CHAPTER 7

General discussion & future perspectives
GENERAL DISCUSSION

The brain has a primary role in energy homeostasis. From animal studies it is known that insulin has a satiating effect on the brain and, therefore, is implicated in energy balance and body weight regulation (1-16). Studies relating insulin’s effects in the brain to body weight in humans are scarce (17-22). The studies presented in this thesis were performed to gain more insight into the effects of insulin, and more specifically different insulin formulations, on CNS regions involved in eating behavior in men. Type 1 diabetic patients were used as an insulin-deficient model to reliably study the actions of exogenously administered insulin. In addition, the use of well-controlled type 1 diabetes allowed for characterising diabetes related changes in brain perfusion and glucose metabolism and for a comparison with healthy controls.

Analysis of $[^{15}O]H_2O$ and $[^{18}F]FDG$ PET data in healthy volunteers (Chapter 2)

PET allows for the measurement of CBF (using $[^{15}O]H_2O$ as tracer) and CMR$_{glu}$ (using $[^{18}F]FDG$). Using full kinetic modeling, quantitative CBF and CMR$_{glu}$ values can be obtained in humans. After the initial development of the compartment model to measure individual rate constants (23), however, CMR$_{glu}$ measurements have increasingly been based on the assumption of fixed rate constants in combination with static scans (24). Therefore, no recent estimates of rate constants obtained using state-of-the-art high-resolution PET scanners are available for human studies. In addition, very few studies have measured regional CBF using $[^{15}O]H_2O$ and a state-of-the-art high-resolution PET scanner. Therefore, in Chapter 2 fully quantitative methods for measuring CBF and CMR$_{glu}$ were implemented, based on dynamic data acquired in healthy volunteers on a high resolution PET scanner. Values of CBF and CMR$_{glu}$ were obtained for several brain regions by non-linear regression (NLR) analysis of regional time activity curves. In addition, CBF and CMR$_{glu}$ data were obtained using parametric methods (BFM for CBF data and Patlak analysis for CMR$_{glu}$ data). Parametric results correlated well with those obtained by NLR analyses for both CBF and CMR$_{glu}$. Furthermore, use of an IDIF, as a non-invasive alternative for a BSIF, resulted in similar results for CMR$_{glu}$ when using the Patlak approach. This is a major advantage, as omission of radial artery cannulation increases clinical applicability of this methodology. Unfortunately, an IDIF did not produce accurate CBF values and therefore an arterial input function remains necessary in cerebral $[^{15}O]H_2O$ PET studies. A clear reason for this discrepancy between $[^{15}O]H_2O$ and $[^{18}F]FDG$ could not be identified. A possible explanation may be, apart from slight inaccuracies in scatter and decay correction, that for both $[^{15}O]H_2O$ and $[^{18}F]FDG$ studies an accurate input curve is needed for the whole duration of the scan, whereas for $[^{15}O]H_2O$ studies, the peak area is of greater importance. Therefore, optimalisation of duration of tracer injection and optimalisation of frame length, may lead to a better match between IDIF and BSIF in $[^{15}O]H_2O$ studies. This needs to be investigated in future studies. It should be noted that validity of an IDIF for
[\textsuperscript{\textbf{18}}\text{F}]FDG studies does not apply automatically to data acquired on another PET scanner. This still needs validation for every single tracer, every single scanner type, and each acquisition and data analysis protocol separately. The exact effects of the different options (e.g. iterative reconstruction algorithm, frame duration and duration of tracer injection) cannot be predicted and need to be investigated (25-29) in a way similar to that described in Chapter 2.

**CEREBRAL CHANGES IN TYPE 1 DIABETES (CHAPTERS 3 & 5)**

*Decreased cerebral blood flow and glucose metabolism in type 1 diabetes (Chapter 3)*

