrews & Walker, 1999). Cortisol works via both ways. As already described, it promotes gluconeogenesis and thereby impairs insulin-dependent down-regulation of hepatic glucose release. Other researchers found that the cortisol-induced insulin resistance in man is due to the decrease in both hepatic and extrahepatic sensitivity to insulin (Rizza *et al*., 1982).This decrease in insulin action can be explained by the mechanism described above.

When insulin resistance occurs, due to elevated levels of cortisol, glycogenesis is no longer stimulated and glucose will not be taken up by the liver to produce glycogen. This contributes to the hyperglycemic effects of cortisol.

11β-hydroxysteroid dehydrogenase type 1

Hyperglycemia, due to increased gluconeogenesis and glycogenolysis, insulin resistance and “stress eating” might result in obesity and the development of metabolic syndrome (Vicennati *et* al., 2009; Gallagher *et al*., 2010). Obesity in turn also has an influence in cortisol metabolism. Within certain tissues, active cortisol can be converted into the inactive cortisone and vice versa. For this conversion two different enzymes are known to be important. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol into the inactive cortisone, which occurs mainly in the kidney. Dysfunction of this enzyme results in hypertension (Edwards *et al*., 1988). The other enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the opposite conversion, from the inactive cortisone to cortisol. This process mainly occurs in the liver and adipose tissue (Kotelevstev *et al*., 1997). Several research groups have found that people with obesity have an increased activity of 11β-HSD1 within the liver and adipose tissue and therefore an elevated level of active cortisol (Stewart *et al*., 1999; Rask *et al*., 2001; Anagnostis *et al*., 2009). The increase in cortisol in turn stimulates hyperglycemia and insulin resistance and a continuous positive feedback loop is created. A scheme of this loop is visualised in **figure 4**.

