Hypoglycaemia in diabetes
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Citation for published version (APA):
CHAPTER 10

18F-FLUORODEOXYGLUCOSE UPTAKE IN BROWN ADIPOSE TISSUE DURING INSULIN-INDUCED HYPOGLYCAEMIA IN NON-DIABETIC ADULTS

Submitted
ABSTRACT

Objective
Hypoglycaemia is associated with hypothermia despite increased heat production. The latter is likely to be mediated by sympathetic innervation. Brown adipose tissue is activated by cold exposure and stimulated by norepinephrine released from the sympathetic nervous system. We therefore examined the effect of hypoglycaemia on uptake of the labelled glucose analogue $^{18}$F-fluorodeoxyglucose in brown adipose tissue using positron emission tomography and computer tomography.

Methods
In nine healthy adults $^{18}$F-fluorodeoxyglucose uptake as measure of brown adipose tissue activity was assessed in a cold environment (17°C) during euglycaemia (blood glucose 4.5 mmol/L) and hypoglycaemia (2.5 mmol/L) using a hyperinsulinemic glucose clamp.

Results
Brown adipose tissue activity was observed in all participants. No difference was observed in the median (range) maximal standardized uptake values of $^{18}$F-fluorodeoxyglucose in brown adipose tissue between euglycaemia and hypoglycaemia: 4.2 (1.0-7.7) vs. 3.1 (2.2-12.5) g/mL ($p=0.7$).

Conclusions
This study shows that there is a similar amount of $^{18}$F-fluorodeoxyglucose uptake in brown adipose tissue during hypoglycaemia when compared to euglycaemia. It is therefore not likely that brown adipose tissue activity is a major determinant of the increased heat production that occurs during hypoglycaemia.
INTRODUCTION

Hypothermia has been associated with acute hypoglycaemia since the earliest use of insulin to treat diabetes. Many diabetic patients presenting as an emergency with insulin-induced hypoglycaemia have concomitant hypothermia. In the early 1980s Gale and colleagues showed that during cold induced shivering and concomitant hypoglycaemia, core temperature fell by 0.3-1.2°C, even though energy expenditure (i.e. heat production) increased. With worsening hypoglycaemia, fewer subjects were observed shivering and shivering was totally inhibited at a blood glucose level of 2.5 mmol/L, but this did not fully abolish hypoglycaemia-induced heat production. This unexplained persistence in thermogenesis is likely to be mediated by sympathetic activation, because it could be abolished by pre-treatment with propranolol.

Metabolic imaging with fluorodeoxyglucose (18F-FDG) using positron emission tomography (PET) and computed tomography (CT) has shown that active brown adipose tissue (BAT) is present in adult humans in quantities that may contribute to non-shivering thermogenesis. BAT is activated by cold exposure and stimulated by norepinephrine released from the sympathetic nervous system, while propranolol reduces BAT activity. BAT is therefore a potential candidate to contribute to thermogenesis during hypoglycaemia. We therefore examined the effect of hypoglycaemia on uptake of 18F-FDG in BAT using PET-CT.

MATERIALS AND METHODS

Subjects

Healthy male volunteers were recruited through public advertisement. Inclusion criteria consisted of being of Caucasian origin, aged between 18–50 years and having a BMI between 20–28 kg/m². Exclusion criteria were the use of β-blockers, smoking, significant renal impairment and family history of type 2 diabetes for first and second degree relatives. The institutional ethics committee approved the study. All participants gave their written informed consent.

