

## Glucagon-like-peptide-1 inhibits blood-brain glucose transfer in humans

Susanne Lerche MD<sup>1</sup>, Birgitte Brock MD, PhD<sup>1</sup>, Jørgen Rungby MD, DMSc<sup>1</sup>,  
Hans E. Bøtker MD, DmSc<sup>6</sup>, Niels Møller MD, DmSc<sup>2</sup>, Anders Rodell M.Sc., PhD<sup>3</sup>,  
Bo Martin Bibby M.Sc. PhD<sup>5</sup>, Jens J. Holst MD, PhD<sup>4</sup>,  
Ole Schmitz MD, DMSc<sup>1</sup>, Albert Gjedde MD, DMSc<sup>3</sup>

<sup>1</sup>Institute of Pharmacology, University of Aarhus,  
University Park 1240, 8000 Aarhus C

<sup>2</sup>Department of Endocrinology M, Aarhus University Hospital,  
Noerrebrogade 44, 8000 Aarhus C

<sup>3</sup>PET-Center, Aarhus University Hospital  
Noerrebrogade 44, 8000 Aarhus C

<sup>4</sup>Department of Medical Physiology, University of Copenhagen  
Panum Institute, Blegdamsvej 3B, 2200 Copenhagen

<sup>5</sup>Department of Biostatistics, University of Aarhus  
Vennelyst Park 6, 8000 Aarhus C

<sup>6</sup>Department of Cardiology, Aarhus University Hospital, Skejby  
Brendstrupgaardsvej, 8200 Aarhus N

**Running title:** GLP-1 and human blood-brain glucose transfer

### Corresponding author

Susanne Lerche, MD  
Institute of Pharmacology  
University of Aarhus  
The Bartholin Building  
University Park 1240  
8000 Aarhus C, Denmark  
lerche@ki.au.dk

Received for publication 16 August 2007 and accepted in revised form 3 November 2007.

## ABSTRACT

*Objective:* Glucagon-like-peptide-1 (GLP-1) has many effects on glucose homeostasis and GLP-1 receptors are broadly represented in many tissues including the brain. Recent research in rodents suggests a protective effect of GLP-1 on brain tissue. The mechanism is unknown.

*Hypothesis, Research design and Methods:* To test whether these neuroprotective effects could relate to changes of glucose transport and consumption we studied 10 healthy men in a randomized, double-blinded placebo-controlled cross-over experimental design. We used Positron Emission Tomography to determine the acute insulin independent effect of GLP-1 on unidirectional glucose transport into the brain during a pituitary-pancreatic normoglycaemic (plasma glucose  $\approx$  4.5mM) clamp with 18-fluoro-deoxy-glucose (FDG) as tracer.

*Results:* On average, GLP-1 reduced cerebral glucose transport by 27% in total cerebral grey matter ( $P=0.05$ ) and by 25-30% in individual grey matter regions ( $P=0.02-0.06$ ). The same regions revealed a uniform trend towards similarly reduced cerebral glucose metabolism. Consequently, the intracerebral glucose concentration remained constant in all regions, with and without GLP-1.

*Conclusions:* Therefore we have demonstrated that a hormone involved in postprandial glucose regulation also limits glucose delivery to brain tissue and hence provides a possible regulatory mechanism for the link between plasma glucose and brain glucose. Because GLP-1 reduces glucose uptake across the intact blood brain barrier at normal glycaemia, GLP-1 may also protect the brain by limiting intracerebral glucose fluctuation when the plasma glucose is increased.

**ABBREVIATIONS.** **GLP-1**(glucagon-like-peptide-1), **FDG**(18-fluoro-deoxy-glucose), **PET**(positron emission tomography), **BBB**(blood-brain-barrier), **GLP-1R**(GLP-1 receptor), **LC**(lumped constant),  **$J_{\text{glucose}}$** (unidirectional glucose transfer),  **$\text{CMR}_{\text{glucose}}$** (cerebral metabolic rate for glucose),  **$C_t$** (brain tissue glucose concentration),  **$K_{1\text{glc}}$**  (unidirectional clearance for glucose),  **$K_1^*$** ( unidirectional clearance for FDG),  **$k_2^*$** ( efflux coefficient for FDG) ,  **$k_3^*$** ( phosphorylation rate constant for FDG),  **$K_{\text{glc}}$** (net clearance of glucose),  **$K^*$** (net clearance of FDG), **GIR**(glucose infusion rate).