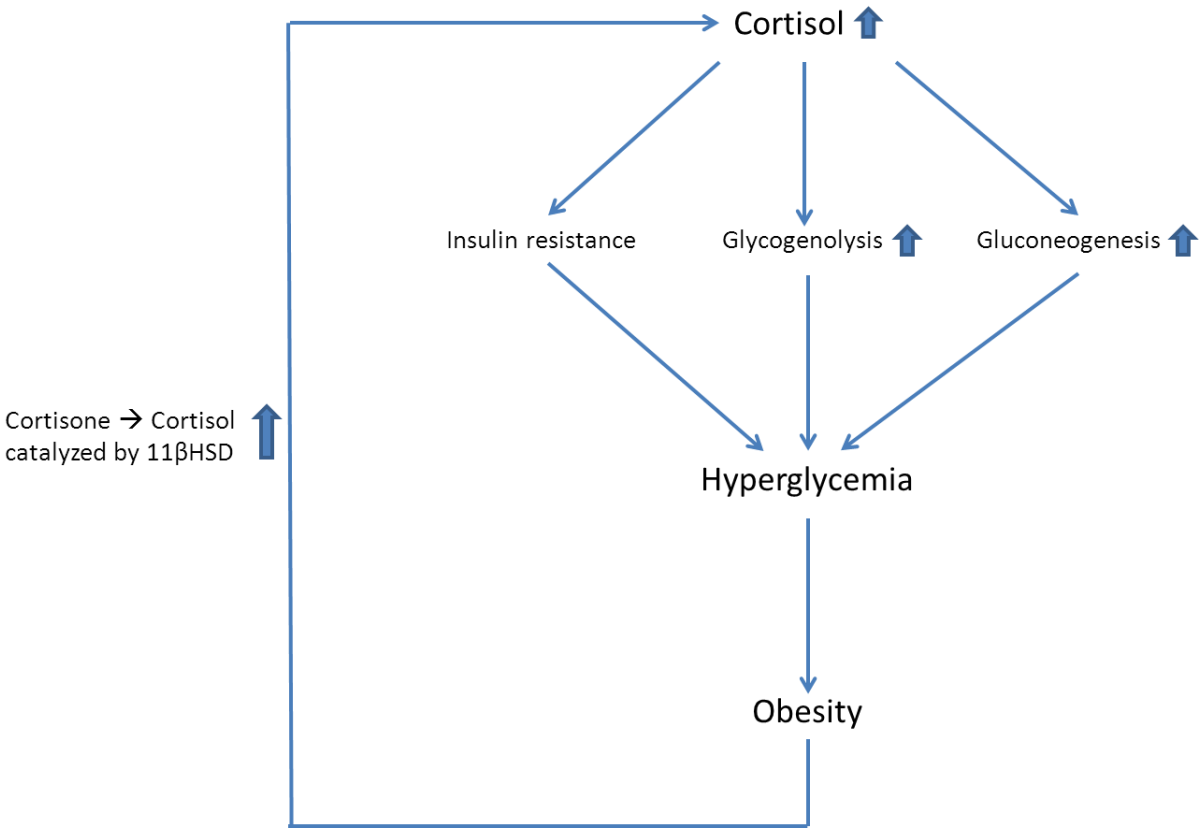

Figure 4. Positive feedback loop of the influence of cortisol on the glucoce metabolism. Cortisol stimulates glycogenolysis and gluconeogenesis and causes insulin resistance. These three processes result in hyperglycemia. Hyperglycemia together with "stress eating" result in obesity. Obesity in turn changes cortisol metabolism resulting in higher levels of the glucocorticoid.

Figure 4 suggests an elevated level of cortisol in plasma , which can be measured. However multiple studies do not find this elevation in the plasma (Rask *et al.*, 2001; Liu *et al.*, 2013). One of the studies addressed this to a tissue specific dysregulation of the cortisol metabolism. They conclude that the conversion of cortisone to cortisol by 11β-HSD1 is impaired in the liver, but normal/increased in adipose tissue (Rask *et al.*, 2001). This results in a compensation and no elevated levels of cortisol in plasma.

Another explanation might be that, because of the continuous positive feedback loop, the substrate for active cortisol (cortisone) is no longer available. Then the conversion from the inactive form to the active form is no longer possible and cortisol levels will return to normal again. This could be investigated by measuring the levels of cortisone over a longer period at subjects with stress induced obesity.

Other compensational mechanism might be possible too. It might be that other parts of the body will start to counteract the cortisol induced hyperglycemia by taking up glucose or inhibiting glycogenolysis and gluconeogenesis. This might slightly prevent obesity and thereby the conversion of cortisone into cortisol.

Stress and the lipid metabolism

In this chapter the influence of cortisol on the lipid metabolism will be described. Before starting with this the normal lipid metabolism will be explained.

The normal lipid metabolism

The normal fat digestion and absorption is shown in **figure 5**. Large lipid droplets are emulsified by bile salts secreted by the liver, resulting in smaller micelles. Pancreatic lipases enzymatically digest triacylglycerol (TAG) into free fatty acids (FFAs) and monoacylglycerol (MAG). Next, FFAs and monoglycerides diffuse across the apical membrane of the small intestine and move towards the smooth endoplasmatic reticulum (sER) and recombine into triglycerides. The triglycerides join cholesterol and proteins to form large droplets called chylomicrons. Chylomicrons leave the cell by exocytosis and via the lymphatic system they enter into the cardiovascular system. Via this system chylomicrons arrive at primarily adipose tissue and muscle tissue. There they bind to membrane – bound lipoprotein lipase (LPL) and TAGs are once again degraded into FFA and MAG for transport into the tissue. TAG is resynthesized inside the cell and stored (Silverthorn, 5th edition; Berg, 6th edition).

