CHAPTER 1

General introduction
1.1 OBESITY: EPIDEMIOLOGY AND PATHOPHYSIOLOGY

1.1.1 Obesity and body weight regulation

The global epidemic of obesity is increasing rapidly (1) and overweight is not just a cosmetic issue, but causes or exacerbates many health problems. Obesity is associated with a high risk of type 2 diabetes, coronary heart disease and cancer. Obesity is the leading preventable cause of illness and premature death worldwide (2). Due to the current overwhelming availability of food and the lack of physical activity, excess energy is stored for times when it might be needed – but these times never come. Numerous studies are being performed to gain more insight into the pathophysiological mechanisms leading to chronically inappropriate eating behavior, in excess of energy expenditure, in order to find preventive or therapeutic methods to stop the rising incidence of obesity.

The physiology of human energy homeostasis is based on the first law of thermodynamics, stating that energy can only be transformed from one state to another and cannot be created or destroyed. To maintain energy balance, the amount of food and fluid consumed needs to match the amount of energy loss, by radiant, conductive and convective heat loss, any activity performed, and heat loss from evaporation. Any imbalance between intake and utilization results in an alteration of body weight and body composition (3). Although this seemingly simple balance between food intake and energy expenditure should offer simple methods to maintain or even lose body weight by simply adjusting, most likely by decreasing food intake or increasing physical activity, the failure of dieting and weight-loss programs shows that most people are not capable to successfully maintain their body weight, but relapse in their habitual behavior.

Food intake involves both food-seeking behavior, important for meal initiation, and satiety perception, important in meal termination (4). Meal initiation is, among others, influenced by the nowadays wide availability of energy dense food, which in Western societies is less expensive than healthy food and aggressively promoted in commercials. Moreover, “liking” and “wanting” of palatable food can overcome feelings of satiety and lead to food intake despite feelings of fullness or not being hungry (3). Satiation occurs at the time of eating and represents the inhibitory effects induced by food ingestion (sensory, cognitive, digestive and hormonal), and terminates eating; satiety starts after the end of an eating episode and prevents further eating before the return of hunger (5). The inhibitory effects induce satiation and satiety by direct or indirect effects on the brain.

1.1.2 Body weight regulation: the brain

The central nervous system (CNS) plays a major role in energy homeostasis and food intake regulation. Both anorexigenic and orexigenic hormone signals, derived from both peripheral organs (adipose tissue, pancreas, gut) and the brain, are integrated in the brain through complex signaling networks to establish and maintain energy homeostasis. Several hormones are known to have a satiating
effect on the brain (a.o. choleystokinin, amylin, peptide YY, and glucagon-like peptide-1, GLP-1) and so far only ghrelin, secreted by the stomach, is known to stimulate appetite and increase meal size. Many of these satiety signals are produced in the periphery, signaling to the brain via the vagus nerve, but several other signals are also synthesized in the brain. Nutrients such as glucose, fatty acids, and amino acids also act as signaling molecules informing the CNS about the energy status of the organism (7).

To date, only two hormones meet the criteria for negative feedback adiposity signal: insulin and leptin. They both circulate at levels proportional to body fat content and energy balance, enter the brain and act on neuronal systems implicated in energy homeostasis. Interference with signaling of either hormone results in increased food intake and body weight (8) (Figure 1.1). During weight loss, low levels of insulin and leptin in the brain promote anabolic neuronal pathways, stimulating eating behaviour, decreasing energy expenditure, and

Figure 1.1 Insulin and leptin in the central nervous system (adapted from (6)).
Stimulating effects are indicated by ‘+’ and inhibiting effects by ‘−’. High insulin and leptin levels, indicators of an anabolic state, inhibit orexigenic and stimulate anorexigenic brain pathways, decreasing appetite, food intake, and hepatic glucose production, and increasing energy expenditure. By this negative feedback mechanism, energy homeostasis is accomplished. AgRP, Agouti-related peptide; FFA, free fatty acids; NPY, neuropeptide Y; POMC, proopiomelanocortin.
inhibiting catabolic pathways that cause anorexia and weight loss. Food intake results in the generation of neural and hormonal satiety signals to the hindbrain. Insulin and leptin sensitive pathways interact with hindbrain satiety circuits to regulate meal size, thereby modulating food intake and energy balance (8). Both hormones act mainly in the arcuate nucleus, located in the mediobasal hypothalamus, where anabolic and catabolic neuronal pathways are located that affect food intake, energy expenditure, and glucose homeostasis (6). Neurons belonging to the anabolic pathway co-express neuropeptide Y (NPY) and Agouti-related peptide (AgRP), two peptides that are able to stimulate food intake and reduce energy expenditure, and thereby promote weight gain (9-11). These neurons are inhibited by leptin and insulin (8); consequently, high insulin or leptin levels lead to reduced hypothalamic signaling and therefore to decreased anabolism. In comparison, anabolic effects of AgRP arise from antagonism of neuronal melanocortin receptors that serve to limit food intake and body weight. In parallel to the response to NPY administration, chronic blockade of central melanocortin receptors causes weight gain and insulin resistance (12). Melanocortins are peptides derived from the precursor proopiomelanocortin (POMC), and POMC neurons in the arcuate nucleus innervate the same hypothalamic areas supplied by fibers from NPY/AgRP neurons. POMC neurons are stimulated by input from insulin and leptin (13;14), which leads to a catabolic state: inhibition of food intake and promotion of weight loss (15). When neuronal input from leptin and insulin is reduced, POMC neurons are inhibited, whereas NPY/AgRP neurons are activated, responses that in turn can cause hyperphagia, insulin resistance, and glucose intolerance (6).