**CLINICALTRIALS.GOV IDENTIFIER.** NCT00256256

**G**lucagon-like-peptide-1 (GLP-1) is an incretin hormone with several beneficial effects on glucose homeostasis. GLP-1 is not solely produced by the entero-endocrine L-cells in the gut, but also in the brain by neurons in the nucleus of the tractus solitarius and thus functions as a neuropeptide (1). It stimulates insulin secretion and inhibits glucagon secretion in the presence of glucose, inhibits gastric emptying and reduces appetite and food intake(2,3). Furthermore, it acts as a  $\beta$  cell growth factor by stimulating  $\beta$  cell proliferation and islet neogenesis and by reducing  $\beta$  cell apoptosis (4). The glucose dependency of GLP-1 action on insulin and glucagon secretion results in a low risk of hypoglycaemic episodes (5). These effects makes GLP-1 attractive as an anti diabetic medication, and several studies prove the beneficial effect on glucose homeostasis in the type 2 diabetic patient (6,7).

Although GLP-1 receptors (GLP-1R) are predominantly located on the  $\beta$ ,  $\delta$ , and presumably  $\alpha$  cells of the pancreas, receptors in other tissues may mediate GLP-1's effects outside the pancreas (8,9), where gastric emptying and appetite are well-known targets as are other tissues including heart, kidney and lungs (8). GLP-1R expression has been demonstrated in human brain (10), where receptors are particularly abundant in the paraventricular nucleus and the arcuate nucleus in the hypothalamus (11). Infusion of GLP-1 dose-dependently enhances satiety and reduces food intake in normal and obese subjects including patients with type 2 diabetes and neural mechanisms appear to be involved (3).

The abundance of receptors in other locations in the brain, i.e., the thalamus, brainstem, lateral septum, subfornical organ, area postrema, the cerebral cortex, cerebellum and caudate-putamen, as well as in the hippocampus suggests that GLP-

1 may have additional effects in the central nervous system as a whole (10,12). This is also evident from GLP-1R-knock-out mice who have seriously disturbed learning abilities (13).

In rodents recent studies provide evidence of both neuroprotective and neurotrophic effects of GLP-1 (13,14), including improvement of learning and memory (13). The mechanism of these putative protective effects of GLP-1 in the central nervous system is unknown. Applied directly to the rodent brain, GLP-1 affects the peripheral glucose metabolism (15), implying that interaction exists between GLP-1, brain and glucose homeostasis.

In the brain, intracellular and interstitial glucose concentrations are maintained by the stereospecific and non-energy-demanding mechanism of facilitated diffusion, mediated by the GLUT1 transporter, across the blood-brain barrier (BBB). BBB glucose transfer is down-regulated during hyperglycaemia (16,17) and stimulated during hypoglycaemia (18). As insulin has no measurable effect on blood-brain glucose transfer (19,20), it remains uncertain how the non-insulin-dependent glucose transport across the BBB is regulated. GLP-1 itself has been shown to cross the BBB by passive diffusion (21).

As the role of glucose delivery and metabolism in neuroprotection is unclear, we tested the hypothesis that an interaction exists between GLP-1 and delivery of glucose by measuring the transport and metabolism of the glucose tracer FDG during positron emission tomography (PET) under the influence of GLP-1.

## **RESEARCH DESIGN AND METHODS**

Eleven non-smoking, healthy, Caucasian, males were included in the

study, 1 subject was withdrawn due to uncompleted PET scan. Mean age was 25  $\pm$  (SD) 3 years, body mass index was 22.6  $\pm$  0.6 kg/m<sup>2</sup>. They had no history of diabetes or cardiovascular disease and received no medication. All had a normal physical examination. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the local ethics committee of the County of Aarhus. All participants received both oral and written information and signed an approved informed consent form before entering the study.

The study was carried out as a randomized, double-blinded, placebo controlled, crossover study. Each subject was studied twice in random order with GLP-1 and placebo infusion. PET sessions were separated by an interval of 4-6 weeks.

Both sessions commenced at 8.00 hours after an overnight fast. The subjects did not exercise 24 hours prior to the sessions.

Subjects were placed in bed and two catheters were inserted for infusion of clamp hormones and for the infusion of GLP-1 or placebo. A third catheter was placed in an arterialized (heated) dorsal hand vein for blood sampling.

The randomization was performed by the pharmacy at Aarhus University Hospital and the randomisation list was kept at the pharmacy during the study. Sealed envelopes containing information about the code were delivered together with the study medication at day 1 and kept unbroken in the case report form. The code was broken after all data analysis was completed.

A pancreatic-pituitary clamp was performed according to principles previously described (22). In brief somatostatin (Ferring GmbH, D-24109 Kiel) was infused at a rate of 300  $\mu$ g/h to suppress endogenous insulin, glucagon,

growth hormone (GH) and GLP-1 production. Human glucagon (Glucagen, Novo Nordisk A/S, Copenhagen, Denmark) 0.6 ng/kg/min, growth hormone (GH) (Genotropin Miniquik 0.2mg, Pfizer ApS, Ballerup, Denmark) 2 ng/kg/min and Insulin (Actrapid, Novo Nordisk A/S, Copenhagen, Denmark) 0.12 mU/kg/min were infused with the aim of maintaining near basal levels (0-360 min). Glucose (200 g/Liter) was infused at a variable rate to clamp plasma glucose (PG) close to 4.5mM. Euglycaemia was maintained in order to assess a physiological role of GLP-1 at the BBB independent of insulin and glucagon levels and independent of a direct effect of PG on GLP-1 action.