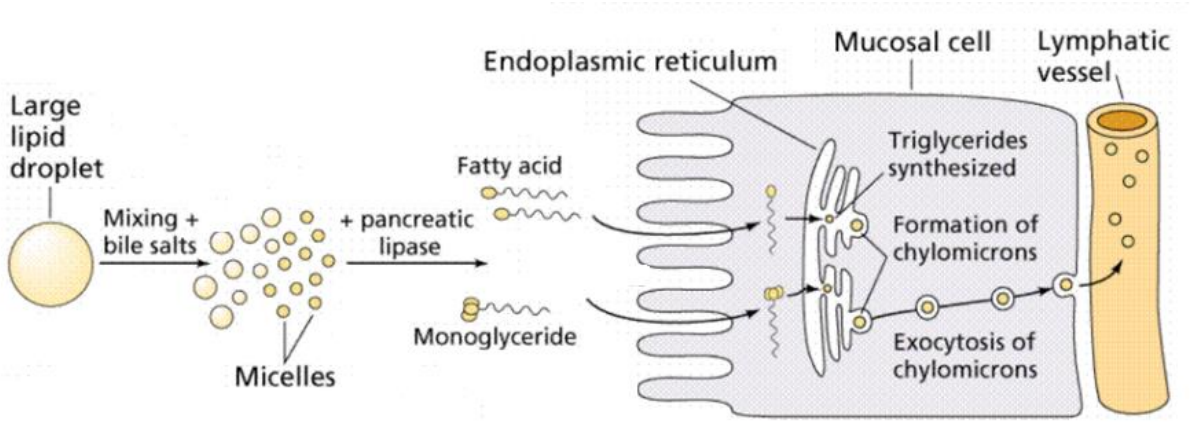

Figure 5. Fat digestion and absorption. Large lipid droplets get emulsified to smaller micelles by bile salts. Pancreatic lipases convert TAGs into FFA and monoglycerides, which enter the cell via diffusion. Triglycerides are formed again in the sER and are packed, together with cholesterol and proteins, into chylomicrons. Chylomicrons are released into the lymphatic system via exocytosis and finally get into the blood. Adapted from ridge.icu.ac.jp/biobk/biobookdigest.

Lipolysis

TAGs can be catabolized into FFAs when energy for the body is needed. This biochemical catabolism is also called lipolysis. Lipolysis occurs in all tissues, however most abundant in white adipose tissue (WAT) and brown adipose tissue (BAT) (Lass *et al*., 2011). Three enzymes are implicated in the hydrolysis of TAG molecules (shown in **figure 6**): adipose triglyceride lipase (ATGL) performs the first and rate-limiting step hydrolyzing TAGs to generate diacylglycerols (DAGs) and FFAs (Zimmermann *et al*., 2004). Hormone-sensitive lipase (HSL) is the rate-limiting enzyme for DAG catabolism (Osuga *et al*., 2000; Haemmerle *et al*., 2002). Finally, monoglyceride lipase (MGL) cleaves MAG into glycerol and FFAs (Karlsson *et al*., 1997).

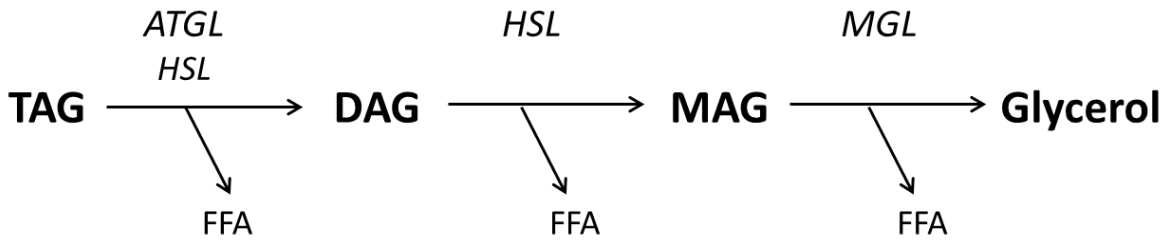

Figure 6. Lipolysis. Hydrolysis of TAG into FFAs and glycerol. Adapted from Lass *et al*., 2011

Cortisol is known to have a major influence on lipolysis. Short-term administration of cortisol *in vivo* showed to promote adipose tissue lipolysis (Divertie *et al*., 1991). One of the causes might be that this glucocorticoid creates a resistance to the inhibition of lipolysis by insulin (Dinneen *et al*., 1993). Another group defined that this resistance probably appears due to a combination of increased LPL activity and the increase of lipolysis itself (Samra *et al*., 1998). Increased LPL activity involves an increased level of LPL mRNA, resulting in an increase in LPL synthesis, and additional posttranslational regulation (Ottoson *et al*., 1994).