Insulin is transported into the brain via an insulin receptor mediated, saturable pathway in brain capillary endothelial cells (16) and increases in plasma insulin levels may result in increased cerebrospinal fluid (CSF) insulin levels (17). Relatively high levels of the insulin receptor are found in the olfactory bulbs and arcuate nucleus of the hypothalamus, but also in several other regions, including cerebral cortex, cerebellum, hippocampus and choroid plexus, as well as other hypothalamic areas and regions of the lower brainstem (18-21). Animal studies have shown that disrupted intracerebral insulin signaling causes weight gain and that intracerebroventricular (ICV) insulin administration reduces food intake and body weight (22-25). In humans, central insulin action was studied by the administration of intranasal insulin, which is immediately taken up by the brain without systemic (hypoglycemic) effects (26); acute intranasal insulin promotes satiety and reduces snack intake in the postprandial state in women (27) and results in weight loss in the longer term in men (28;29).

Leptin, secreted by adipocytes in proportion to fat mass, enters the brain by a saturable system independent of insulin (30). Within the brain, multiple populations of leptin receptor expressing neurons have been identified, including neurons of the hypothalamic arcuate nucleus, neurons of the ventromedial nucleus of the hypothalamus, dopaminergic neurons in the ventral tegmental area, GABAergic neurons of the lateral hypothalamic area and neurons within the nucleus of the solitary tract (31). ICV leptin infusion reduces food intake and
CHAPTER 1. General introduction

body weight in lean rats, but not in obese Zucker rats (32). Leptin deficiency or the lack of leptin receptors results in hyperphagia and severe obesity in humans (33), with hyperphagia persisting despite high insulin levels. Therefore, leptin which does not have the hypoglycemia inducing side-effect of insulin, has been proposed as therapy for treatment of obesity. In obese subjects, who already have high circulating leptin levels, however, increasing leptin levels by peripheral leptin administration does not result in weight loss. Nevertheless, leptin therapy can be useful in leptin deficient diseases like anorexia nervosa and lipodystrophy syndromes (34-37).

1.2 BRAIN IMAGING

The human brain is protected by the skull and can be imaged by several imaging modalities, two of which are used in this thesis: magnetic resonance imaging (MRI), by which the cerebral anatomy and structure can be visualized with high spatial resolution and without exposure to radioactivity, and positron emission tomography (PET), by which several metabolic and molecular processes of the brain can be visualized and measured with high sensitivity.

1.2.1 Positron emission tomography (PET)

Positron emission tomography (PET) is an imaging technique that makes use of labeled tracers, i.e. molecules labeled with a positron emitting nuclide, that are administered in minute (trace) amounts. Depending on the actual tracer being used, various metabolic and molecular processes, such as blood flow, glucose metabolism and receptor density, can be measured. Positron emitters, radionuclides that decay by the emission of a positron, have a short half-life (e.g. 2 minutes for $^{15}$O and 2 hours for $^{18}$F). Emitted positrons collide with electrons in tissue, after which both particles annihilate, resulting in two gamma rays (photons) of 511 keV, which are emitted in opposite directions (Figure 1.2). Using coincidence detection these (simultaneously emitted) photons can be detected by the PET scanner. After data reconstruction, an image is obtained showing the concentration of the tracer in the part of the body within the field of view of the scanner (e.g. the head) during the time of the scan. Before injection of the tracer, a transmission scan is acquired to account for attenuation (i.e. absorption of photons in the field of view when passing through the patient, so that these photons are not detected) and to be able to correct for scatter (i.e. change of direction of one or both photons due to an interaction in the body before detection by the scanner, leading to an erroneous line of response). To measure the distribution of radioactivity in tissue over time, a dynamic PET scan is acquired. Full tracer kinetic analysis also requires an input function, i.e. the time course of radioactivity in arterial blood that is available for the tissue. This input function can be obtained using an on-line blood sampler (38), which measures whole blood radioactivity concentrations in arterial blood, usually from the radial artery (Figure 1.3). To obtain a plasma input function, additional
arterial blood samples are necessary to measure plasma to whole blood activity ratios. Finally, for quantitative data analysis, a tracer kinetic model is needed that describes the data in terms of underlying physiological parameters. Using a fitting routine, the model equations are then fitted to the time-activity curves of one or more selected regions of interest. Flow measurements using $[^{15}\text{O}]\text H_2\text O$ PET are based on a single-tissue compartment model for diffusible tracers, in which $C_A$, tracer ($[^{15}\text{O}]\text H_2\text O$) concentration in arterial blood, and $C_T$, tracer ($[^{15}\text{O}]\text H_2\text O$) concentration in (brain) tissue, are the measured parameters. The model allows for $K_1$ and $k_2$ to be fitted; in the context of CBF, $K_1 = \text{CBF}$ and $k_2 = \text{CBF}/V_T$ (Figure 1.4). For $[^{18}\text{F}]\text{FDG}$ a two-tissue compartment model is needed (Figure 1.2).

