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Insulin sensitivity : modulation by the brain

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GENERAL INTRODUCTION

As a consequence of the growing obesity prevalence, type 2 diabetes mellitus (T2DM) has become a global epidemic. Currently, this disease affects 171 million individuals globally, with an estimated mortality of about 4 million deaths each year (1). The development of T2DM is the result of insulin resistance of target organs (i.e. liver, adipose tissue, skeletal muscle) and impaired insulin secretion *by* pancreatic β-cells, ultimately leading to hyperglycemia. In addition to glucose metabolism, lipid metabolism is disturbed in T2DM patients, resulting in concomitant dyslipidemia.

GLUCOSE METABOLISM

Dietary carbohydrates enter the body in complex forms, such as disaccharides and polymers (starch). In the gut, these complex carbohydrates are digested into glucose and other simple carbohydrates that can be transported across the intestinal wall to the hepatic portal vein and delivered to liver parenchymal cells (i.e. hepatocytes) and other tissues. The maintenance of narrow-controlled blood glucose concentrations (glucose homeostasis) is essential for a constant provision of glucose to the brain.

Glucose homeostasis is regulated by the balance between endogenous production of glucose, mainly by the liver, and uptake and utilization of glucose by peripheral tissues. To prevent hypoglycemia in the fasted state, glucose is released from the liver (and in small amounts from the kidneys (2)) by two pathways: glycogenolysis and gluconeogenesis. Glycogenolysis is the biochemical breakdown of stored glycogen to glucose, while gluconeogenesis is the process of generating new glucose molecules from non-carbohydrate sources, such as amino acids and the glycerol portion of triglycerides. Glucose disposal takes place in peripheral tissues like skeletal muscle, adipose tissue and heart. Once glucose is transported into the cell, it can undergo glycolysis (catabolism of glucose) to render energy in the form of ATP, e.g. required for muscle contraction, or can be stored either as glycogen in liver or muscle or as the glycerol moiety of TG in adipose tissue.

Glucose homeostasis is controlled primarily by the anabolic hormone insulin. Several catabolic hormones (glucagon, catecholamines, cortisol, and growth hormone) oppose the actions of insulin. After a meal containing carbohydrates, plasma glucose levels rise and as a result, insulin is secreted by pancreatic β-cells (Fig. 1). Insulin inhibits glycogenolysis and gluconeogenesis, and as a result inhibits endogenous glucose production. In addition, insulin stimulates glucose disposal in peripheral tissues. The net result of these actions of insulin is a normalization of plasma glucose levels after a meal.

Insulin initiates its effects on target tissues by binding to the insulin receptor (IR), which results in the tyrosine phosphorylation of insulin receptor substrates (IRS) by the insulin receptor tyrosine kinase. This allows association of IRS with the regulatory subunit of phosphoinositide 3-kinase (PI3K). PI3K activates 3-phosphoinositide-dependent protein kinase-1 (PDK), which activates protein kinase B (PKB)/Akt, a serine kinase. PKB/Akt in turn deactivates glycogen synthase kinase 3 (GSK-3), leading to activation of glycogen synthase and thus glycogen synthesis. Activation of PKB/Akt also results in the translocation of GLUT4 vesicles from their intracellular pool to the plasma membrane, where they allow uptake of glucose into the cell.