Decreased total grey matter CBF and CMR\textsubscript{\textit{glu}} was observed in patients with well-controlled type 1 diabetes under fasting, non-clamped conditions relative to healthy volunteers. Previously, only a single study reported the use of [\textsuperscript{\textbf{18}}\text{F}]FDG PET for comparing CMR\textsubscript{\textit{glu}} in type 1 diabetic patients (with neuropathy) with that in healthy subjects. In line with the present findings, decreased CMR\textsubscript{\textit{glu}} in patients without diabetes-related complications was found, but this decrease was not statistically significant. Groups, however, were small and a semi-quantitative approach for calculating CMR\textsubscript{\textit{glu}} was used (30). Using another tracer, i.e. D-[U-\textsuperscript{\textbf{13}}\text{C}]glucose and PET, others also found decreased CMR\textsubscript{\textit{glu}} in well-controlled type 1 diabetic patients compared with healthy controls (31). In contrast to the present data, Fanelli and co-workers found no differences in blood-to-brain glucose transport between poorly controlled type 1 diabetic patients and healthy volunteers, measured with [1\textsuperscript{-\textbf{11}}\text{C}]glucose and PET (32). The latter studies were performed under hyperinsulinemic clamp conditions during which insulin levels were artificially and acutely raised by an intravenous infusion of insulin, whilst glucose levels were clamped at mild to moderate hypoglycemic levels.

With respect to CBF, only one human PET study, by Fanelli et al., using [\textsuperscript{\textbf{15}}\text{O}]H\textsubscript{2}O PET has compared type 1 diabetic patients with healthy volunteers (32). In contrast to the findings in this thesis, no differences were observed between both groups, but again this study was performed under hyperinsulinemic clamp conditions. Preclinical (33) and clinical (34-36) studies without clamping found, in line with the present data, decreased perfusion (using SPECT) in type 1 diabetic patients compared with healthy controls.

Most trials comparing CBF and CMR\textsubscript{\textit{glu}} between diabetic patients and healthy controls have used intravenous insulin to normalize glycemia in order to evaluate the effects of diabetes per se, i.e. independent of the prevailing plasma glucose levels. In the “real world”, however, patients with type 1 diabetes are generally not euglycemic but are subject to hyperglycemia and (peripheral) hyperinsulinemia most of the day. Therefore, a decreased CMR\textsubscript{\textit{glu}} found in the present PET study at increased glucose levels better reflects the real-life situation of type 1 diabetic patients. Probably, in other studies, clamp-induced normoglycemia and hyperinsulinemia (average insulin levels of diabetic patients in the present studies were 88 pmol/L, whereas in the study by Fanelli et al. (32)
clamped insulin levels were 690 pmol/L) 'masked' the decreased CMR$_{\text{glu}}$ that is present under hyperglycemic conditions most of the day in these subjects. The finding in this thesis of a decreased CMR$_{\text{glu}}$ in type 1 diabetes does not exclude the possibility that alternative substrates may be used by the brain instead of glucose (31;37-41). Use of these substrates may cause total brain metabolism to be unaltered. In addition, clamp-induced normoglycemia and hyperinsulinemia could have 'masked' the decreased CBF that is present under hyperglycemic conditions present most of the day in type 1 diabetic patients. In peripheral tissues, insulin is known to have vasodilatory effects (42-44). The direct effects of insulin on CBF cannot easily be investigated in humans, but in rats topically applied insulin increased cortical blood flow (45), which suggests a direct effect of insulin on the brain vasculature. In addition, it was shown that insulin induced glucose lowering in type 1 diabetic patients resulted in an increase in CBF (46;47). It could be hypothesized that the decreased CBF observed in the present study during hyperglycemia, in the short term, has a protective role by decreasing the glucose load to the brain. Assuming that in daily life, these cerebral alterations persist throughout the day, they ultimately may lead to clinical consequences (e.g. cognitive changes). Clearly, this hypothesis needs to be tested in future studies.

**Increased brain activation in appetite related brain regions in type 1 diabetes (Chapter 5)**

Compared with healthy volunteers, type 1 diabetic patients showed increased activation in appetite related brain regions in response to visual food stimuli, as measured with fMRI. This result was even more pronounced when adjusted for blood glucose levels during MRI, which were, as expected, significantly higher in diabetic patients than in controls. To the best of our knowledge, this is a new finding. It should be noted that the diabetic patients in the present study were not obese (mean body mass index, BMI, of 25.2 versus 25.5 kg/m$^2$ in the group of healthy volunteers). It could be hypothesized that the relatively low portal insulin levels (resulting in increased glucose levels) in diabetic patients in the present study activated a mechanism that increased food craving, via a direct effect on the brain. Type 1 diabetic patients, unlike healthy subjects, need to inject an appropriate amount of insulin before each meal and, therefore, they need to estimate the carbohydrate content of all meals, every day. Most type 1 diabetic patients regularly visit a dietician and are more aware of carbohydrate and fat contents of everything they eat. It is not surprising that eating disorders are more common among men and women with type 1 (and type 2) diabetes (48-53), and manipulation with insulin is an effective way to lose weight at the expense of deteriorating diabetes control (higher A1C). However, in the present study no differences in eating behavior were found between diabetic patients and healthy volunteers, as assessed by the DEBQ, although it is possible that this questionnaire is not sensitive enough.
CHAPTER 7. General discussion & future perspectives