The stimulation of lipolysis results in an elevated concentration of FFAs in the plasma, which has several consequences. The elevated circulating FFAs inhibit insulin-stimulated glucose uptake, glycogen synthesis and glucose oxidation together with an increase in hepatic glucose output (Bergman & Ader, 2000). Inhibition of insulin-stimulated glucose uptake is also called insulin resistance and is found to be caused by protein kinase C (PKC) activation and oxidative stress-activated signalling pathways. FFAs induce the activation of PKC which increases the phosphorylation of serine 307 of IRS-1. This inhibits IRS-1 tyrosine phosphorylation by IkappaB-kinase (IKK) or c-jun N-terminal kinase (JNK). The same study found that FFAs also activate several stress kinases: S6 kinase p70 (p70SK), stress-activated protein kinase (SAPK) and p38 MAP kinase (p38MAPK). This suggests the involvement of oxidative stress-activated signalling pathways (Ragheb *et al*., 2009).

TNF-α, resistin and adiponectin

As already described in the paragraph discussing the effects of cortisol on the glucose metabolism, insulin resistance results in hyperglycemia. Hyperglycemia together with “stress eating” results in obesity, which in turn has other effects. One of the already described effects is the increased turnover of cortisone into active cortisol catalysed by the enzyme 11β-HSD1. Other effects of obesity are increases in tumour-necrosis factor-α (TNF-α) and resistin together with a decrease in adiponectin (Saltiel & Kahn, 2001).

It was found that the expression of TNF-α is increased in fat of obese rodents and humans. This induces phosphorylation of insulin receptor substrate 1 (IRS-1), resulting in reduced insulin receptor activity and insulin resistance (Hotamisligil *et al*., 1996). Resistin is a peptide hormone secreted by adipose tissue and was found to be elevated in obese mice. The use of anti-diabetic drugs and the administration of anti-resistin antibody seemed to improve blood sugar and insulin action in mice with diet-induced obesity, suggesting that elevated resistin levels contribute to insulin resistance (Saltiel & Kahn, 2001). Another peptide derived from adipose tissue is adiponectin. Obese mice and human were found to have a decreased expression of adiponectin mRNA. Treatment of mice with this peptide decreased insulin resistance, the concentration of FFAs in plasma and the triglyceride content of muscle and liver (Yamauchi *et al*., 2001). These findings suggest that increased TNF-α,

increased resistin and decreased adiponection, due to obesity, result in insulin resistance and thereby close the positive feedback loop, because the insulin resistance results again in hyperglycemia and obesity.

A summary of the effects described above is shown in the positive feedback loop of **figure 7**.

