Insulin sensitivity : modulation by the gut-brain axis

Heijboer, A.C.

Chapter 8

Summary and conclusions

Maintenance of plasma glucose concentration is highly important for normal body physiology, particularly for the central nervous system, which uses glucose as the obligatory fuel and has no endogenous glucose stores. The pancreatic hormone insulin is important in tightly regulating glucose homeostasis. Insulin inhibits endogenous glucose production and stimulates glucose uptake by peripheral tissues, like skeletal muscle and adipose tissue, and thereby lowers glucose levels. In conditions of insulin resistance, these tissues are less sensitive for insulin action, which is reflected in decreased inhibition of endogenous glucose production and decreased tissue glucose uptake at the same concentration of insulin. Initially, pancreatic beta cells compensate for insulin resistance by increasing insulin secretion. However, when this compensatory mechanism fails, hyperglycaemia and type II diabetes will ensue. Insulin resistance is a dominant feature in subjects with obesity and type II diabetes. Without treatment hyperglycaemia progresses in time, making these patients prone for developing secondary complications.

Up till now, only scarce data are available upon physiological regulation of insulin sensitivity by feeding status, in interaction with gastrointestinal hormones and the brain. Feeding status seems to impact insulin sensitivity. Recent studies showed intensive interactions of gut hormones and brain centres in regulating food intake. The hypothalamic arcuate nucleus plays a central role in this interaction. Since fine-tuning of glucose homeostasis is essential to survive times of scarcity, the present thesis is focussed on physiological modulation of insulin sensitivity, with special emphasis on the role of feeding status (fasting, high fat diet), gut hormones (PYY, ghrelin), brain and gut-brain interactions.

Feeding status and insulin sensitivity

During the postabsorptive state and prolonged fasting it is extremely important to keep glucose concentrations high enough for functioning of the brain. During fasting, adipose tissue lipolysis increases, resulting in increased supply of FFA to the liver and muscle. In the liver and muscle, FFA can either be used for β-oxidation or TG storage. By β-oxidation, the liver transforms FFA in ketone bodies, which can be used as energy source by the brain. If tissue FFA uptake exceeds β-oxidation, excessive TG storage will be the consequence. In obesity, increased FFA concentrations and excessive tissue TG storage are associated with tissue insulin resistance. Fatty acid derivatives are ligands for nuclear transciption factors like PPARs and SREBPs, which alter the expression of enzymes/proteins involved in glucose and lipid metabolism and thus interact with insulin effects. The impact of fasting on tissue insulin sensitivity is unknown. Therefore, we studied the effects of 16 hours of fasting (prolonged fasting) versus 4 hr of fasting (postprandial state) on hepatic and muscle insulin sensitivity in wild-type mice in vivo in relation to tissue TG accumulation and changes in mRNA expression of transcription factors and related proteins involved in glucose and lipid

metabolism **(chapter 2).** Whole-body and hepatic insulin sensitivity were measured by hyperinsulinaemic euglycaemic clamp in combination with D-[^{14}C]glucose infusion. Tissue specific insulin sensitivity was measured by use of 2-deoxy-D[^{3}H]glucose. Sixteen hr of fasting did not impact hepatic insulin sensitivity in terms of glucose production in the presence of increased hepatic triglyceride content (71±19 versus 12±7 µg/mg protein, $p<0.01$). In muscle, however, fasting resulted in increased insulin sensitivity with increased muscle glucose uptake (4.0±2.6 versus 1.3±0.3 % glucose uptake/ g tissue, $p<0.01$) without changes in muscle triglyceride content (25±7 versus 29±13 µg/mg protein, ns). In the liver, fasting resulted in increased mRNA expression of genes promoting gluconeogenesis (PGC1 and PEPCK) and triglyceride synthesis (PPARγ, DGAT1 and DGAT2), but in decreased mRNA expression of genes involved in glycogenolysis (GP) and fatty acid synthesis (SREBP1c, FAS and ACC1). In muscle, increased mRNA expression of genes promoting glucose uptake (PGC1 and GLUT-4), as well as lipogenesis (PPARγ, FAS, ACC1, DGAT-1 and -2) and β-oxidation (PPARα) was found. We conclude from this study that 16 hours of fasting does not induce hepatic insulin resistance, although it causes liver steatosis, whereas muscle insulin sensitivity increases without changes in muscle triglyceride content. Therefore, fasting induces differential changes in tissue-specific insulin sensitivity and liver and muscle TG content are unlikely to be involved in these changes.