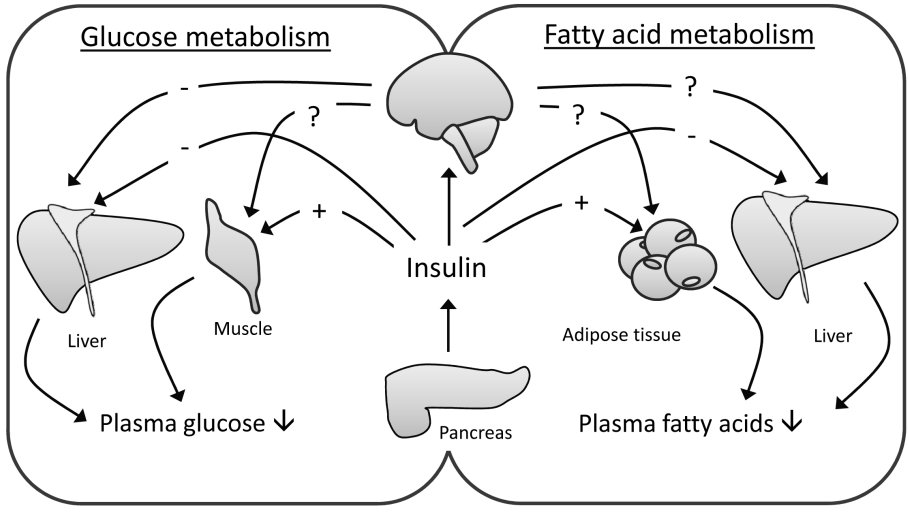

Fig. 1. Schematic representation of insulin's effect in glucose and fatty acid metabolism. Insulin reduces glucose levels by stimulating glucose uptake by peripheral tissues (e.g. muscle) and inhibiting glucose production by the liver. The latter involves insulin action in the brain. Insulin reduces fatty acid levels by stimulating fatty acid uptake by white adipose tissue and by inhibiting triglyceride secretion by the liver.

Furthermore, activated PKB/Akt phosphorylates the nuclear protein Proline-rich Akt substrate of 40 kDa (PRAS40), of which the precise function is still under debate.

The golden standard for investigating and quantifying insulin sensitivity in a clinical setting, but also in experimental setting in rodents, is the hyperinsulinemic-euglycemic clamp. During the hyperinsulinemic-euglycemic clamp, a constant concentration of insulin is infused intravenously. In order to prevent hypoglycemia induced by the insulin rise, glucose is infused to maintain plasma glucose levels at euglycemic levels. The rate of glucose infusion necessary to maintain euglycemia, as determined by checking plasma glucose levels every 5-10 minutes, is a measure for insulin sensitivity. A high glucose infusion rate indicates sensitivity to insulin action, whereas a low glucose infusion rate indicates that the body is resistant to insulin action. The information obtained by the hyperinsulinemic-euglycemic clamp can be extended by the use of glucose tracers. Glucose can be labeled with either stable or radioactive atoms, including [1-^{14}C]glucose or [3-^{3}H]glucose (3;4). Prior to beginning the hyperinsulinemic period, a tracer infusion enables quantification of the basal rate of glucose production. During the clamp, the plasma tracer concentrations enable the calculation of whole-body insulin-stimulated glucose disposal, as well as the endogenous glucose production (5). The hyperinsulinemic-euglycemic clamp can be further extended using radioactive-labeled 2-deoxyglucose. This tracer is administered during the clamp as a bolus when steady-state euglycemia is reached to determine organ-specific glucose uptake in organs that are unable to glycolyse 2-deoxyglucose (all organs except liver and kidney) (6).

In addition to insulin, other hormones affect glucose metabolism. For example, the thyroid hormones (TH) thyroxine (T_4) and triiodothyronine (T_3), are tyrosine-based hormones produced by the thyroid gland. TH production by thyroid gland is regulated by thyroid-stimulating

hormone (TSH) produced by the anterior pituitary, which itself is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus. TH are crucial regulators of metabolism, as illustrated by the profound alterations in metabolism during hyper- and hypothyroidism (7). Hyperthyroid animals develop elevated plasma glucose and insulin levels, due to increased endogenous glucose production, including increased gluconeogenesis and glycogenolysis, and decreased glucose clearance and disposal (8;9). Conversely, in hypothyroid animals, basal endogenous glucose production is reduced (10;11), whereas glucose utilization and turnover in skeletal muscle and adipose tissue is decreased (12).

FATTY ACID METABOLISM

The most common lipids in our diet are cholesterol and triglycerides (TG). Since lipids are hydrophobic, they are transported in the circulation in water-soluble spherical particles called lipoproteins. These lipoproteins carry TG and esterified cholesterol in their core, surrounded by a shell of phospholipids, free cholesterol and proteins termed apolipoproteins. Based on their composition and origin, lipoproteins can be divided into five major classes i.e. chylomicrons, very low lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) (13).