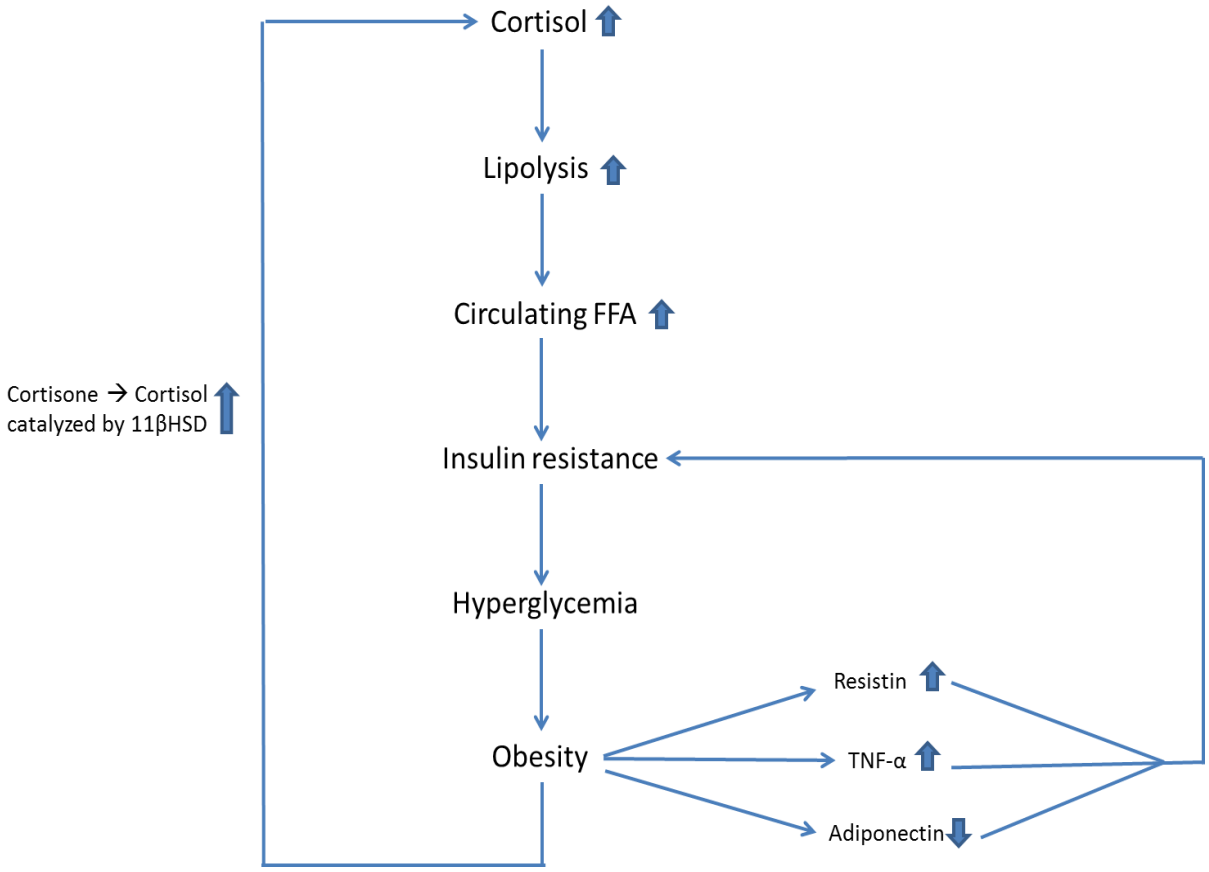

Figure 7. Positive feedback loop of the influence of cortisol on the lipid metabolism. Cortisol stimulates lipolysis causing insulin resistance via an increase in circulating FFAs. This results in hyperglycemia and finally obesity. Obesity changes cortisol metabolism resulting in higher levels of the glucocorticoid and increases the levels of resistin and TNF-α, together with a decrease in adiponectin. These three changes result in insulin resistance, closing the positive feedback loop.

De novo lipogenesis

Besides the big effect of cortisol on lipolysis, it also has an effect on *de novo* lipogenesis (DNL). This process consists of the endogenous production of FFAs from dietary carbohydrates (Hellerstein, 1999). Glucocorticoids increase rates of hepatic DNL and thereby reduce the contribution from the stored cytosolic TAG pool. This might result in a fatty liver (hepatic steatosis) and an increased export of TAGs to adipose tissue (Dolinsky *et al*., 2004; Macfarlane *et al*., 2008). Normally FFAs would inhibit DNL (Hillgartner *et al*., 1995), but the glucocorticoids probably overrule this negative feedback and therefore stimulate an overall increase in DNL.

Adipogenesis

Another process glucocorticoids have influence on is adipogenesis, the development of mature adipocytes. Cortisol stimulates the differentiation of pre-adipocytes into mature adipocytes (Halvorsen et al., 2001) and thereby an increase in adipose tissue is observed. A mechanism for adipgenesis stimulation is discovered by Lindroos et al. and shown in **figure 8**. Cortisol upregulates the glucocorticoid-dependent gene LIM domain only 3 (LMO3), which also showed to have a tight correlation with expression levels of 11β-HSD1 (meaning this level also increases). Next, LMO3 modulates adipocyte differentiation via PPARγ which in turn regulates a set of adipocyte specific genes (Lindroos *et al*., 2013).