With regard to the physiological relevance of the increase in muscle insulin sensitivity during fasting, a state dominated by low insulin levels, it can be speculated that this mechanism might serve as an extremely efficient manner to prepare peripheral tissues metabolically to shift to glucose uptake, when the next meal arrives and insulin levels increase, thereby preventing glucose peaks after meals.

High fat feeding, insulin sensitivity and the brain

At present, the western type of diet contains too much fat. This high fat diet is a risk factor for overweight, insulin resistance, and, thereby, for type II diabetes. Studies in rats and dogs on a high fat diet show the induction of hepatic insulin resistance as an early event, followed by muscle insulin resistance (1;2). The arcuate nucleus integrates a multitude of behavioural and metabolic adaptations to food intake and starvation, necessary to maintain fuel homeostasis despite profound environmental variations in nutrient availability. Two types of neurons in the arcuate nucleus of the hypothalamus are of major importance for the control of these processes: the orexigenic neurons co-expressing AgRP and NPY, and the anorexigenic neurons expressing POMC/ α-MSH. α-MSH binds to and stimulates melanocortin (MC) receptors. Recent data from other groups and our own group showed

involvement of the arcuate NPY/POMC circuitry in the modulation of insulin sensitivity, in addition to its impact on food intake.

The impact of a high fat diet on tissue specific insulin sensitivity in mice and triglyceride accumulation in relation to changes in mRNA expression levels of NPY, POMC, AgRP and CART in the hypothalamus is unknown. Therefore, we studied the effects of a 2 weeks high fat diet in wildtype mice on these parameters **(chapter 3)**. Whole-body and hepatic insulin sensitivity were measured by hyperinsulinaemic-euglycaemic clamp in combination with D-[^{14}C]glucose infusion. Tissue specific insulin sensitivity was measured by use of 2-deoxy-D[^{3}H]glucose. Two weeks of high fat diet did show hepatic insulin resistance with regard to inhibition of hepatic glucose production (35±20% versus 61±23%, $p<0.05$) and in the presence of increased hepatic TG accumulation (32 ±10 versus 12±6 µg/mg protein, $p<0.05$). Under hyperinsulinemic conditions, whole body glucose uptake was not different between the HF fed group and the control group (66±10 µmol/kg.min versus 59±8 µmol/kg.min, ns), also reflected by unaltered muscle-specific glucose uptake in the HF fed group compared to the control group (1.3±0.6 versus 1.3±0.3% per g tissue). Muscle TG content was not significantly different either (27±9 versus 23±7 µg/mg protein, ns). We did not observe changes in hypothalamic mRNA expression levels of NPY, AgRP, POMC and CART after 2 weeks of high fat diet compared to control mice.

From this study, we conclude that 2 weeks of high fat feeding in mice does not affect mRNA expression levels of NPY, AgRP, POMC or CART in the whole hypothalamus, despite induction of hepatic, but not peripheral, insulin resistance. Since in our study mRNA levels were measured in whole hypothalamus without regional assessment within the different nuclei, we cannot exclude involvement of certain specific hypothalamic area's in the regulation of hepatic insulin resistance during a high fat diet. In addition, we did not measure the expression levels of the relevant peptide levels. Additional studies have to be performed to evaluate the potential role of the respective nuclei within the hypothalamus in mediating peripheral insulin sensitivity during high fat feeding.