**Combined PET and fMRI results in type 1 diabetes (Chapter 3 & 5)**

Results of \[^{15}O\]H\(_2\)O PET, \[^{18}F\]FDG PET and fMRI combined, i.e. global reduced CBF and CMR\(_{glu}\) together with increased activation in appetite related brain regions, in type 1 diabetic patients compared with healthy volunteers may seem contra-intuitive, since an increase in neuronal activation usually is accompanied by an increase in blood flow (which increases blood oxygenation and therefore decreases deoxyhemoglobin concentration and increases the BOLD signal). The BOLD signal, however, is known to be fast and intense at low baseline CBF (54-56).

It should be noted that differences between groups in BOLD response remained significant after adjustment for global CBF, as measured with PET. In addition, PET measurements were performed during a resting baseline condition (eyes closed, warm, quiet environment), whereas fMRI was performed while actively viewing attractive food related pictures (eyes open, noisy scanner). Moreover, the generally decreased resting-state CBF and CMR\(_{glu}\) increased only locally in appetite related brain regions in response to visual food stimuli. Future studies using \[^{15}O\]H\(_2\)O PET should confirm the increased CBF in appetite regulating brain regions in response to food cues during a similar food related task.

**EFFECTs OF insulin detemir on THE HuMAN BRAIN (CHAPTER 4 & 5)**

**Current mechanisms for decreased body weight gain after insulin detemir treatment**

Treatment with insulin detemir has been shown to result in less weight gain or even weight loss compared to treatment with other basal insulin therapies (57-65). In the present thesis, studies were described in which the focus was on differences in action on the brain between insulin detemir and NPH insulin. However, other mechanisms leading to differences in body weight have been proposed. It has been hypothesized that insulin detemir causes less hypoglycemia and, therefore, less defensive snacking and less weight gain (59;61;66-70).

Indeed, compared with NPH insulin, insulin detemir treatment resulted in less (nocturnal) hypoglycemia (66;68;70-73), but this is unlikely to fully explain the weight loss, as comparable hypoglycemia frequencies after treatment with NPH insulin (67;74;75), and insulin glargine (76) did not result in a comparable weight loss. In addition, no significant relationship between hypoglycemia and weight gain was seen with insulin detemir, whereas this was the case for NPH insulin (77). In addition, weight gain could simply indicate success in avoiding hypoglycemia (60;77).

Another mechanism explaining the stability of body weight during insulin detemir treatment, is via a preferential effect of insulin detemir on hepatic metabolism. In comparison with other insulin preparations, insulin detemir has been shown to have a relatively larger effect on the liver than on peripheral muscle and adipose tissue. Because no significant barrier exists between blood plasma in the

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sinusoid and the hepatocyte plasma membrane, insulin detemir, both free and albumin-bound, is taken up continuously by hepatocytes. In contrast, binding to albumin retards the transfer of insulin detemir from the circulation into adipose tissue and skeletal muscle. Therefore, albumin binding of detemir increases the hepatic-peripheral insulin gradient, decreasing peripheral hyperinsulinemia and mimicking the physiological, non-diabetic state. Thus, in order for detemir to exert similar blood glucose control, its effect on the extraction by the liver may in relative terms be higher, resulting in reduced endogenous glucose production, whereas its peripheral action, including anti-lipolytic activity, is lower (60;78;79). A third mechanism could be that insulin detemir therapy results in increased energy expenditure. However, in a randomized cross-over study in which type 1 diabetic patients were treated for 16 weeks with insulin detemir and NPH insulin, respectively, no differences in total energy expenditure, resting energy expenditure, diet-induced thermogenesis or activity energy expenditure were observed between treatments (80). Therefore, differences in weight gain between insulin detemir and NPH insulin cannot be explained by differences in effects on energy expenditure. From the above-mentioned studies, no definite conclusion could be drawn and all suggested the brain for an alternative or additional target for insulin detemir to have its weight reducing effects.