In the intestine, dietary lipid is emulsified by the action of bile and TG are hydrolyzed into monoacylglycerol and fatty acids (FA) by pancreatic lipase. Cholesterol, monoacylglycerol and FA are absorbed by the intestinal cells where FA are re-esterified to TG. The TG and cholesterol are packaged in chylomicrons. Upon entering the circulation, chylomicrons are processed by lipoprotein lipase (LPL) (14). LPL hydrolyzes TG, thereby delivering FA to peripheral tissues, where it can be used as energy source (skeletal muscle and heart) or can be stored in adipose tissue. The resulting TG-depleted remnant chylomicrons are taken up by the liver. The liver can secrete cholesterol and TG, packaged in VLDL particles for transport to peripheral tissues. After VLDL enters the circulation, the TG content of VLDL can be hydrolyzed into FA by LPL and used as energy source. The processing of VLDL by LPL results in the formation of IDL (or VLDL-remnant), which can be further processed to become cholesterol-rich LDL. IDL and LDL can be cleared by the liver. High levels of chylomicrons, VLDL, IDL and LDL can lead to accumulation of lipids in the vascular wall and the development of atherosclerosis. HDL, as formed by the liver and intestine, can remove excess cholesterol from peripheral tissues. Next, HDL-derived cholesterol can be taken up by the liver. In the liver, excess cholesterol is secreted in the bile, thereby maintaining cholesterol homeostasis (15).

FA are important metabolic substrates. The uptake, transport and storage of FA is intensively regulated and plasma FA levels show little variation. TG in TG-rich lipoproteins are hydrolyzed into FA by the action of LPL, located at the capillary endothelium. The released FA are taken up by the underlying tissue via passive diffusion across cell membrane or via active transport, which is facilitated by FA transporters, such as the FA translocase CD36 (16). In WAT, these FA can be re-esterified and stored as TG in intracellular lipid droplets. In heart and skeletal muscle, these FA are metabolized to produce ATP. Excess FA are complexed to serum albumin and transported back to the liver.

In brown adipose tissue (BAT), FA is metabolized by uncoupling FA oxidation from ATP generation, thereby resulting in dissipation of energy as heat. Only recently, it was shown that BAT is also present and functional in human adults (17). At birth, human newborns have considerable amount of BAT, mostly surrounding the vasculature and organs, to defend the body from cold exposure. In adults, BAT is mainly present in the supraclavicular and neck region (18;19). BAT burns FA stored as TG in lipid droplets as a response to sympathetic innervation. BAT comprises only a small percentage of total body weight and lipids stored in BAT can sustain thermogenesis for only a short time. A recent study revealed that TG-rich lipoproteins are an important substrate for heat production by BAT (20). Cold exposure accelerates clearance of plasma TG as a result of increased uptake into BAT, a process dependent on LPL activity and CD36 (20).

Insulin exerts direct effects on VLDL production, by accelerating the degradation of apoB (the principal apolipoprotein of VLDL), thereby acutely decreasing VLDL secretion by the liver (21;22) (Fig. 1). In adipose tissue, insulin stimulates TG storage in adipose tissue in three ways. First, insulin activates LPL in the capillary walls of adipose tissue, thereby stimulating lipolysis (breakdown of TG into FA and glycerol), enabling FA uptake by adipocytes. Second, insulin stimulates glucose transport into adipocytes, providing substrate for the glycerol portion of TG. Third, insulin inhibits hormone-sensitive lipase (HSL), thereby inhibiting hydrolysis (breakdown of TG into FA and glycerol) in adipocytes. The inhibition of lipolysis in adipose tissue by insulin decreases the flux of FFA towards the liver, contributing to decreased hepatic VLDL secretion.

The role of TH in the regulation of lipid metabolism has been extensively studied ever since the link between thyroid function and body weight has been recognized. Plasma concentrations of cholesterol and TG are inversely correlated with TH levels. TH stimulate hepatic LDL receptors, thereby promoting uptake of (V)LDL. Therefore, hypothyroidism results in decreased LDL receptors and increased plasma cholesterol and TG levels. Hypermetabolism is one of the hallmarks of hyperthyroidism, reflected in an increase of resting energy expenditure (REE). It is unknown whether this increase in REE coincides with an increase in spontaneous physical activity. FA are important substrates fuelling the increase in REE during hyperthyroidism (23). The FA are provided by several mechanisms. First, lipolysis (breakdown of TG into FA and glycerol) is stimulated by TH (24). Second, hepatic *de novo* lipogenesis is promoted by T3 via induction of lipogenic enzymes (25). Third, TH increase the amount of available nutrients, including FA, by increasing food intake. Although TH stimulate lipolysis, it is still unknown how FA fluxes are modulated by TH status in metabolically relevant tissues *in vivo*.