**Figure 1.2 PET principle.** Decay of an injected radionuclide will result in the emission of positrons, which in tissue will collide with electrons. When a positron has lost its energy, it will combine with an electron after which both particles will annihilate, resulting in two gamma rays (photons) of 511 keV travelling in opposite directions. Subsequently, these simultaneously emitted photons (so-called coincidence photons) can be detected by the PET scanner.
1.5. \([^{18}\text{F}]\text{FDG}\) first crosses the blood brain barrier (BBB) by facilitated transport and enters the first tissue compartment at a rate \(K_f\). Once in tissue, it is either phosphorylated by hexokinase at a rate \(k_3\) or it is transported back to the blood at a rate \(k_2\). Since \([^{18}\text{F}]\text{FDG}\) cannot be further metabolized after phosphorylation, it is trapped, and measured by PET. FDG metabolism can be calculated from \(K_i (= K_f \cdot k_3/(k_2 + k_3))\) and the arterial plasma glucose level. To calculate glucose metabolism from FDG metabolism, a lumped constant (LC) is applied to account for the differences in transport and phosphorylation between glucose and FDG.

Figure 1.3 PET study set-up. Acquisition of a brain PET scan, where the patient is positioned such that the head is within the centre of the field of view of the PET scanner. Lights are dimmed and eyes are closed, noise is minimized. Tracer injection takes place through a venous line. The time course of radioactivity in arterial blood is measured continuously using an on-line blood sampler, withdrawing blood from the radial artery.
General introduction. CHAPTER 1

Figure 1.4 Single-tissue compartment model for [15O]H₂O PET data. $C_A$, tracer ([15O]H₂O) concentration in arterial blood, in Bq/mL; $C_T$, tracer ([15O]H₂O) concentration in (brain) tissue, in Bq/mL; BBB, blood brain barrier; $F$, flow, in mL/cm³ per minute (= $K_1$); $V_T$, volume of distribution (unitless); $F/V_T = k_f$.

Figure 1.5 [18F]FDG PET model (adapted from (39)). Two-tissue compartment model. In the lower panel (below the dotted line), FDG metabolism is depicted, whereas glucose metabolism is shown in the upper panel. BBB, blood-brain-barrier; $K_1$, rate of transport from blood to brain; $k_2$, rate of transport from brain to blood; $k_3$, rate of phosphorylation by hexokinase; $k_4$, rate of desphorylation by glucose-6-phosphatase (for an irreversible model, $k_4 = 0$). FDG and glucose parameters are marked with and without an asterisk, respectively. G-6-P, glucose-6-phosphate; G-1-PO₄, glucose-1-phosphate; G-6-PO₄, glucose-6-phosphate; F-6-PO₄, fructose-6-phosphate.
1.2.2 Magnetic resonance imaging (MRI)
Magnetic resonance imaging (MRI) is a non-invasive imaging technique, by which the anatomy can be visualized with high spatial resolution. Magnetic resonance arises from the interaction of nuclei that have a magnetic moment within an external magnetic field (40). This (constant) magnetic field is generated by the magnet of the MRI scanner. MRI makes use of the natural properties of hydrogen, which makes up 75-80% of the human body, as part of either water or lipids. Differences in the relative proton density provide contrast between structures, e.g. brain has high water content (high MRI signal) and bone low water content (low MRI signal) (41). In a magnetic field, nuclei of many atoms with a nuclear ‘spin’ can behave as magnetic dipoles and can assume either a high-energy state (behaving as oriented against the field applied) or a low-energy state (as if aligned with the magnetic field applied). Transitions between energy states result in absorption or emission of energy in the radiofrequency range, which are detected by MRI (40).