In addition to the impact of NPY on food intake, icv administration of NPY acutely hampers the capacity of insulin to inhibit hepatic glucose production. The POMC pathway also seems to be involved in regulating insulin sensitivity. Chronic (7 days) icv infusion of α-MSH enhanced peripheral and hepatic insulin sensitivity in rats (3) and POMC gene overexpression ameliorates insulin resistance in leptin-deficient mice (4). In the latter studies, the effects on insulin sensitivity occur in the presence of a concomitant reduction in food intake and fat mass, which precludes distinction of putative direct effects of POMC/α-MSH on

insulin sensitivity from indirect effects via feeding and body composition. To document the direct effects of activation of MC3/4 receptors on insulin sensitivity, we injected melanotan II (MTII), an agonist of these MC3/4 receptors icv, and quantified hepatic and peripheral insulin sensitivity of glucose metabolism in mice without access to food **(chapter 4)**. We performed this study in 24 hour fasted mice, which received 3 times an icv injection with MTII during this period of time. Whole-body and hepatic insulin sensitivity were measured by hyperinsulinaemic euglycaemic clamp in combination with D-[^{3}H]glucose infusion. A real time polymerase chain reaction (RT-PCR) was used to measure mRNA expression levels of GLUT-4 in skeletal muscle. Hepatic insulin sensitivity was unaltered as hyperinsulinaemia suppressed hepatic glucose production to a similar extent in MTII- vs. vehicle-treated animals (45 ± 27% vs. 50 ± 20%, ns). However, glucose disposal rate was significantly higher in MTII treated animals (151 ± 20 vs. 108 ± 20 µmol/min/kg, resp., $p<0.01$), in the presence of increased GLUT-4 mRNA expression in skeletal muscle in the MTII treated group compared to vehicle-treated mice (307 ± 94 vs. 100 ± 56 %, $p<0.01$).

In conclusion, this study shows that activation of central melanocortin-3/4 receptors by MTII enhances insulin sensitivity of whole body glucose disposal, independently of food intake and fat mass, whereas it does not affect insulin's ability to suppress EGP. These observations are in line with the emerging notion, that neural circuits, apart from their effects on feeding, modulate insulin sensitivity to adapt metabolic conditions in the face of environmental fluctuations in nutrient availability.

Gastrointestinal hormones and insulin sensitivity

Gastrointestinal hormones vary according to feeding status and are known to affect food intake. For instance, PYY_{3-36} concentration is low during fasting and increased in the fed state, whereas ghrelin concentration is high after fasting, and peaks just before a meal. Accordingly, PYY_{3-36} inhibits food intake whereas ghrelin stimulates food intake. Gastrointestinal hormones are known to influence appetite by regulating neuropeptides in the hypothalamus. As these neuropeptides can affect insulin sensitivity, gastrointestinal hormones might be involved in regulating insulin sensitivity as well.

PYY_{3-36}

In view of the fact that PYY_{3-36} inhibits NPY- and activates POMC neuronal activity, we wondered whether PYY_{3-36} can improve insulin sensitivity independently of its effects on food intake and body weight. Therefore, we infused PYY_{3-36} intravenously and quantified glucose and lipid metabolism during a hyperinsulinemic euglycemic clamp in mice that were fed a high fat diet for 2 weeks **(chapter 5)**. To measure insulin sensitivity of hepatic and peripheral glucose metabolism, a hyperinsulinaemic euglycaemic clamp with radioactive labelled

glucose and 2-deoxy-glucose was performed. To measure insulin sensitivity of FFA metabolism, a hyperinsulinaemic euglycaemic clamp, in combination with ^{14}C-palmitate was performed. PYY_{3-36} enhanced insulin-induced glucose disposal (103.8 ± 10.9 vs. 76.1 ± 11.4 µmol/min/kg, respectively, p=0.001). Accordingly, 2-DG uptake in muscle and adipose tissue in hyperinsulinaemic conditions was higher during PYY_{3-36} infusion, although the difference with control reached statistical significance only for muscle (2.1 ± 0.5 vs. 1.5 ± 0.5 %/ g tissue, $p<0.05$). In contrast, PYY_{3-36} did not significantly impact insulin's capacity to inhibit endogenous glucose production (62 ± 29 vs. 42 ± 18%, ns). Insulin action on FFA metabolism was not affected by PYY_{3-36} either, as indicated by similar lipolysis rates during hyperinsulinaemia (22.4 ± 12.3 vs. 21.3 ± 10.9 µmol/min/kg, ns) in PYY_{3-36} and saline infused animals. In conclusion, this study shows that PYY_{3-36} reinforces insulin action in mice maintained on a high fat diet, independent of its effects on food intake and body weight. In this context, PYY_{3-36} appears to predominantly impact insulin mediated glucose disposal, whereas it leaves insulin action on glucose production largely unaffected. These data suggest that PYY_{3-36} or synthetic analogues of this peptide may be valuable assets to our armamentarium of drugs designed to battle insulin resistance and type II diabetes mellitus.