ROLE OF THE BRAIN IN GLUCOSE AND FATTY ACID METABOLISM

The first evidence that the brain is involved in control of peripheral glucose homeostasis already dates back from 1855, when the French physiologist Claude Bernard showed in rabbits that punctures in the floor of the fourth ventricle resulted in hyperglycemia. Several brain regions from cortex to brainstem are involved in the regulation of homeostasis, but the hypothalamus is considered the main integrator and processor of peripheral metabolic information. The hypothalamus consists of several nuclei (collection of neuronal cells) involved in metabolism,

including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamic area (LHA), the ventromedial nucleus (VMH), and the dorsomedial nucleus (DMH).

One of the critical neuronal structures involved in the regulation of metabolism is the ARC, situated around the base of the third ventricle in the hypothalamus. The ARC contains neurons that exert potent effects on food intake, energy expenditure and glucose and FA metabolism. Both hormonal and nutrient-related signals regulate these neurons. One group of neurons co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP), peptides that potently stimulate food intake and reduce energy expenditure, thereby promoting weight gain. Another group of neurons co-express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), peptides that decrease food intake and increase energy expenditure, thereby promoting weight loss. The expression of these orexigenic and anorexigenic neuropeptides is dependent on the fed status and a balance between the neuropeptides is essential for maintaining energy homeostasis.

Studies have shown that the brain is an insulin-sensitive organ and insulin receptors are widely distributed in the brain (26). Insulin crosses the blood-brain barrier through insulin-receptor mediated active transport (27;28). The origin of insulin in the brain is mostly peripheral and only a modest amount is synthesized locally (29). By activating its receptors, insulin decreases the expression of NPY and stimulates expression of POMC, both contributing to a decrease in food intake and increase in energy expenditure. Activation of insulin signaling in the brain plays an important role in the regulation of glucose metabolism (Fig. 1). Central administration of insulin, resulting in activation of insulin signaling in the brain without elevating plasma insulin levels, is sufficient to decrease plasma glucose levels by inhibiting endogenous glucose production (30). Conversely, blockade of central insulin action results in decreased ability of circulating insulin to suppress endogenous glucose production (30;31). Insulin may obtain its effects on endogenous glucose production by decreasing NPY neuronal activity, as it has been shown that central NPY administration impairs the ability of circulating insulin to suppress endogenous glucose production (32). The effect of central insulin action on endogenous glucose production is dependent on ATP-dependent potassium (K_{ATP}) channel activation in the brain. Central administration of sulfonylureas (K_{ATP} channel blockers) abolishes the central effects of insulin on endogenous glucose production and prevents in part the suppression of endogenous glucose production by circulating insulin (30). Moreover, K_{ATP} channel activation *per se* is sufficient to lower plasma glucose levels via inhibition of glycogenolysis and gluconeogenesis (33). Although it is established that insulin signaling in the brain is required for inhibitory effect of circulating insulin on endogenous glucose production, it is unknown whether the central effects of insulin are involved in the stimulation of disposal of glucose by circulating insulin.

Recently, it was shown that central insulin action is not only involved in peripheral glucose metabolism, but also in FA metabolism. Insulin administration in the brain promotes lipogenesis and suppresses intracellular lipolysis in white adipose tissue (WAT), which results in increased fat mass and adipocyte cell size upon chronic insulin administration (34;35). Furthermore, suppression of VLDL secretion by circulating insulin can in part be prevented by central administration of NPY (32). However, it is unknown whether the central effects of insulin are also involved in the stimulation of uptake of (F)FA and TG by adipose tissue from plasma.

In addition to insulin, the effects of TH on glucose metabolism are partly mediated via a central mechanism. Comparable to peripheral administration, TH administration directly into the PVN of the brain increases endogenous glucose production and induces hepatic insulin resistance (36;37). Furthermore, both the peripheral as well as the central effects of TH on glucose production depend on sympathetic projections to the liver (37;38).