**CBF and CMR\textsubscript{glu} in type 1 diabetic patients after treatment with insulin detemir (Chapter 4)**

Diabetic patients treated with NPH insulin showed decreased CBF and CMR\textsubscript{glu} compared with healthy volunteers (Chapter 3). In Chapter 4 it was investigated whether treatment with insulin detemir would result in an increase in CBF and/or CMR\textsubscript{glu}.

CBF responses to a meal specifically in appetite regulating regions in formerly obese subjects were similar to those in obese subjects, but differed from those in lean subjects (81) and an increased CBF in appetite regulating brain regions was observed in response to meal consumption in successful dieters compared to non-dieters (82). These studies suggest a role of the brain on body weight, but did not investigate the role of insulin on CBF. Partly based on these studies we hypothesized that insulin detemir would have a stronger effect on CBF in appetite regulating brain regions than NPH insulin. Indeed, compared with NPH insulin, treatment with insulin detemir increased CBF in appetite related brain regions, and global CBF was similar to that in healthy controls.

Previously, glucose entry into the brain was assumed to be completely insulin independent and no effect of increased insulin levels on cerebral glucose uptake was observed in rat and human studies (83-85). However, insulin-sensitive glucose transporters were demonstrated at the blood-brain barrier and on glial cells (86-90). In addition, in human studies using \[^{18}\text{F}]\text{FDG PET, it has been shown that the brain can respond to insulin with respect to its action on glucose uptake and metabolism (91;92). Moreover, effects of insulin on CMR}_{\text{glu}} (measured with \[^{18}\text{F}]\text{FDG PET) in men with peripheral insulin resistance were blunted compared with insulin sensitive men, specifically in regions implicated in appetite and**
reward (93), thereby suggesting a role of central insulin on energy homeostasis and body weight. Based on these studies we hypothesized that insulin detemir treatment would increase CMR$_{\text{glu}}$. In contrast to the initial hypothesis, CMR$_{\text{glu}}$ was not significantly increased and it remained significantly lower than in healthy volunteers. Although these findings may be due to a lack of power, more paired $[^{18}\text{F}]$FDG than $[^{15}\text{O}]$H$_2$O scans (24 versus 18) were available for analysis, and test-retest variability is better for CMR$_{\text{glu}}$ than for CBF (10% versus 20%, respectively; (83;94-96)). Apparently, insulin detemir had only an effect on CBF. The exact (clinical) consequences of these changes in the longer term are not known.

**Blunted activation in appetite related brain regions in response to visual food stimuli in type 1 diabetic patients after treatment with insulin detemir (Chapter 5)**

Results of fMRI analyses showed that the increased brain activation in response to the presentation of food related pictures, as seen in patients treated with NPH insulin, was less pronounced after treatment with insulin detemir and more resembled the pattern in healthy volunteers (Chapter 5). This effect could contribute to the observed weight loss after treatment with insulin detemir, but whether this blunted activation is cause or consequence of the observed weight loss could not be determined from this study. Two previous prospective studies (97;98) however, have demonstrated that increased brain responses to visual food cues can predict future weight gain. Furthermore, a direct effect of insulin detemir on the brain was observed in studies using systemic infusions in both mice (99) and humans (100;101), which resulted in acute changes in EEG and MEG as well as ensuing reductions in food intake. All these data combined suggest that an enhanced satiating effect of insulin detemir on the brain could play a causal role in body weight loss.

**Combined PET and fMRI findings after treatment with insulin detemir (Chapter 4 & 5)**

PET and fMRI findings together suggest that insulin detemir and NPH insulin have differential effects on the brain, particularly in response to stimulation with food related pictures compared with non-food items. These differences in brain activation measured using fMRI could be due, at least in part, to differences in resting-state CBF (54-56). However, after adjustment for the modest difference in total grey matter CBF (measured with PET) between treatments, differences in food related activation (measured with fMRI) were similar. It should be noted that an fMRI measured increase in brain activation was found in response to the presentation of food stimuli, whereas PET measurements were obtained in a non-stimulated baseline condition. Future studies using $[^{15}\text{O}]$H$_2$O PET should confirm CBF changes in response to visual food stimuli in these appetite-related brain regions following both treatments.
METHODOLOGICAL CONSIDERATIONS AND POTENTIAL LIMITATIONS

Study design and participants
The strength of the present study was, in addition to using state-of-the-art neuroimaging methods, the randomized cross-over design by which patients were their own control. Therefore less participants were needed than in a parallel design study. Another strong point is the inclusion of a run-in period of at least 4 weeks to reduce the effect of the initiation of study participation. In addition, only type 1 diabetic patients were included who had been diagnosed at least one year before randomization. Consequently, these patients were accustomed to diabetes self-management. 