Study protocol

Each participant was studied on two separate occasions in the morning after an overnight fast, separated by at least 1 week, using a modified hyperinsulinemic glucose clamp technique. The subject, dressed in briefs only, rested in a supine position in an air-conditioned chamber (17°C) for two hours. Body temperature was measured at 10-minute intervals with a tympanic thermometer (Cresta TH838, Radiant Innovation Inc., Hsinchu, Taiwan). At both visits a cannula was inserted into the antecubital vein of the
left arm. This cannula was used to infuse human soluble insulin (Actrapid; Novo Nordisk) and dextrose. A second cannula was inserted in a retrograde direction into a vein on the right hand, which was placed into a heated-hand box to arterialize the venous blood for blood sampling. Infusion of insulin was started at 1.5 mU/kg/min and 20% dextrose was infused at a variable rate to achieve the desired blood glucose concentrations. During each study session, arterialized blood glucose concentration was stabilized at 4.5 mmol/L for a period of 30 min, after which it was lowered to 2.5 mmol/L over 20 min. One hour after the start of each glucose clamp study, when the subject during the hypoglycaemia condition had been hypoglycaemic for at least 15 min, 200 MBq of \(^{18}\)F-FDG was administered intravenously and the experimental condition was maintained for a second hour. Blood glucose was always restored to 4.5 mmol/L at the end of the clamp. An upper-body (from the base of the skull to the inguinal area) static PET acquisition was performed at each occasion. PET-CT images were acquired with a Gemini
time of flight multidetector helical PET-CT scanner (Philips, Medical Systems, Eindhoven, The Netherlands). In areas where uptake of $^{18}$F-FDG was identified by PET and the presence of fat was identified by CT (Hounsfield units between -250 and -50) \(^{11}\), the maximal standardized uptake values (SUV\(_{\text{max}}\)), defined as the activity in Bq/ml within the region of interest divided by the injected dose in Bq/g (body weight), were determined (Hybrid Viewer, Hermes Medical Solutions, Stockholm, Sweden). Anatomical regions of interest were the cervical, supraclavicular, and superior mediastinal fat depots. In the regions of interest a SUV\(_{\text{max}}\) of $^{18}$F-FDG of ≥2.0 g/mL was considered to indicate the presence of active BAT \(^{13}\). Participants received a meal after scanning had been completed.

**Biochemical assays**

Blood glucose level was measured at bedside using the HemoCueβ-glucose system (HemoCue AB, Angelholm, Sweden). Plasma samples for hormones were obtained at baseline, 50, 70, 90 and at 110 min. Catecholamines were determined with an in-house high-performance liquid chromatography method \(^{14}\), glucagon with the LINCO \(^{125}\)I radioimmunoassay (LINCO Research, Inc., St. Charles, MO), cortisol with a chemiluminescent immunoassay (Immulyte 2000; Diagnostic Products Corp., Los Angeles, CA), growth hormone by solid phase two-site fluoroimmunometric assay (IFMA) (Perkin ElmerWaltham, MA USA; detection limit 0.1 mU L\(^{-1}\)) and insulin concentration was determined with a chemiluminescent immunometric assay (Immulyte 2000).

**Statistical analysis**

The data were not normally distributed and therefore differences between groups were analyzed using the Wilcoxon Signed Rank Test. The Friedman Test was used for analysis of variance. To assess linear relationship between two variables a Spearman correlation coefficient was calculated. A \(p\)-value <0.05 was considered significant.

**RESULTS**

Nine healthy males were recruited (median (range) age 22 (19-30) years, body-mass index 22 (20-23) kg/m\(^2\)). Their mean (SD) arterialized blood glucose was 4.5 (0.3) mmol/L during euglycaemia and 2.7 (0.3) mmol/L during hypoglycaemia and steady-state plasma free insulin levels were 398 (79) and 370 (78) pmol/L (\(p=0.40\)), respectively. Plasma epinephrine levels were significantly lower during euglycaemia: areas under the curve (AUC) from 50 to 110 min median (range) 11.7 nmol/L(5.5-40.7) compared with hypoglycaemia 95.7 nmol/L(12.2-218.5) (\(p=0.01\)). Plasma norepinephrine levels showed no difference in AUC 50-110 min for the two conditions: 111.1 nmol/L (77.6-199.1) during euglycaemia compared with 139.0 nmol/L (97.1-198.8) during hypoglycaemia (\(p=0.12\)). AUCs for glucagon, cortisol and growth hormone
**Figure 2** - The glucose counterregulatory hormones during the euglycaemic and hypoglycaemic conditions.
were significantly higher during hypoglycaemia (Figure 2). There was no change in body temperature from baseline across ten time points during euglycaemia (from baseline to 100 min, every 10 min, \( p=0.9 \)). However, during hypoglycaemia temperature changed from baseline, \( p<0.01 \). A greater fall in temperature was observed during hypoglycaemia when compared to euglycaemia 80 min from baseline (after 40 min of hypoglycaemia): -0.1 (-0.5-0.8) °C during euglycaemia vs. -0.3 (-1.1-0.1) °C (\( p=0.02 \)) during hypoglycaemia and this difference continued at 90, 100 and 110 min (Figure 3). Subjects were not shivering during either clamp condition.

BAT activity was observed in all subjects. No difference was observed in the SUV\(_{\text{max}}\) of \(^{18}\text{F}-\text{FDG}\) in BAT between euglycaemia and hypoglycaemia: 4.2 (1.0-7.7) vs. 3.1 (2.2-12.5) g/mL (\( p=0.73 \)). Further, no difference was found for the SUV\(_{\text{max}}\) in skeletal muscle between the conditions: 4.1 (2.2-6.5) vs. 3.4 (1.7-5.5) g/mL (\( p=0.26 \)) (Figure 4). No correlations existed between the SUV\(_{\text{max}}\) of \(^{18}\text{F}-\text{FDG}\) in BAT and levels of counterregulatory hormones.