1.2.3 Functional MRI
In contrast to MRI, functional MRI (fMRI) measures brain activation. This method makes use of the blood oxygen level dependent (BOLD) contrast, in which imaging contrast results from the ratio of oxy- to deoxyhemoglobin in venous blood (42). When a particular part of the brain is activated by a specific task (e.g. choosing whether a picture that is shown represents an indoor or outdoor image), the increased consumption of oxygen by neurons during this activation is accompanied by a disproportionate increase in the supply of fully oxygenated blood, resulting in a decrease in the concentration of deoxyhemoglobin downstream from the site of activation and therefore an increase in MRI signal (41). Consequently, an increased BOLD signal represents increased brain activation and this can be related to specific (cognitive) tasks applied.

1.2.4 Arterial spin labelling (ASL)
Arterial spin labelling (ASL) is another MRI technique used as an alternative method for measuring cerebral perfusion. By using magnetically labeled protons in arterial blood as diffusible tracer, ASL does not require the injection of a positron emitting radionuclide. At the level of the carotid arteries, an appropriate series of radiofrequency pulses is applied, to magnetically label water protons in arterial blood, i.e. before it enters the capillary bed. When entering the capillary bed, magnetization of the tissue is altered in a way that can be measured quantitatively (43). Both a labeled and a control image are acquired and the perfusion-weighted image is generated by the subtraction of these images (41).
1.3 TYPE 1 DIABETES AND INSULIN THERAPY

1.3.1 Type 1 diabetes
Type 1 diabetes is a common and a chronic metabolic disorder, characterized by the failure of insulin production, due to an auto-immune mediated destruction of the insulin producing beta cells of the pancreas. Consequently, insulin levels ultimately fall and glucose levels rise. Due to hyperglycemia, patients develop symptoms of polyuria, polydipsia, body weight loss and blurred vision. Until the discovery of insulin by Banting and Best in 1922 (44), for which they received the Nobel Prize in 1923, type 1 diabetes was a lethal disease. Since then, insulin therapy has become the cornerstone of type 1 diabetes treatment and, in most patients, lifelong insulin treatment is required in order to mimic normal metabolic physiology thus mitigating the (acute) symptoms of hyperglycemia listed above and, in the longer term, to reduce the harming effects of hyperglycemia and its associated complications.

1.3.2 Insulin: structure and physiology
Insulin is a 51-amino acid hormone, with a molecular weight of 5808 Da. Insulin is synthesized as proinsulin, then processed and secreted by pancreatic beta cells of the islets of Langerhans into the portal circulation via the hepatic vein. Subsequently, the liver extracts approximately 60% before it enters the systemic circulation. The circulating form of endogenous insulin is a monomer consisting of two chains, an A-chain of 21 amino acids and a B-chain of 30 amino acids, linked by two disulfide bridges. The A-chain contains an intra-chain disulfide bridge between A7 and A11. At micromolar concentrations, insulin dimerises and, in the presence of zinc ions, further associates into hexamers (45). In order to maintain euglycaemia (i.e. plasma glucose levels between 3.5 – 7.0 mmol/L), insulin release occurs at a basal rate and in short-lived bursts in response to stimuli related to nutrient intake (Figure 1.6). Basal insulin secretion occurs in the fasting state to inhibit hepatic glycogenolysis, ketogenesis and gluconeogenesis and accounts for approximately 40% of total insulin output per 24 hours. Stimulated insulin secretion occurs when plasma glucose levels exceed 4.4-5.6 mmol/L, particularly after meals, to restore euglycemia by promoting peripheral glucose uptake and fuel storage. In addition, insulin secretion in response to a meal occurs in 2 phases: an initial transient surge (first phase) followed by a prolonged more steady increase (second phase). Although glucose is the most potent secretagogue, other stimuli, including dietary nutrients (e.g. amino acids), gut-derived incretin hormones and neural signals are also involved in insulin secretion. In healthy individuals, plasma glucose and insulin excursions occur in parallel and are tightly coupled throughout the day, thus maintaining strict glucoregulation (46;47).

1.3.3 Insulin formulations and analogs
After the discovery of insulin in 1922, insulin therapy significantly evolved.
While at first insulin was extracted from bovine or porcine pancreata, later (1978) recombinant DNA technology became available and insulin analogs were designed (46). NPH (Neutral Protamine Hagedorn) insulin was the first long (intermediate) acting insulin available - the protracting effect of protamine on insulin was originally discovered in 1936 - and it still is widely used for both type 1 and type 2 diabetes and in pregnancy. Its use, however, is often accompanied by (nocturnal) hypoglycemia and unpredictable uptake from subcutaneous tissue, resulting in a varying glucose-lowering effect, partially caused by the solution (suspension) that needs to be mixed properly before injection. More recently, two other basal insulin analogs have been introduced and are currently available: insulin glargine (Lantus®) in 2000 (49) and insulin detemir (Levemir®) in 2004 (50). The ultra-long acting insulin degludec (Tresiba®) currently awaits full FDA approval. Prandial insulins currently available are human insulin (Actrapid®), insulin aspart (NovoRapid®), lispro (Humalog®) and glusiline (Apidra®). Specific action profiles including duration of action for several insulin preparations are listed in Table 1.1.