Unfortunately, no baseline PET session was not performed (and therefore no baseline fMRI session). A baseline scan would have made it possible to calculate differences (in brain activation and CBF and CMR\textsubscript{glu}) between start and end of insulin treatment. However, additional PET scans were not possible, as this would lead to too high radiation dose.

PET and fMRI measurements were all performed in the same order (i.e. fMRI first), but for logistical reasons, not on the same day. MRI scans were performed between 8:00 and 9:00 h and PET scans between 9:30 and 11:00 h. Ideally, studies should have been performed on the same time or, even better, during a single session. However, at both days, patients used the same insulin regimen and were carefully instructed the evening preceding the scan. Furthermore, for both insulin types, average glucose and insulin levels did not differ between MRI and PET measurements. A final issue is that CBF and CMR\textsubscript{glu} were not obtained simultaneously, as this is not possible with PET. Both scans were acquired, on average, 25 min apart (to allow for decay of \textsuperscript{15}O), but were performed under stable resting conditions after an acclimatisation period of at least 20 minutes. Therefore changes in CBF and/or CMR\textsubscript{glu} during the 25 minutes between both measurements were highly unlikely.

NPH insulin was chosen as active comparator, since this insulin is the standard basal human insulin worldwide and, therefore, the best active comparator. In addition, NPH insulin has been used as comparator in most previous studies that reported decreased body weight after treatment with insulin detemir. Blinding was not possible, since NPH insulin is a cloudy suspension that needs to be thoroughly stirred before injection, whereas insulin detemir is a clear, colorless solution that does not require stirring. It is very unlikely that knowledge of the insulin type had an effect on brain activation during fMRI, CBF or CMR\textsubscript{glu} during PET.

It should be noted that glucose levels during data acquisition were relatively high in the majority of patients, in spite of careful instructions and telephone calls the night before. Rising glucose levels during the night and/or morning of the test, possibly due to psychological stress, could not be treated with (short-acting) insulin since this would have confounded results. In addition, the fasting
condition is not exactly a ‘normal-life’ situation, but this was by far the best option available (e.g. even a light breakfast would have necessitated an injection of prandial insulin, which would have affected results).

Weight gain associated with insulin treatment may be relevant for type 1 diabetic patients, but weight gain and obesity are especially important for patients with type 2 diabetes. This study was performed in type 1 diabetic patients since these patients are insulin-deficient, allowing for assessment of effects of exogenously administered insulin per se. In addition, human type 1 diabetes may be regarded as a ‘clean model’ of hyperglycemia, since these patients are not typically characterized by confounding factors such as obesity, hypercholesterolemia, hypertension, and the use of drugs potentially interfering with the measurements. Whether the present results can be extrapolated to type 2 diabetic subjects cannot be determined from our study. Further research in these patients is needed, especially since central insulin resistance may play a role in type 2 diabetes.

The present findings in male subjects may not be readily extrapolated to women. The choice of including only men was deliberate. Firstly, this resulted in a relatively homogenous group. Secondly, cerebral flow and glucose metabolism in women are different (102-104) and menstrual-cycle dependent (105), which would make cycle matching necessary. Finally, pooling of data probably would not have been valid. Consequently, gender specific analyses would have been required, resulting in the inclusion of twice as many subjects.

Other factors influencing body weight

Although no between-treatment differences in eating behavior, appetite or food preferences were observed, as measured with questionnaires, the possibility of differences in caloric intake cannot be excluded, as patients did not keep food diaries. Furthermore, frequency and intensity of physical activity were not measured and changes during the study could have affected the data. However, as self-reported diaries are a potential source of inaccuracy (106) and are time consuming for both patient and researcher, these were not included in the study protocol. In theory, seasonal influences could have an effect on eating behavior and body weight (107), but data were collected for three consecutive years and all patients were included in the study for 24 weeks, which makes a seasonal effect not likely. In addition, no differences in mood, which could affect food preferences and body weight (108) were observed between treatments.

fMRI for measuring brain activation

fMRI was used to measure differences in brain activation between groups during a food-related encoding task (Chapter 5). To prevent the effect of habituation, two different sets of food pictures were designed and session order was perfectly randomized between treatments. It is therefore very unlikely that habituation has affected results.

fMRI is based on the BOLD effect, which depends on changes in local deoxyhemoglobin concentrations in the brain as a result of neuronal activity,
leading to alterations in MRI signal intensity (55;109). Since diabetes is
characterized by microvascular changes and decreased total grey matter
CBF (Chapter 3) this could have affected fMRI results (54-56). Nevertheless,
adjustment for CBF did not change fMRI data.
Since a block design was used, consisting of blocks with a minimum duration
of 16.5 to 19.5 seconds, it is very unlikely that a possible difference between
diabetic and healthy subjects in hemodynamic response function used to model
responses to visual stimuli in the fMRI study, has affected the data.