The value of PYY_{3-36} as a new drug depends on the duration of its effects. Therefore, we investigated the long-term effects of PYY_{3-36} (administration of 7 days either continuously via subcutaneous mini-pumps or intermittent via subcutaneous injections once a day) on insulin sensitivity **(chapter 6)**. Whole-body and hepatic insulin sensitivity were measured by hyperinsulinaemic euglycaemic clamp in combination with D-[^{14}C]glucose infusion. Tissue specific insulin sensitivity was measured by use of 2-deoxy-D[^{3}H]glucose. Long term PYY_{3-36} administration did not seem to have any adverse or counterregulating effects, as long term administration showed the same effect as acute administration: increased peripheral insulin sensitivity. Notably, there was no difference between continuous and intermittent administration.

Ghrelin

As ghrelin acts in the hypothalamus where it promotes NPY and orexin gene expression and inhibits POMC/αMSH expression via activation of the GHS-receptor, we wondered whether ghrelin might affect insulin sensitivity via the GHS-receptor. To address this hypothesis, we intravenously administered vehicle, ghrelin, or growth hormone releasing peptide 6 (GHRP-6, a specific agonist of the GHS-R), and measured insulin sensitivity in wildtype mice **(chapter 7)**. Until recently, acylation of the serine-3 residue of the ghrelin molecule was believed to be required for bioactivity. However, recent data suggest that des-ghrelin may counteract ghrelin in the control of energy balance (5), and in vitro experiments revealed opposing effects of ghrelin and des-ghrelin on glucose production by primary hepatocytes (6). To further explore

the role of des-ghrelin in the regulation of fuel flux, we studied the effects of this peptide in the same experimental context. Whole-body and hepatic insulin sensitivity were measured by hyperinsulinaemic euglycaemic clamp in combination with D-[^{14}C]glucose infusion. Tissue specific insulin sensitivity was measured by use of 2-deoxy-D[^{3}H]glucose. Hyperinsulinaemia suppressed EGP significantly less in animals that received ghrelin compared to controls (control: 71±11 %, ghrelin: 46±22 %, GHRP-6: 70±22 %; $p<0.05$). In contrast, glucose disposal rate was significantly higher in animals that received ghrelin compared to the control group (control: 59±8 µmol/kg/hour, ghrelin: 77±16 µmol/kg/hour, GHRP-6: 60±9 µmol/kg/hour; $p<0.05$), in accordance with insulin-mediated 2-DG uptake in muscle and adipose tissue, which was higher in ghrelin-treated animals, compared to the control group (muscle: control: 8.6 ± 4.4; ghrelin: 26 ± 21; GHRP-6: 7.1 ± 3.4 µmol/g tissue, $p<0.05$) although this difference did not reach statistical significance in adipose tissue (adipose tissue: control: 2.6 ± 1.7; ghrelin: 7.9 ± 11; GHRP-6: 3.6 ± 1.5 µmol/g tissue, $p=0.09$). With regard to des-ghrelin, during the hyperinsulinaemic period, insulin stimulated glucose disposal and tissue specific glucose uptake did not differ from the control group. In contrast, hyperinsulinaemia suppressed hepatic glucose production significantly less in animals that received des-ghrelin compared to controls (47±13 versus 71±11 %, $p<0.01$). In conclusion, ghrelin differentially affects tissue-specific insulin action, hampering its ability to inhibit endogenous glucose production while reinforcing its impact on glucose disposal. These effects occur acutely and they are not secondary to ghrelin's well-known influence on feeding and body weight. The GHS-R is not likely to mediate ghrelin's metabolic effects. Des-ghrelin also appears to modulate fuel flux and may counteract ghrelin in the control of glucose metabolism.