METABOLIC DISTURBANCES IN TYPE 2 DIABETES MELLITUS

T2DM is a multifactorial, chronic disease characterized by hyperglycemia and often accompanied by dyslipidemia. T2DM is no immediate life threatening disease, but it does increase the risk for developing complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy. The diagnosis of diabetes mellitus is based on WHO recommendations in 2006. Criteria include fasting plasma glucose level ≥ 7.0 mmol/l or a 2 h-plasma glucose level of ≥ 11.1 mmol/l following a glucose load.

The development of T2DM is the result of both a deficient insulin secretion by pancreatic ß-cells and insulin resistance. In the insulin resistant state, skeletal muscle, liver and adipose tissue display a decreased responsiveness to insulin mediated glucose disposal. Furthermore, hepatic insulin resistance results in decreased insulin-mediated suppression of endogenous glucose production. In the early stage of the development of T2DM, insulin resistance leads to an increase in insulin secretion by the β-cells to overcome the reduced sensitivity of peripheral organs, thereby maintaining normal glucose levels. In a later stage, β-cells fail to compensate, leading to progressive hyperglycemia and ultimately T2DM.

FA metabolism is also disturbed in T2DM patients. Insulin resistance is associated with increased intracellular lipolysis of TG in adipose tissue by HSL, resulting in an increased FA flux from adipose tissue to liver, skeletal muscle and heart where it is stored as TG, contributing to the development of insulin resistance in these tissues (39). The increased storage of TG in liver in combination with hepatic insulin resistance results in increased secretion of VLDL (40;41). The increased plasma levels of TG in VLDL are a hallmark of the dyslipidemia associated with insulin resistance and T2DM.

As mentioned before, central insulin actions are critical for the maintenance of glucose and energy homeostasis. Obesity has been shown to result in elevated insulin levels in cerebrospinal fluid (42). Recently it was shown that high-fat feeding induces central insulin resistance already following short exposure to the diet, as demonstrated by reduced insulin signaling in the brain (43). A high-fat diet induces central insulin resistance independently of adiposity as the development of central insulin resistance occurs within days of exposure to the high-fat diet. The attenuated insulin signaling in neurons occurs through three mechanisms: first, the inactivation of IRS-1 by serine phosphorylation, second, the proteasomal degradation of IRS-1, and third, the lysosomal degradation of the IR (44). Since central insulin actions are critical in the regulation of glucose and FA metabolism, the central effects of insulin resistance require more attention.

CIRCADIAN RHYTHM AND INSULIN SENSITIVITY

A recently described environmental trigger associated with development of T2DM is disturbance of circadian rhythms, which can be due to environmental light pollution, reduction of sleep duration and/or quality, jet lags and shift work. Circadian rhythms are 24 h cycles generated by the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus. The SCN induces daily rhythms in body temperature, heart rate, blood pressure, feeding behavior, glucose levels and many more. This circadian clock is synchronized to the environmental cycle by light-dark information perceived by the eyes. Many hormones involved in metabolism, such as insulin, glucagon, cortisol and leptin have been shown to exhibit circadian oscillation (45-49). In addition, the circadian clock has been reported to regulate metabolism and energy homeostasis, by mediating expression and/or activity of certain metabolic enzymes and transport systems involved in e.g. cholesterol metabolism and glycogen and glucose metabolism. Disruption of circadian rhythms due to shift work can result in hormone imbalance, psychological and sleep disorders, cancer proneness and reduced life span (50;51). Furthermore, shift work and reductions in sleep duration result in impaired glucose tolerance (52-55) and increased adiposity (56).

The rhythmic expression and activity of the metabolic pathways is mainly attributed to the coordinated circadian oscillation of clock genes (*Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2)* that are expressed in each organ and cell. By phenotyping clock gene mutant and knockout mice, a direct link was made between the circadian clock and metabolic disorders. Homozygous *Clock* mutant mice have greatly attenuated diurnal feeding rhythm, are hyperphagic and obese and develop a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis and hyperglycemia (57). *Bmal*$^{-/-}$ mice exhibit suppressed diurnal variations in glucose and TG levels as well as abolished gluconeogenesis (58). Mice knockout for *Clock* and *Bmal1* in pancreatic β-cells show impaired glucose tolerance, hypoinsulinemia, and defects in size and proliferation of pancreatic islets, suggesting that the loss of the pancreatic clock inhibited the β-cells from secreting insulin (59).