**PET for measuring cerebral blood flow and glucose metabolism**

Initially, Sokoloff et al (23) introduced the method to measure the rate of
glucose consumption in rat brain *in vivo* using 2-deoxy-D-[14C]glucose and
autoradiography. This method was implemented for human [18F]FDG PET studies
by Reivich and colleagues (110), and adapted for the use of dynamic [18F]FDG
PET data by Huang et al. (111). Using this model, CMR<sub>glu</sub> could be derived from
\[ K_1 \cdot \text{glucose}/LC, \]
where \( K_1 \) is the net influx rate and LC is the lumped constant.
This method was broadly implemented by assuming fixed rate constants in
combination with static acquisition protocols. These fixed rate constants may
however not be valid in certain clinically relevant patient populations, such as
those having diabetes; therefore the full kinetic model was used in the studies
in diabetic patients presented in this thesis (Chapter 3 & 4). Brooks et al. (112)
and Hasselbalch et al. (113), also used rate constants derived from dynamic
[18F]FDG scans (in type 1 diabetic patients and in healthy subjects, respectively)
during normoglycemia and clamp-imposed hyperglycemia (13 mmol/L and 15.7
mmol/L, in the respective studies). In line with the present data (Chapter 3),
they found a decreased \( K_1 \) in hyperglycemia according to the Michaelis-Menten
equation, which describes competition between glucose and FDG and is valid
in both normoglycemia and hyperglycemia, i.e. for plasma glucose values that
are well within the range encountered in the present patients (plasma glucose
5.0-16.4 mmol/L). It should be noted that hypoglycemic conditions, i.e. plasma
glucose levels < 3.8 mmol/L, were not encountered. Such conditions would
have imposed a different problem, as the transport step would become the rate
limiting step due to limited glucose supply and the LC would increase (114). \( k_3 \)
values in the present diabetic patients were probably reduced compared with
healthy subjects due to a primary effect (reduced hexokinase activity) in diabetes.
In contrast, \( k_2 \) was not affected by plasma glucose levels (Chapter 3).

There is limited yet consistent literature suggesting a mild decrease in LC at
higher glucose levels (23;115;116). To account for the increased glucose levels in
type 1 diabetic patients compared with healthy controls, two LC scenarios were
applied (Chapter 3): a fixed LC and an LC decreasing with increasing plasma
glucose levels (116). Using either LC scenario, CMR<sub>glu</sub> was significantly lower in
patients with type 1 diabetes at their fasting glucose and insulin levels. It should
be noted that the equation adopted in the second LC scenario was derived from
data obtained in hyperglycemic rats and not in humans. Furthermore, this LC
was based on measurements using [14C]DG and not [18F]FDG. Nevertheless, as the
LC takes into account differences between FDG and glucose, and since absolute values between LC of \([^{18}F]FDG\) and \([^{14}C]DG\) do not significantly differ and behave similarly in humans and animals (117), it does not change interpretation of the data. In the calculation of CMR\(_{glu}\) after both insulin treatments (Chapter 4), a fixed LC was used, as no significant difference in glucose level was found during PET data acquisition.

A further issue in analysing the PET data is the use of predefined regions in which possible partial volume effects (PVE) were not accounted for. As PVE correction is based on grey matter (GM) and white matter (WM) segmentation, however, even a small mismatch in co-registration could have large impact on the absolute values derived from the small regions of interest and PVE corrections would therefore only add uncertainties. In addition, only relative values were needed (i.e. diabetic compared with healthy subjects and insulin detemir treated versus NPH insulin treated subjects) and GM/WM ratios were not expected to differ between groups. To assess effects of PVE, in Chapter 2 additional, manually drawn, GM and WM regions were used to minimise PVE. Using these regions, GM/WM ratios increased from 1.5 to 3.0 for \([^{18}F]FDG\) and from 1.4 to 3.3 for \([^{15}O]H_2O\) data, which is in line with the generally accepted 3 to 1 ratio (118).