1.3.4 Insulin therapy in type 1 diabetes
Patients with type 1 diabetes need life-long substitution therapy with insulin. In general, the treatment regimen consists of a combination of a basal (intermediate or long-acting) insulin, injected once or twice daily, and a prandial (short-acting) insulin, injected with meals, to mimic ‘normal’ physiology as closely as
General introduction. CHAPTER 1

Table 1.1 Pharmacokinetic properties of available insulin preparations

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Onset</th>
<th>Peak</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rapid acting</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lispro</td>
<td>Humalog</td>
<td>Eli Lilly</td>
<td>0.2-0.5</td>
<td>0.5-2</td>
<td>3-4</td>
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<tr>
<td>Aspart</td>
<td>NovoRapid</td>
<td>Novo Nordisk</td>
<td>0.2-0.5</td>
<td>0.5-2</td>
<td>3-4</td>
</tr>
<tr>
<td>Glusiline</td>
<td>Apidra</td>
<td>Sanofi-Aventis</td>
<td>0.2-0.5</td>
<td>0.5-2</td>
<td>3-4</td>
</tr>
<tr>
<td><strong>Short acting</strong></td>
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<td></td>
</tr>
<tr>
<td>Regular</td>
<td>Humulin R</td>
<td>Eli Lilly</td>
<td>0.5-1</td>
<td>2-4</td>
<td>6-8</td>
</tr>
<tr>
<td></td>
<td>Actrapid</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intermediate acting</strong></td>
<td></td>
<td></td>
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<tr>
<td>Isophane insulin (NPH)</td>
<td>Humulin NPH</td>
<td>Eli Lilly</td>
<td>1.5-4</td>
<td>4-10</td>
<td>Up to 20</td>
</tr>
<tr>
<td></td>
<td>Insulatard</td>
<td>Novo Nordisk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insuman basal</td>
<td>Sanofi-Aventis</td>
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<tr>
<td><strong>Long acting</strong></td>
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<td></td>
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<td>Glargine</td>
<td>Lantus</td>
<td>Sanofi-Aventis</td>
<td>1-3</td>
<td>no</td>
<td>Up to 24</td>
</tr>
<tr>
<td>Detemir</td>
<td>Levemir</td>
<td>Novo Nordisk</td>
<td>1-3</td>
<td>no</td>
<td>Up to 24</td>
</tr>
<tr>
<td><strong>Premixed insulin analogs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPL/lispro</td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>75%/25%</td>
<td>Humalog Mix 75/25</td>
<td>Eli Lilly</td>
<td>0.2-0.5</td>
<td>1-4</td>
<td>24</td>
</tr>
<tr>
<td>50%/50%</td>
<td>Humalog Mix 50/50</td>
<td>Eli Lilly</td>
<td>0.2-0.5</td>
<td>1-4</td>
<td>24</td>
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<tr>
<td>IAP/aspart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%/30%</td>
<td>Novo Mix 70/30</td>
<td>Novo Nordisk</td>
<td>0.2-0.5</td>
<td>1-4</td>
<td>24</td>
</tr>
<tr>
<td>75%/25%</td>
<td>Humalog Mix 75/25</td>
<td>Eli Lilly</td>
<td>0.2-0.5</td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

Adapted from (46). IAP, insulin aspart protamine; NPH, neutral protamine Hagedorn; NPL, neutral protamine lispro.
possible. An alternative for this multiple daily insulin injections (MDII) regimen is continuous subcutaneous insulin infusion (CSII) with short-acting insulin (‘pump’ therapy). Initially, insulin was injected only once or twice daily, but in the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study, it was shown that intensive insulin therapy (MDII or CSII with frequent blood glucose testing), aimed at maintaining normoglycemia as closely as possible, reduced the risk of diabetes associated complications and improved long-term diabetes outcome (51;52). Intensive insulin therapy, however, is also associated with an increased risk of hypoglycemia (53) and body weight gain (54). In theory, the latter may offset the benefits of intensified insulin treatment, as weight gain is linked to insulin resistance, hypertension and dyslipidemia (55). Nevertheless, in spite of the link between these obesity associated factors and macrovascular disease, the DCCT/EDIC studies showed long-term improvement in cardiovascular outcome in the intensive treatment group (56).