The increased prevelance of obesity and T2DM in shift workers can be the result of discrepancy between behavior and endogenous phase, as shift workers consume energy at times of their cycles when intake is normally absent and energy expenditure levels are low (60-63). Studies in mice and rats have confirmed that food intake restricted to the resting phase results in abdominal obesity (64-67). However, a disturbed circadian rhythm can alter insulin sensitivity without affecting adiposity. For example, a single night of partial sleep deprivation is already sufficient to induce insulin resistance in patients with type 1 diabetes mellitus as well as in healthy subjects (68;69). Disruption of circadian rhythms accelerates the development of diabetes through pancreatic β-cell loss and function, as shown in a diabetes-prone rat model (70). In elderly, the SCN is decreased in volume and cell number, thereby losing rhythmicity (71-73). This may underlie the high prevalence of profound disturbances of sleep and hormonal circadian rhythms, common in the elderly (74-77), which may contribute to the development of T2DM. More insight into the role of SCN loss and the role of desynchronized central and peripheral clocks in the development of insulin resistance would lead to better understanding of the pathophysiology of T2DM in shift workers and elderly.

THERAPIES FOR TYPE 2 DIABETES MELLITUS

T2DM imposes a major health risk, due to increased morbidity and mortality. Lifestyle changes, such as restricted caloric intake and exercise, can often significantly improve the outcome of the disease. However, the need for pharmacological strategies remains. The major goal of therapeutic intervention in T2DM is to ameliorate hyperglycemia and insulin resistance, but also increased risk factors including dyslipidemia and hypertension should be taken into account. Sulfonylureas and biguanides are the most prescribed drugs used to treat hyperglycemia in T2DM. Insulin therapy is necessary when medication alone is unable to control glucose levels and when β-cells fail to produce sufficient insulin. There is much interest in drugs that not only improve glucose metabolism, but also have beneficial effects on body weight and dyslipidemia.

Biguanides are the first-line treatment drug of choice for the treatment of T2DM, in particular in overweight patients. Metformin is the sole agent clinically used in this class. The molecular mechanisms of action underlying its beneficial effects on glucose metabolism are not fully known, although activation of AMP-activated protein kinase (AMK) in the liver appears critically involved (78;79). Metformin decreases hepatic gluconeogenesis, inhibits glycogenolysis and improves insulin sensitivity especially in skeletal muscle. As it does not affect insulin secretion, it is not associated with hypoglycemia when used as monotherapy. Metformin has modest improving effects on LDL cholesterol and TG levels, the underlying mechanism(s) not being understood. It does not reduce body weight, but is also not associated with body weight gain.

Sulfonylureas act mainly by stimulating insulin release from the β-cells of the pancreas. They bind to K_{ATP} channels on the cell membrane of β-cells. This inhibits a tonic, hyperpolarizing efflux of potassium, thus causing the electric potential over the membrane to become more positive. This depolarization opens voltage-gated calcium channels. The rise in intracellular calcium leads to increased fusion of insulin granules with the cell membrane, and therefore increased secretion of insulin. There is some evidence that sulfonylureas also sensitize β-cells to glucose, limit endogenous glucose production, decrease lipolysis (breakdown and release of FA by adipose tissue) and decrease clearance of insulin by the liver. Hypoglycemia is unfortunately a common side-effect as a result of increased insulin release and decreased insulin clearance. Since sulfonylureas increase appetite and weight gain, they are not the first choice for the management of T2DM in obese patients.

Glucagon-like peptide-1 (GLP1) is a cleavage product of the proglucagon molecule which is secreted by the intestinal L-cells and in the brain (80;81). It is released in response to food intake in proportion to caloric content to inhibit endogenous glucose production and to stimulate glucose-dependent insulin secretion (80;82). In addition, GLP-1 exerts multiple other effects, including inhibition of food intake, slowing of gastric emptying and inhibition of glucagon secretion (83;84). GLP-1 is known to beneficially affect glucose metabolism in T2DM patients (84;85). However, GLP-1 is easily degraded by the enzyme dipeptidyl-peptidase IV (DPP-IV), limiting its therapeutic efficacy (86). Therefore, pharmaceutical companies are developing GLP-1 analogues resistant to inactivation by DPP-IV. Clinical studies have shown that these analogues are very effective in reducing body weight, improving glucose metabolism and reducing plasma lipids in patients with T2DM. The anorexic effect and the hepatic insulin sensitizing effect of GLP-1 are in part mediated via GLP-1 receptors in the brain (83;87;88).