In Chapter 6, GM/WM ratios were low (average 1.4 for all subjects and both methods investigated) and no partial volume correction was applied to these data either. Nevertheless, potential errors should be similar for both methods, as identical regions were used (PVElab) and spatial filtering was applied to guarantee equal spatial resolution for both methods.

**ASL to measure cerebral blood flow**

Although \([^{15}O]H_2O\) PET is the gold standard for measuring CBF in humans, this method also has some disadvantages. In addition to a (low) level of radiation exposure, scanning protocols require dedicated facilities, including a cyclotron to produce \(^{15}O\), that are located in close proximity to the scanner given the half-life of only two minutes. Furthermore, the procedure necessitates insertion of an arterial line to obtain the arterial input function. In Chapter 2 it was shown that use of an IDIF, although possible for \([^{18}F]FDG\), was not successful for \([^{15}O]\) \(H_2O\). Consequently, an alternative non-invasive method to measure CBF would be welcome.

As described in Chapter 6, quantification of CBF using ASL MRI resulted in comparable average CBF values to those obtained with \([^{15}O]H_2O\) PET. Unfortunately, also significant differences between methods were observed, most prominently in anterior and posterior cingulate cortex/corpus callosum. The reasons for these regional differences are still unclear. It should be noted that CBF measurements using ASL are based on several assumptions, such as fixed arterial transit time, fixed T1 value of blood and tissue and a fixed partition coefficient of water, which do not have to be valid under all conditions. For example, it is known that the partition coefficient of water differs between grey and white matter and that its value is lower than that based on water content measurements (119;120). However, fixing this value to 0.90 in PET analyses as
well, did not improve agreement between methods.
A test-retest design was not performed and no intervention (e.g. hypercapnia (121)) was included, which limited the range of CBF values.
A possible explanation for global differences between methods could be that PET and MRI were not performed on the same day, but on average 21 days apart. Global differences between methods could also be caused by the time of scanning, since all MRI scans were acquired between 8 and 9 AM and all PET scans somewhat later, between 9:30 and 10 AM. Although diurnal variations in CBF have been shown previously (122-124), it is unlikely that this small difference in time of scanning is responsible for the observed CBF differences measured during stable supine conditions (125). In addition, the PET scan could have caused more stress due to arterial sampling, whereas the ASL MRI was acquired in between a memory task and accompanied by noise. Unfortunately, stress signals, e.g. cortisol levels or pulse rate during, were not measured.
In addition to the differences between methods observed, using the same (fixed) parameter values in ASL MRI for diabetic and healthy subjects may have resulted in some bias, although differences between methods appeared to be the same for both subject groups.

**CSF insulin**
From animal studies it is known that insulin is transported into the brain via an insulin receptor mediated, saturable pathway in brain capillary endothelial cells (126). Increases in plasma insulin levels result in increases in CSF insulin levels (127). Insulin measured in CSF has likely passed through the brain and will be removed by insulin receptors within the choroid plexus (128). In the brain, relatively high levels of insulin receptors are found in the olfactory bulbs and the arcuate nucleus of the hypothalamus (129-132), regions important in energy homeostasis and body weight regulation. Animal studies have shown that disrupted intracerebral insulin signaling causes weight gain and that intracerebroventricular insulin administration reduces food intake and results in weight loss (1;2;9;133).

Previous studies in mice, investigating whether or not insulin detemir crosses the blood-brain barrier have used acute infusions and showed conflicting results (99;134). In **Chapter 5** results of CSF insulin samples were presented, obtained in a subgroup of patients that consented to undergo a lumbar puncture (LP). Increased CSF insulin levels were found in patients treated with insulin detemir, but serum insulin levels were higher in that group as well. Nevertheless, the CSF to serum insulin ratio was higher after treatment with insulin detemir (0.12 for insulin detemir versus 0.075 for NPH insulin), indicating that insulin detemir enters the brain more readily than NPH, or that its clearance from the CSF is slower. Observed ratios were in line with previous data from non-diabetic humans (135), demonstrating that on average 10% of circulating serum insulin was present in CSF. CSF is produced at a rate of 0.35 mL/min in healthy individuals (136) which means that the total amount (~150 mL) is formed and re-absorbed several times a day and, therefore, any conclusion concerning the present CSF
data could only be made about the non-steady-state at the time of LP. In addition, two different insulin assays were used to measure insulin in serum and CSF, but unfortunately no assay is available that can measure the total range of insulin levels as reported in the present study.