Several mechanisms play a role in weight gain with insulin therapy. Subcutaneously injected insulin in diabetic patients results in higher circulating insulin levels and elevated insulin concentrations in peripheral tissues compared with healthy volunteers, as peripherally injected insulin does not primarily pass the liver. Therefore, in diabetic patients, peripheral tissues are continuously exposed to higher insulin levels than in healthy subjects, leading to increased lipogenesis in adipose tissue and protein synthesis, collectively resulting in an anabolic state and weight gain. Furthermore, a very well-known (side)-effect of insulin treatment is hypoglycemia. Hypoglycemia needs to be solved or prevented by carbohydrate consumption, even when no feelings of hunger or appetite are present, and this promotes weight gain as well.

1.3.5 Insulin therapy: insulin detemir

In 2004, insulin detemir (Lys-B29 (N-tetradecanoyl) des (B30) human insulin) was launched on the market (50). It was the first clinically available acetylated basal insulin analog; the amino acid threonine at B30 is removed and a 14-carbon, myristoyl fatty acid is acylated to lysine at B29 (59) (Figure 1.7). The protracted action of detemir is primarily achieved through slow absorption into blood. Dihexamerization and albumin binding of hexameric and dimeric detemir prolongs residence time at the injection depot. Further retention of detemir occurs in the circulation where albumin binding causes an insulin buffer. Insulin detemir is associated with less weight gain and even body weight loss when compared with other insulin preparations (60-67). In Table 1.2 and Figure 1.8 studies concerning insulin detemir and change in body weight in type 1 diabetes are listed.

To date, no convincing explanation for these weight differences exists, although four possible mechanisms have been described. The first mechanism by which insulin detemir causes decreased weight gain, is via a reduction in the frequency of hypoglycemia and therefore less defensive snacking (62;64;68-72). An alternative mechanism for the weight stability of insulin detemir is
via a preferential effect on hepatic metabolism of insulin detemir. It has been shown that, in comparison with other insulin preparations, insulin detemir has a relatively larger effect on the liver than on peripheral muscle and adipose tissue. Because no significant barrier exists between blood plasma in the sinusoid and the hepatocyte plasma membrane, insulin detemir, both free and albumin-bound, is taken up continuously by the hepatocytes. In contrast, binding to albumin results in delayed transfer of insulin detemir from the circulation into adipose tissue and skeletal muscle. Therefore, albumin binding of detemir increases the hepatic-peripheral insulin gradient, mimicking the physiological, non-diabetic state. Thus, in order for detemir to exert similar blood glucose control, its effect on and extraction by the liver may be relatively higher, resulting in reduced endogenous glucose production, whereas its peripheral action, including anti-lipolytic activity, is lower (63;73;74). A third mechanism is that insulin detemir therapy results in increased energy expenditure (75). The last explanation for the weight-stability of insulin detemir could be via enhanced effects of insulin detemir on the brain, where insulin is known to act as a satiety hormone. In mice it has been shown that insulin detemir infusion results in higher brain insulin levels and increased brain activity compared with human insulin (76). In humans, insulin detemir infusion results in increased brain activity and decreased food intake compared with human insulin (77;78). To further elucidate the mechanisms by which insulin (detemir) acts on the human brain, the studies described in the present thesis were performed.
Figure 1.8 Studies in which effect of insulin detemir on body weight in type 1 diabetes is reported. White bars, NPH insulin; black bars, insulin detemir; striped bars, insulin glargine.
### Table 1.2 Studies in which insulin detemir effect on body weight in type 1 diabetes is reported