DISCUSSION

The present study has demonstrated that insulin-induced hypoglycaemia in healthy adults does not change the uptake of \(^{18}\text{F}-\text{FDG}\) in brown adipose tissue during exposure to mild cold while body temperature was observed to fall. Since \( \beta \)-adrenoreceptor blockade
Figure 4 - Impact of hypoglycaemia on $^{18}$F- fluorodeoxyglucose (FDG) uptake in brown adipose tissue (BAT) and muscle.
Maximum-intensity projections show no difference in $^{18}$F-FDG uptake in BAT between euglycaemia (A) and hypoglycaemia (H) conditions. It is noted that there is profound uptake of $^{18}$F-FDG in muscle due to the hyperinsulinaemic conditions. Transversal PET images show similar uptake of $^{18}$F-FDG in BAT and muscle during euglycaemia (B) and hypoglycaemia (E). Uptake is superimposed on adipose tissue in correlated CT images (C, D and F, G respectively). Subheading I and J: changes in the uptake of $^{18}$F-fluorodeoxyglucose recorded in individual subjects. $\text{SUV}_{\text{max}}$ = maximal standardized uptake value.
attenuates the uptake of $^{18}$F-FDG into BAT, it seems likely that sympathetic stimulation of β-adrenoreceptors during hypoglycaemia will activate BAT. However, the β-adrenergic stimulation that was induced by acute hypoglycaemia in the current study did not enhance $^{18}$F-FDG uptake in BAT. The cause of the sympathetic mediated persistence in increased thermogenesis during hypoglycaemia remains unclear.

This study has its limitations. First, it was assumed that injection of equivalent amounts of $^{18}$F-FDG would result in similar activity of $^{18}$F-FDG in plasma during hypoglycaemia when compared to euglycaemia. Blood glucose was approximately 40% lower at hypoglycaemia, theoretically leading to a 1.7 times higher initial plasma $^{18}$F-FDG specific activity and this could result in relatively more uptake of $^{18}$F-FDG in BAT, while its real metabolic activity was lower. This, however, would strengthen rather than weaken our conclusion that BAT activity does not increase during hypoglycaemia. Second, it is known that fatty acids are the main substrate for BAT, while uptake of $^{18}$F-FDG, a glucose analogue, is used as a measure of BAT activity. It is possible that BAT in a hypoglycaemic condition uses relatively more fatty acids than glucose. Hence, BAT activity might have increased, while this was not visualized. Third, we did not randomize the order of the euglycaemic and hypoglycaemic condition. Although randomization would have been decent, the experiments were separated by at least 1 week and it is unlikely that it has influenced our results. Last, in a study with rats ambient temperature seemed of importance. The modification of the regional blood flow to brown adipose tissue was investigated after exposure to cold (22°C) and thermoneutrality (28-32°C). When the rats were studied during euglycaemic conditions an increased blood flow was noticed to brown adipose tissue after exposure to cold when this was compared to the same rats after exposure to a period of thermoneutrality. However, during the hypoglycaemic condition the rats had a profound decrease in BAT blood flow after cold acclimatization and an increase in blood flow in BAT during hypoglycaemia after acclimatization in thermoneutrality. The authors could not find the same effect of ambient temperature during hypoglycaemia in dogs and suggest that small mammals might have a different strategy for survival during periods of energy shortage. This could explain the decrease in heat production during hypoglycaemia in rats that has been observed by Green and MacDonald, which is the opposite of the increase in heat production during hypoglycaemia that was found in man. We did not perform temperature control experiments to determine the separate contributions of hypoglycaemia and cold. Exposure to cold seems to be an important determinant to visualize BAT in humans and it was doubtful whether we would visualize any BAT during thermoneutral conditions; given the added radiation exposure a control experiment in thermoneutral conditions seemed unethical.
During hypoglycaemia muscle shivering is reduced and with the consequent fall in temperature, metabolism is reduced to protect the brain from neuroglycopenia. Humans are homothermous individuals and temperature is regulated within narrow borders. BAT activity during hypoglycaemia might be necessary to limit the fall in body temperature to protect the individual.

This study shows that there is no increase in $^{18}$F-FDG uptake in BAT during hypoglycaemia. It is therefore not likely that BAT activity is a major determinant of the persistence in heat production that occurs during hypoglycaemia.

REFERENCES


13. Cypess A.M., Lehman S., Williams G. et al. Identification and importance of brown