It has been suggested that insulin is produced locally in the brain (127;137-140); if that were true, our results would be interpreted differently. However, cerebral insulin nowadays is generally accepted to be of pancreatic origin (3;141;142) and to be transported across the blood brain barrier by a saturable transport system (126;141;143-147). Therefore CSF insulin levels are likely to be higher after treatment with insulin detemir.

It should be noted that even a small volume of blood in CSF (e.g. due to a traumatic puncture), could have biased results, but this is not likely to have occurred since all samples used were completely clear at visual inspection. Furthermore, such increased CSF insulin levels should have resulted in outliers and these were not encountered. All first of three tubes were sent to the chemistry lab and some did have increased red blood cells, however, only the last tubes were used to measure insulin. Therefore it is very unlikely that blood mixing would have occurred.

**FUTURE PERSPECTIVES**

The studies presented in this thesis provide new insights into the effects of insulin and the differential effects of two basal insulin formulations on CBF and CMR$_{\text{glu}}$ in the human brain, within the context of systemic glucose and body weight regulation. Firstly, these results may contribute to the understanding of insulin’s central regulation of energy homeostasis and body weight. Secondly, they may lead to the development of new insulin analogs with an even stronger CNS effect. Thirdly, neuroimaging methodology used here may serve as reference for testing these central effects in future studies.

Based on the first aspects, it could be envisioned that future studies should involve measurements of brain activation (including fMRI and/or PET studies) in the resting state and upon presentation of food related stimuli during acute insulin infusion in various populations, e.g. obese, lean, insulin resistant and type 2 diabetic humans of both genders (body weight regulation has been shown to act via insulin in males, whereas leptin plays a more important role in females (17;148)). These experiments should be performed in both fasting and postprandial states to discriminate between effects of pharmacological (fasting) and physiological (postprandial) hyperinsulinemia and between eating behavior regulation in fasted versus satiated conditions (149-151). In addition, differentiation between acute effects of insulin on the brain and secondary effects due to changes in body weight could be made.

Further research may:

1) Explore potential mechanisms relating signaling of peripherally administered insulin to the brain in humans by investigating the role of the autonomic nervous system.
2) Investigate the role of vascular function changes in insulin stimulated CBF
responses.

3) Include other populations (e.g. type 2 diabetes, obese individuals, women etc.) in order to allow generalization of the present findings.

4) Use similar brain imaging methods to assess CNS effects of novel insulin formulations or other regulators of feeding behavior and energy homeostasis, such as gut hormones.

5) Further improve and implement current PET and MRI imaging methods such as IDIF for $^{18}$F-FDG PET and $^{15}$O-H$_2$O at more widely available PET scanners, as well as simultaneous PET and MRI measurements at combined PET/MRI scanners and ASL MRI.

6) Expand current state-of-the-art imaging tools by using other tracers that allow visualization of neurotransmitter pathways implicated in eating behavior and reward, such as serotonin and dopamine systems (152-156).

**CONCLUDING REMARKS**

In conclusion, the reduction in body weight observed after 12 weeks of treatment with insulin detemir versus NPH insulin in T1DM was paralleled by a change in PET measured CBF and fMRI measured activation patterns in appetite related brain regions in response to visual food stimuli. These findings support the hypothesis that insulin detemir may induce its weight sparing effect via an enhanced effect on the brain. In addition, they provide insight into the effects of insulin on the central regulation of energy homeostasis and body weight. In order to allow generalization of these findings, future studies, using the same state-of-the-art technology, should include other populations (e.g. women, patients with obesity and/or type 2 diabetes) and relate CNS alterations to clinically relevant outcome measures.

The PET and MRI studies presented in this thesis provide evidence for cerebral differences in type 1 diabetic men compared with healthy age and BMI matched controls. CBF and CMR$_{glu}$ were decreased in patients with well-controlled type 1 diabetes when scanned at real life (fasting) glucose and insulin levels. At present, it is not known whether these differences in cerebral blood flow and metabolism, which may persist throughout the day, are linked to cognitive functional alterations that have consistently been found in type 1 diabetic patients (157-162). Consequently, in order to appreciate the clinical relevance of these findings, future large-scale prospective studies in well-characterized type 1 diabetic patient cohorts are warranted.
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