Conclusions

The hypothalamic arcuate nucleus has a central role in the regulation of appetite and food intake. During fasting, expression of neuropeptides involved in the regulation of food intake in the arcuate nucleus change: expression of the orexigenic neuropeptide NPY increases, whereas expression of the anorexigenic neuropeptide POMC decreases, resulting in stimulation of food intake. Under fed conditions, expression of POMC increases, whereas expression of NPY decreases, resulting in a inhibition of food intake.

Recent studies showed that the brain regulates appetite and food intake in crosstalk with the gut. Secretion of gastrointestinal hormones varies according to feeding status. During fasting, secretion of ghrelin, an activator of NPY neurons and inhibitor of POMC neurons, increases, whereas secretion of PYY_{3-36}, an agonist of the presynaptic NPY Y2 receptor and thereby inhibitor of NPY neurons and activator of POMC neurons, decreases, resulting in increased food intake. During feeding, opposite changes occur.

Until recently, it was thought that regulation of glucose metabolism was the result of crosstalk between the liver, muscle and adipose tissue with the pancreas and adrenals, producing glucoregulatory hormones, with a central role for insulin. Recent studies, however, changed this “peripheral” view to a more “central” concept with a prominent role for the brain regulating glucose homeostasis. Recent studies of Rossetti et al. showed that chronic blockade of hypothalamic insulin receptor signalling caused hepatic insulin resistance of glucose production, whereas acute depletion of insulin receptors in the liver failed to alter the effect of physiological hyperinsulinemia on the rate of glucose production (7-9). These studies show that brain insulin action is required for intact glucose homeostasis.

The studies performed in this thesis show that the brain not only regulates food intake but also insulin sensitivity of glucose metabolism in liver and peripheral tissues, like skeletal muscle and adipose tissue (see figure 1), dependently and independently of feeding status and likely via related changes in gastrointestinal hormones. In this way, at every moment, tissue insulin sensitivity can be fine-tuned, dependently and independently of feeding status. We showed that fasting itself resulted in an increase in muscle insulin sensitivity. Since intravenous administration of ghrelin in mice was shown to increase muscle insulin sensitivity as well, it can be speculated that the effects of fasting on peripheral insulin sensitivity are mediated by increased secretion of ghrelin, probably (partly) in crosstalk with the arcuate nucleus NPY/POMC neuronal circuitry. Interestingly, all intervention studies performed by others and in this thesis showed tissue specific effects of the different interventions on insulin sensitivity. Van den Hoek et al. showed that central infusion of NPY decreased insulin action on hepatic glucose production, without affecting peripheral insulin sensitivity (10). Central injections of MTII (stimulating the POMC pathway) resulted in an increase in peripheral glucose disposal without affecting hepatic insulin sensitivity. Intervenous administration of ghrelin increased muscle insulin sensitivity but decreased hepatic insulin sensitivity. Intravenous infusion of PYY_{3-36} increased peripheral insulin sensitivity as well, but had no impact on hepatic insulin sensitivity. Since, for example, fasting and ghrelin increase peripheral insulin sensitivity whereas NPY, which is also activated by fasting and ghrelin, has no effect on peripheral insulin sensitivity but induces hepatic insulin resistance, the effects of fasting and ghrelin on peripheral insulin sensitivity cannot merely be explained by modulation of NPY neurons. In contrast, the induction of hepatic insulin resistance by ghrelin might be (partly) the result of activation of NPY neurons by ghrelin.

Although it has been shown by others that regulation of food intake by gastrointestinal hormones is mediated by the brain, this remains speculative for the regulation of body insulin sensitivity. Receptors for gastrointestinal hormones are found throughout the body, both centrally and peripherally. From the studies in this thesis no conclusions can be drawn about the site of action of gastrointestinal hormones in the central nervous system. Therefore, more

research, like peripheral and central administration of gastrointestinal hormones in combination with blockers of the NPY/POMC system and denervation studies, is needed to draw conclusions about the contribution of the brain and site of action in the brain with regard to the regulation of insulin sensitivity due to changes in gastrointestinal hormone levels.