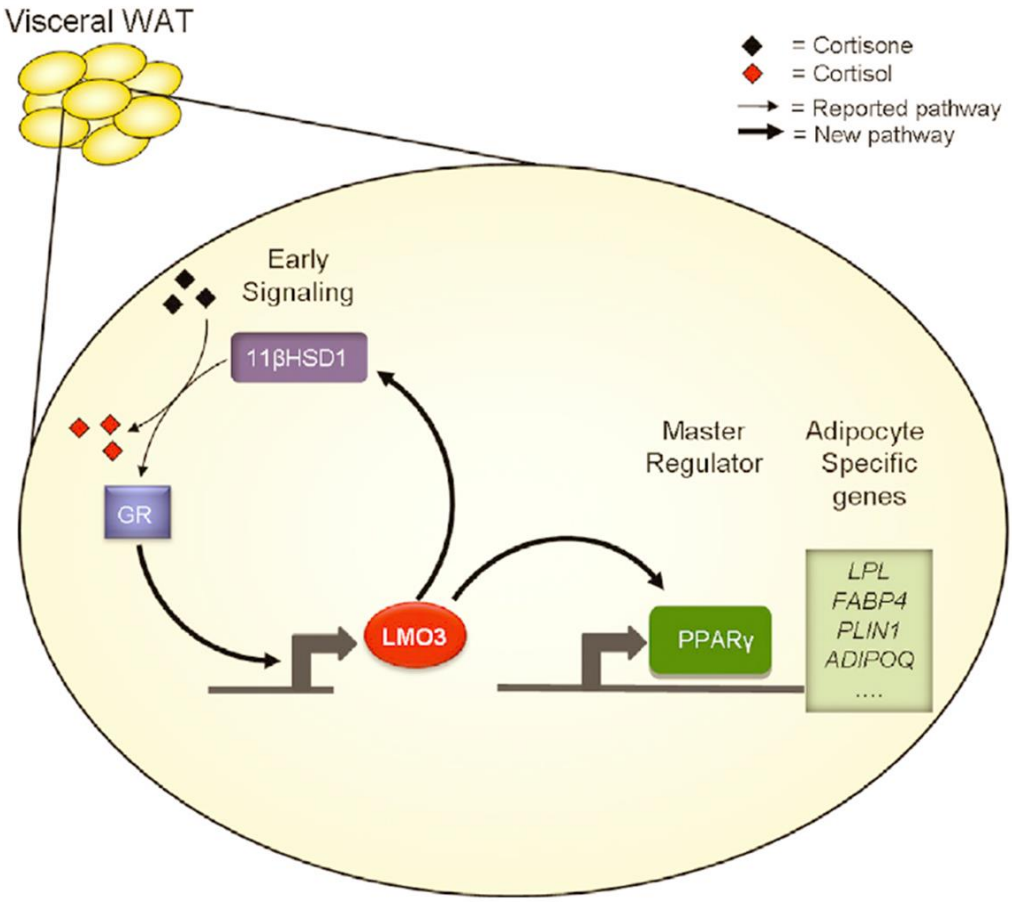

Figure 8. LMO3 is an important factor in cortisol-induced adipogenesis. Adapted from Lindroos *et al*., 2013

Stress and the central nervous system

In the introduction is explained how stress induces the secretion of cortisol via the HPA axis. Besides the effects of cortisol on different peripheral tissues, it has also effects on the central nervous system itself. In this chapter those effects will be described.

Chronic stress has effects on the morphology and chemistry of different brain regions such as the hippocampus, prefrontal cortex and amygdala (McEwen, 2008). Chronic stressors induce structural remodelling of the hippocampus (synaptic plasticity) by suppressing neurogenesis (development of neurons) and cell survival (Gould *et al*., 1997), and remodelling of dendrites (McKittrick *et al*., 2000). It was found that remodelling of the hippocampus results in impairment of several hippocampal dependent memory tasks (Coburn-Litvak *et al*., 2003). Chronic stress also has effect on the prefrontal cortex and amygdala. In the prefrontal cortex it causes shortening of dendrites and in the amygdala it stimulates dendritic growth of the neurons. The hyperactivity in the amygdala enhances fear-conditioning and aggression (McEwen, 2008).

Acutely (within hours), cortisol directly inhibits the HPA axis, but chronically this glucocorticoid has other effects on the brain. Chronically high concentrations of cortisol increase the expression of corticotropin-releasing factor (CRF) mRNA in the central nucleus of the amygdala. This results in the activation of a chronic stress-response network (Dallman *et al*., 2006). Besides, cortisol is thought to increase the need to perform pleasurable activities (eating sugar and fat, using drugs or working out). This might also result in eating comfort food. In rats, chronic stress and high cortisol levels result in decreases body weight gain. On the other hand, in humans chronic stress induces either increased "stress eating" and body weight gain or decreased food intake and body weight loss. It has been found that patients with chronic stress, and as a result overeating, have decreased levels of cerebrospinal CRF and therefore less HPA axis activity. From this might be concluded that people might overeat in an attempt to inhibit the chronic stress-response network (Dallman, 2010).