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Number</th>
<th>Insulin</th>
<th>Duration</th>
<th>Weight</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartley et al. (79)</td>
<td>2008</td>
<td>497</td>
<td>ID vs NPH (+IA)</td>
<td>24 mo</td>
<td>+1.7 vs +2.7 kg</td>
<td>-0.94 vs -0.72%</td>
</tr>
<tr>
<td>Chacra et al. (80)</td>
<td>2010</td>
<td>381</td>
<td>ID vs IL susp (+IL)</td>
<td>32 wk</td>
<td>+ 0.62 vs + 1.59 kg</td>
<td>-0.59 vs -0.69%</td>
</tr>
<tr>
<td>Heller et al. (81)</td>
<td>2009</td>
<td>443</td>
<td>ID vs IG (+IA)</td>
<td>52 wk</td>
<td>No difference*</td>
<td>-0.53 vs -0.54%</td>
</tr>
<tr>
<td>Hermansen et al. (70)</td>
<td>2004</td>
<td>595</td>
<td>ID (+IA) vs NPH (+HI)</td>
<td>18 wk</td>
<td>- 0.95 vs + 0.07 kg</td>
<td>-0.50 vs -0.28%</td>
</tr>
<tr>
<td>Home et al. (72)</td>
<td>2004</td>
<td>408</td>
<td>ID (bf + bt vs 12h) +IA vs NPH +IA</td>
<td>16 wk</td>
<td>+ 0.02 vs +0.24 vs +0.86 kg</td>
<td>-0.8 vs -0.96 vs -0.58%</td>
</tr>
<tr>
<td>Johanssen et al. (82)</td>
<td>2009</td>
<td>73</td>
<td>ID vs IG (from NPH) (+any)</td>
<td>~ 18 mo (retrosp.)</td>
<td>+0.3 vs + 0.8 kg</td>
<td>-0.4 vs -0.3%</td>
</tr>
<tr>
<td>Kabadi et al. (83)</td>
<td>2008</td>
<td>45</td>
<td>ID vs IG (+IA)</td>
<td>retrosp.</td>
<td>No difference*</td>
<td>+0.9 vs +0.2%</td>
</tr>
<tr>
<td>Kolendorf et al. (84)</td>
<td>2006</td>
<td>130</td>
<td>ID vs NPH (+IA)</td>
<td>16 wk</td>
<td>-0.3 vs -1.0 kg (p1) vs -0.2 vs +1.3 kg (p2)</td>
<td>-0.3 vs -0.3%</td>
</tr>
<tr>
<td>De Leeuw et al. (69)</td>
<td>2005</td>
<td>428</td>
<td>ID vs NPH (+ IA)</td>
<td>12 mo</td>
<td>-0.1 vs +1.2 kg</td>
<td>-0.64 vs -0.56%</td>
</tr>
<tr>
<td>Pieber et al. (85)</td>
<td>2005</td>
<td>400</td>
<td>ID (m + d vs m + bt) vs NPH (+IA)</td>
<td>16 wk</td>
<td>-0.6 vs + 0.1 vs + 0.7 kg</td>
<td>-0.34 vs -0.48 vs -0.35%</td>
</tr>
<tr>
<td>Pieber et al. (86)</td>
<td>2007</td>
<td>320</td>
<td>ID vs IG (+IA)</td>
<td>26 wk</td>
<td>+0.52 vs +0.96 kg</td>
<td>-0.6 vs -0.5%</td>
</tr>
<tr>
<td>Robertson et al. (87)</td>
<td>2007</td>
<td>347 ch</td>
<td>ID vs NPH (+IA)</td>
<td>26 wk</td>
<td>BMIZ score vs -0.7 vs +0.1</td>
<td>-0.8 vs -0.8%</td>
</tr>
<tr>
<td>Russel-Jones et al. (88)</td>
<td>2004</td>
<td>747</td>
<td>ID vs NPH (+HI)</td>
<td>6 mo</td>
<td>-0.23 vs +0.31 kg</td>
<td>-0.06 vs +0.06%</td>
</tr>
<tr>
<td>Standl et al. (89)</td>
<td>2004</td>
<td>289</td>
<td>ID vs NPH (+HI)</td>
<td>6+6 mo</td>
<td>-0.3 vs +1.4 kg</td>
<td>7.88 vs 7.78%</td>
</tr>
<tr>
<td>Vague et al. (68)</td>
<td>2003</td>
<td>448</td>
<td>ID vs NPH (+IA)</td>
<td>6 mo</td>
<td>-0.2 vs +0.7 kg</td>
<td>-0.58 vs -0.47%</td>
</tr>
<tr>
<td>Zachariah et al. (75)</td>
<td>2011</td>
<td>23</td>
<td>ID vs NPH (+IA)</td>
<td>32 wk</td>
<td>-0.69 vs +1.7 kg</td>
<td>7.8 vs 7.5%</td>
</tr>
</tbody>
</table>

Bf, breakfast; bt, bedtime; ch, children; d, dinnertime; HI, human insulin; ID, insulin detemir; IG, insulin glargine; IA, insulin aspart; IL, insulin lispro; m, morning; mo, months; susp, suspension; wk, weeks; * no values reported.
1.4 AIM OF THE STUDY AND OUTLINE OF THE THESIS

The aim of the studies described in this thesis was to test the hypothesis that the weight sparing effect of subcutaneous treatment with insulin detemir, as compared with a standard regimen, i.e. treatment with NPH insulin, is associated with more pronounced CNS effects. To this end, a randomized, comparator-controlled, open-label, cross-over trial was performed in men with type 1 diabetes, entitled “Effects of INsulin dEtemiR and neutral protaminE hagedorn (nph) insulin on BRain glucOse metabolism: a study in persons with type 1 diabetes” (INcEREBRO study), measuring cerebral blood flow, glucose metabolism and neuronal activation in brain regions associated with appetite regulation. In this trial the following research questions were addressed:

1. Does a 12-week treatment with insulin detemir, compared with a 12-week treatment with NPH insulin, lead to increased blood flow and/or glucose metabolism in appetite-related brain regions?
2. Does a 12-week treatment with insulin detemir, compared with a 12-week treatment with NPH insulin, lead to:
   a. differences in brain responses in appetite-related regions when actively viewing pictures of food items and non-food items?
   b. increased insulin concentrations in CSF?
   c. less body weight gain?