As the brain uses glucose as the obligatory fuel, the regulation of feeding and insulin sensitivity is of major importance to the brain. It is therefore not surprising that the brain itself is involved in regulating these processes tightly to secure survival.

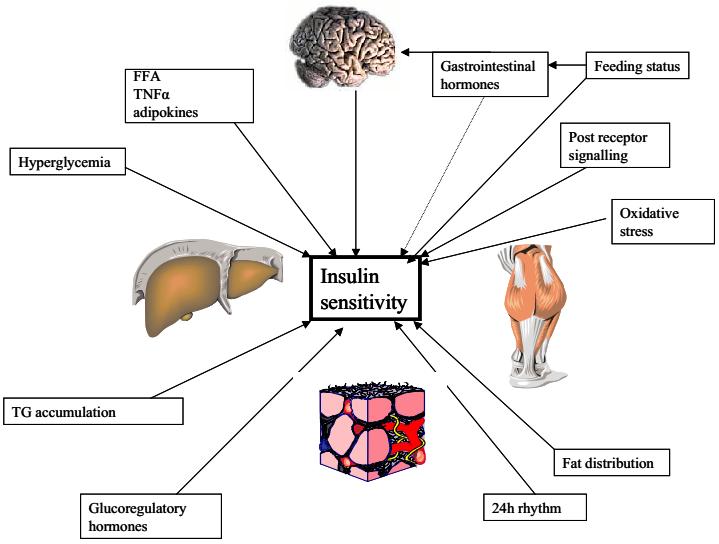

Figure 1. A role for gastrointestinal hormones and the brain in the regulation of insulin sensitivity

Reference List

1. Kim,SP, Ellmerer,M, Van Citters,GW, Bergman,RN: Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. *Diabetes* 52:2453-2460, 2003

2. Kraegen,EW, Clark,PW, Jenkins,AB, Daley,EA, Chisholm,DJ, Storlien,LH: Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 40:1397-1403, 1991

3. Obici,S, Feng,Z, Tan,J, Liu,L, Karkanias,G, Rossetti,L: Central melanocortin receptors regulate insulin action. *J Clin.Invest* 108:1079-1085, 2001

4. Mizuno,TM, Kelley,KA, Pasinetti,GM, Roberts,JL, Mobbs,CV: Transgenic neuronal expression of proopiomelanocortin attenuates hyperphagic response to fasting and reverses metabolic impairments in leptin-deficient obese mice. *Diabetes* 52:2675-2683, 2003

5. Asakawa,A, Inui,A, Fujimiya,M, Sakamaki,R, Shinfuku,N, Ueta,Y, Meguid,MM, Kasuga,M: Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 54:18-24, 2005

6. Gauna,C, Delhanty,PJ, Hofland,LJ, Janssen,JA, Broglio,F, Ross,RJ, Ghigo,E, van der Lely,AJ: Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. *J Clin.Endocrinol.Metab* 90:1055-1060, 2005

7. Obici,S, Zhang,BB, Karkanias,G, Rossetti,L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat.Med.* 8:1376-1382, 2002

8. Obici,S, Feng,Z, Karkanias,G, Baskin,DG, Rossetti,L: Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat.Neurosci.* 5:566-572, 2002

9. Buettner,C, Patel,R, Muse,ED, Bhanot,S, Monia,BP, McKay,R, Obici,S, Rossetti,L: Severe impairment in liver insulin signaling fails to alter hepatic insulin action in conscious mice. *J Clin.Invest* 115:1306-1313, 2005

10. van den Hoek,AM, Voshol,PJ, Karnekamp,BN, Buijs,RM, Romijn,JA, Havekes,LM, Pijl,H: Intracerebroventricular neuropeptide Y infusion precludes inhibition of glucose and VLDL production by insulin. *Diabetes* 53:2529-2534, 2004