Possible treatments for obesity induced by stress

By thinking of possible treatments for obesity induced by stress it might be helpful to go through all the processes contributing to obesitas. Inhibiting some of these processes and their pathways might be a solution for the problem. In this chapter some of the possible solutions will be given.

Mineralocorticoid & Glucocorticoid receptor antagonists

One of the first possibilities for treatment might be inhibiting/blocking the receptors cortisol acts on. It would not be desirable to inhibit components of the HPA axis, because this axis is also responsible for other processes (Buckley & Schatzberg, 2005; Karalis *et al*., 1997) and blocking them might deregulate the body. In this paragraph a number of examples will be given.

The receptor antagonists might be used for are the MR and GR. Both possibilities have been tested. To test the effect of blocking the MR a group used both obese mice and an adipocyte cell line. The obese mice were treated for 3 weeks with the MR antagonist eplerenone. This treatment resulted in a significantly reduced insulin resistance, suppresses macrophage infiltration and reactive oxygen species (ROS) production in the adipose tissue and a correction of the mRNA levels of obesity-related genes. The adipocytes were first treated with aldosterone and H_2O_2 to increase ROS and next eplerenone was added to see if this antagonist reverses the amount of ROS. Indeed, eplerenone seemed to decrease the levels of ROS again and also a dysregulation, of mRNAs of various genes involved in ROS and and cytokines, was corrected (Hirata *et al*., 2009).

Inhibition of the GR was tested by using a recently developed mixed glucocorticoid receptor agonist/antagonist named CORT 108297. For this experiment normal mice were put on a high-fat, high-sugar diet and were extra given the antagonist (two different concentrations), mifepristone (another antagonist known to reverse weight gain) or a vehicle. Compared to the mice administered with the vehicle, the mice given mifepristone or CORT 108297 showed significantly less weight gain. The mice receiving CORT 108297 also had significantly lower steady plasma glucose, compared to the vehicle. This lowering in steady plasma glucose was not correlated with the reduction in weight gain which suggests that the effect of CORT 108297 on insulin sensitivity might be independent of its effect on weight gain (Asagami *et al*., 2011).

Another group used a GR antagonist specifically in the liver. A-348441 was the first liver-selective glucocorticoid receptor antagonist (LSGRA) with anti-diabetic activity. This antagonist inhibits hepatic genes upregulated by glucocorticoids. In insulin-resistant rats on high fat diet and fasted dogs, A-348441 reduces hepatic glucose output and therefore might be a promising treatment (Jacobson *et al*., 2005)

Inhibition of 11β-hydroxysteroid dehydrogenase type 1

When looking at the effects of cortisol on the glucose metabolism it is explained that the enzyme 11β-HSD1 converts the inactive form of cortisol (cortisone) into the active form and that the activity of this enzyme is increased in obese people. Therefore it might be a good treatment to inhibit this enzyme. One group investigated the effect of an 11β-HSD1 inhibitor on dogs. Administration of the inhibitor resulted in decreased hepatic glucose production by reduction of glycogenolysis, an increase of whole body glucose utilization and suppression of lipolysis (Winnick *et al*., 2013).

A different group tested an 11β-HSD1 inhibitor on rats with metabolic syndrome. In normal rats the inhibitor decreased 11β-HSD1 activity in both adipose tissue and liver. In rats with metabolic

syndrome it decreased mean arterial pressure, glucose intolerance, insulin resistance, hypertriglyceridemia, and plasma renin activity and thereby improved symptoms of metabolic syndrome (Schnakenberg *et al*., 2013).

Both groups show that inhibiting 11β-HSD1 is a promising treatment for obesity induced by stress. Very recently it was found that green tea, especially a specific compound, inhibits 11β-HSD1 activity. This group found that the specific compound Epigallocatechin gallate (EGCG) inhibits 11β-HSD1 strongly by direct competition with substrate and/or cofactor binding (Hintzpeter *et al*., 2014).