Therefore, type 1 diabetic patients were randomly assigned to start with either a 12-week period of insulin detemir treatment or a 12-week treatment with NPH insulin, both combined with insulin aspart at mealtimes after a run-in period of at least four weeks, during which their own insulin therapy was optimized. After each treatment period patients underwent (f)MRI and PET studies, and a subgroup had a lumbar puncture to obtain CSF (Figure 1.9). In order to make a comparison with the normal situation, a group of gender, age and BMI matched healthy volunteers was also included; these subjects had one fMRI and one PET session.

In Chapters 2 and 3, the validation of PET imaging and data analysis methods are described, for both healthy volunteers and type 1 diabetic patients, who differ from healthy volunteers mainly in their higher and fluctuating blood glucose and insulin levels, which can affect the interpretation of some imaging modalities. In the following chapters, these imaging techniques are used to investigate the effects of the two different insulin therapies.

Chapter 2 describes the implementation of $^{[15]O}H_2O$ and $^{[18]F}FDG$ scans on a high resolution research tomograph (HRRT) PET scanner for measuring cerebral blood flow and glucose metabolism, respectively, in healthy volunteers. Furthermore, use of image derived input functions (IDIF), as non-invasive alternatives for arterial blood sampling, is investigated. In Chapter 3, results of $^{[15]O}H_2O$ and $^{[18]F}FDG$ studies in type 1 diabetic patients and healthy volunteers
are described. In Chapters 4 and 5, effects of 12 weeks insulin detemir treatment of type 1 diabetic patients are compared with 12 weeks NPH insulin treatment. In Chapter 4 results of $^{15}$O$\text{H}_2\text{O}$ and $^{18}$F$\text{FDG}$ studies are presented and in Chapter 5 results of fMRI studies. $^{15}$O$\text{H}_2\text{O}$ PET is the gold standard method for quantitative measurements of CBF in humans, but this method requires a cyclotron for $^{15}$O$\text{H}_2\text{O}$ production close to the PET scanner. Thus alternative methods to non-invasively measure CBF would be very welcome. Therefore, in Chapter 6, measurements of CBF in healthy volunteers and diabetic patients are compared between ASL MRI and $^{15}$O$\text{H}_2\text{O}$ PET. In Chapter 7 results are summarized and discussed, and placed into context of the existing literature.

![Figure 1.9 Study design of INcEREBRO, a randomized controlled cross-over study.](image-url)
CHAPTER 1. General introduction

REFERENCE LIST

7. Sandoval, D, Cota, D, Seeley, RJ. The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation. Annu Rev Physiol 2008;70:513-535
21. van, HM, Posner, BI, Kopriwa, BM, Brawer, JR. Insulin binding sites localized to nerve terminals in rat median eminence and arcuate nucleus. Science 1980;207:1081-1083
44. Banting, FG, Best, CH. The internal secretion of the pancreas. J Lab Clin Med 1922;7:251-266
47. Ferrannini, E. Physiology of glucose homeostasis and insulin therapy in type 1 and type 2 diabetes. Endocrinol Metab Clin North Am 2012;41:25-39
52. Mattila, TK, de, BA. Influence of intensive versus conventional glucose control on microvascular and macrovascular complications in type 1 and 2 diabetes mellitus. Drugs 2010;70:2229-2245
57. Morales, J. Defining the role of insulin detemir in Basal insulin therapy. Drugs 2007;67:2557-2584
CHAPTER 1. General introduction

60. Chapman,TM, Perry,CM. Insulin detemir: a review of its use in the management of type 1 and 2 diabetes mellitus. Drugs 2004;64:2577-2595
69. De,L, I, Vague,P, Selam,JL, Skeie,S, Lang,H, Draeger,E, Elte,JW. Insulin detemir used in basal-bolus therapy in people with type 1 diabetes is associated with a lower risk of nocturnal hypoglycaemia and less weight gain over 12 months in comparison to NPH insulin. Diabetes Obes Metab 2005;7:73-82

34


77. Hallschmid, M, Jauch-Chara, K, Korn, O, Molle, M, Rasch, B, Born, J, Schultes, B, Kern, W. Euglycemic infusion of insulin detemir compared with human insulin appears to increase direct current brain potential response and reduces food intake while inducing similar systemic effects. Diabetes 2010;59:1101-1107