Topiramate, a sulfamate-substituted derivative of the monosaccharide D-fructose (89), is a broad-spectrum antiepileptic drug with potentially additional neurotherapeutic applications such as bipolar disorder and migraine (90;91). Its precise mechanism of action is unknown, although topiramate is considered to produce its antiepileptic effects through at least six mechanisms of action in the central nervous system: enhancement of GABA-ergic activity (92;93), inhibition of kainite/AMPA receptors (94), inhibition of voltage-dependent sodium channels (95), inhibition of high-voltage-activated calcium channels (96), increase in potassium conductance (97) and inhibition of carbonic anhydrase (98). Besides its antiepileptic action, topiramate is associated with a decrease in body weight. The reduction in body weight upon topiramate treatment has been shown in epileptic patients as well as in obese persons (99-101). Although loss of appetite resulting in reduced caloric intake can account for initial reductions in body weight, other mechanisms might be involved in the long-term effects of topiramate on weight, as weight loss continued after caloric intake returned to baseline levels (102). Several studies in lean as well as in obese mouse and rat models have confirmed that topiramate also reduces food intake and body weight (103;104). Interestingly, studies in obese, diabetic rats demonstrate that topiramate treatment reduces plasma glucose levels and improved insulin sensitivity independent of weight loss (105;106). It is not known whether the enhancement in insulin sensitivity is the result of improved hepatic insulin sensitivity or improved peripheral insulin sensitivity. It is also unknown if topiramate improves insulin sensitivity via actions in the brain. These observations are of interest for target discovery of new antidiabetic drugs.

OUTLINE OF THIS THESIS

The aim of the present thesis was to gain more insight into the role of the brain in the regulation of insulin sensitivity. Increasing evidence suggests a link between circadian rhythms and insulin sensitivity. Shift workers display a higher incidence of T2DM and already one night of partial sleep deprivation leads to reduced insulin sensitivity. Mice mutant for circadian clock genes, that as a consequence have a disturbed circadian rhythmicity, develop obesity affected glucose homeostasis. Since the causal relation between disturbed circadian rhythm and insulin resistance is not known, we determined the effect of a disturbance in circadian rhythm on energy metabolism and insulin sensitivity. The circadian rhythm was disturbed in mice either by exposure to constant light as this is known to suppress rhythmicity as well as lengthen circadian rhythm (**chapter 2**), or by removal of the SCN by thermic ablation (**chapter 3**). Glucose homeostasis is determined by the balance between endogenous production of glucose and by uptake of glucose by peripheral tissues, which is primarily controlled by insulin. Central effects of insulin are involved in the inhibition of endogenous glucose production by circulating insulin. It is unknown whether central effects of insulin are also involved in stimulation of glucose uptake by peripheral tissues. In addition, the effects of high-fat feeding in these central effects of circulating insulin are unknown. Therefore, we determined the role of central insulin signaling in mice on insulin-stimulated glucose uptake by peripheral tissues and the effect of high-fat feeding (**chapter 4**). The antiepileptic drug topiramate improves insulin sensitivity, but the underlying mechanisms are unclear. Therefore, we studied which organs are responsible

for this effect of topiramate on insulin sensitivity and whether the central nervous system is involved in this effect of topiramate treatment in high fat-fed mice (**chapter 5**). In addition to its role in glucose homeostasis, circulating insulin is involved in FA metabolism as it promotes lipogenesis and suppresses intracellular lipolysis in WAT resulting in increased fat storage. Since the relative contribution of the indirect effects though the brain are unknown, we determined the extent to which central effects of insulin contributed to both TG-derived and albumin-bound FA uptake by WAT. In addition, we determined the effect of high-fat feeding on these central effects of insulin (**chapter 6**). Thyroid hormones (TH) are crucial regulators of glucose and FA metabolism, in part via TH action in the brain. Therefore, we studied the direct effects of thyroid status on whole body energy metabolism and on TG-derived and albumin-bound FA uptake by muscle, liver, WAT and BAT (**chapter 7**). The results of the studies described in this thesis and the future perspectives are discussed in **chapter 8**.

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