Neuropeptide Y

Something completely different is neuropeptide Y (NPY). Scientists stated this peptide as a switch determining if stress results in body weight gain or not (Kuo *et al*., 2007). Stressors will induce the release of NPY by sympathetic nerves. This results in an upregulation of both the peptide and its receptors (NPY2R) which leads to the growth of abdominal fat. This growth is the result of a stimulation of fat angiogenesis, macrophage infiltration and proliferation and differentiation of new adipocytes. Pharmacological inhibition of NPY2R proved to be anti-angiogenic and anti-adipogenic (Kuo *et al*., 2007). Therefore this also is a promising treatment for stress-induced obesity.

Other possibilities

Besides the options described above, other possibilities are left. For example inhibiting/reducing the increased lipolysis by blocking the activity of the involved lipases: ATGL, HSL and MGL. This might finally result in a decreased level of FFAs and thereby improve insulin resistance.

Cytokines can also be targets of treatment. As already described above, the cytokine TNF-α is increased in obese people. This causes insulin resistance by phosphorylation of IRS-1. A treatment decreasing the level of TNF-α therefore might contribute to improvement of insulin sensitivity. Another cytokine changed in obese people is adiponectin. This cytokine is decreased in obese people and treatment with this cytokine in mice already showed to improve insulin sensitivity, the level of FFAs in plasma and the triglyceride content in both muscle and liver (Yamauchi *et al*., 2001).

Reducing stress (for example by exercising), changing diet and regular exercise are also possibilities and together with the clinical possibilities this might contribute to improvement of stress-induced obesity.

Despite all the possible and promising treatments it has to be taken into account that inhibiting any of the processes, cytokines etc. might also affect other processes within the body in a negative way. Therefore all possibilities have to be tested thoroughly before even using them in clinical trials. Another fact is that both the glucose and lipid metabolism consist of pathways closely linked to each other. Both complete metabolisms are also closely linked. Inhibiting one pathway might affect another in the other metabolism and no improvement or even worsening might occur. This also illustrates that extensive research on all possibilities needs to occur. However, it can still be concluded that there are many options to eventually counteract stress-induced obesity.

Conclusion

From this literature study can be concluded that stress can play a very important role in the development of obesity and even other diseases like metabolic syndrome and diabetes. In normal situations the HPA axis already plays an important role, shown by the fact that there are circadian oscillations of the glucocorticoid cortisol. Strong stimulation of this axis due to longer periods of stress therefore results in dysregulation of several processes within the body. From the chapters describing the effects of cortisol on the glucose –and lipid metabolism can be concluded that those metabolisms are very tightly regulated and only a single stimulation or inhibition of a process can result in major consequences. Stimulation of one process, for example gluconeogenesis, results in a cascade of many other changes of processes and before you know the metabolisms are deregulated, in this case resulting in the development of obesity. Another conclusion is that the complete mechanism for cortisol inducing insulin resistance is not very well known, however parts of the mechanism are discovered. It is said that cortisol causes insulin resistance by stimulating processes counteracting insulin induced processes; however how this exactly happens is also not clear. Modification of receptors is one of the mechanisms, but changing enzyme activity via certain (unknown) pathways is also a possible mechanism involved. This accounts for both the glucose –and lipid metabolism and therefore more research is needed to get a clear and total view on these exact mechanisms. It is expected to be very difficult, because as already mentioned, both metabolisms are so closely related that studying them, and especially different parts, is hard. Finally, because of all the different processes cortisol has effects on, there also are different possible treatments for stress-induced obesity. The amount of possibilities is promising; however the risk of inhibiting a process also important for other pathways should be taken into account. The suggested treatments consist of inhibiting processes not only involved in the described mechanisms, therefore partial inhibition might be best. This suggests that many more experiments need to be performed before one of the possibilities might be used for patients with stress-induced obesity.

Despite a lot of knowledge about stress-induced obesity via cortisol, still a lot about this disease is unknown. However, for several years small parts and mechanisms are discovered. It will take a long time and a lot of effort to explain and define all the underlying mechanisms, but every little discovery counts and eventually we will be able to treat one of the worldwide problems and its risks: obesity.

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