The subjects received either intravenous recombinant (r)GLP-1(7-36 amide) or placebo at a rate of 1.2 pmol/kg/min (60-360 min)(23). rGLP-1(7-36 amide) was tested and found positive for sterility and negative for bacterial endotoxins. It was dissolved in a sterile buffer containing 600mg of acetoacid, 50.7g of mannitol and sterile water added up to 1000g and had a pH of 4.5. The concentration of rGLP-1(7-36) was 1mg/ml and stored frozen in vials of 0.25 ml. The test solution consisted of 0.25ml, 1mg/ml rGLP-1, 20 ml of human albumin "ZLB" 5 % and natrium chloride NaCl (9 g/Liter) up to 100ml of solution. The placebo solution consisted of the above mentioned buffer solution containing human albumin and NaCl.

PG was measured in duplicate every 10 min. Blood for measuring insulin, c-peptide, glucagon, GLP-1(total and intact), growth hormone (GH), free fatty acids (FFA), cortisol and ghrelin was drawn every 30 min. Blood for measuring epinephrine and norepinephrine was drawn at 0, 150 and 240 min and every 30 min during the PET scan.

**Assays.** PG was measured immediately after sampling on a Beckman glucose analyzer (Beckman, Palo Alto, CA). All

other blood samples were stored at  $-20^{\circ}\text{C}$  (C-peptide at  $-80^{\circ}\text{C}$ ) until assay.

The assays used for measuring serum insulin, serum C-peptide, serum FFA, serum GH, serum cortisol, plasma epinephrine and plasma norepinephrine are described previously (24). S-ghrelin was measured with an in-house assay (25).

The assay for intact GLP-1 is an enzyme-linked immunosorbent assay using unextracted plasma, which was collected and stored in the presence of a dipeptidyl peptidase IV (DPP IV) inhibitor (valine-pyrrolidide, 0.01mM final concentration added to the blood sample immediately after collection). Total GLP-1 was analyzed using a C-Terminal radioimmunoassay for amidated GLP-1 (26). Glucagon was measured using a previously described assay (27).

**PET.** 18-Fluoro-2-deoxyglucose (FDG) was used as a tracer for brain glucose uptake and was produced in house according to Drug Master File, Danish national marketing authorization number 2165. We used a whole body positron emission tomography model EXACT HR47 (Siemens Medical, Knoxville, USA) with a 15 cm field of view and an acquisition capacity of 47 transaxial planes with a spatial resolution of 4-5 mm at the center of the field of view. All PET data were acquired in 3D mode.

A 15 min transmission scan was performed to correct photon attenuation. Five hours after clamp start and four hours after the GLP-1 or placebo infusion was started, a bolus of 200 MBq of FDG in 10 ml saline was injected intravenously over 10 s.

Dynamic acquisition commenced at the beginning of tracer injection and continued for 45 min to acquire 23 frames (6 x 30 s, 7 x 1 min, 5 x 2 min and 5 x 5 min).

For the extraction of region-specific PET data results, regions of interest were

automatically extracted using a model based segmentation approach (28) based solely on the registration of PET images to a standardized anatomic atlas. Regional tissue time-activity-curves for FDG uptake were extracted for total cerebral grey matter, cerebral cortex, thalamus, striatum, cerebellar cortex, brainstem and white matter.

Image-derived arterial input functions were automatically obtained by localising the carotic artery on the PET images for each subject from each study day. An automated method for identification of the most probable voxel positions for the arteries was developed. Using a 3-compartment model, we obtained values of  $K_1^*$  (unidirectional clearance),  $k_2^*$  (efflux coefficient) and  $k_3^*$  (phosphorylation rate constant) for FDG in each region of interest. The rate of dephosphorylation and the absolute quantity of FDG were both considered negligible during the scanning period and therefore are not part of the model. The lumped constant is the conversion factor between the net uptakes of FDG and glucose as functions of transport across the BBB and the phosphorylation. In the model of competition between native glucose and FDG in brain, both transport across the BBB ( $K_1^*$ ,  $k_2^*$ ) and phosphorylation by hexokinase ( $k_3^*$ ) can be described by the Michaelis-Menten equation. This permits the use of fixed transport ( $\tau=K_1^*/K_1$ ) and phosphorylation ( $\phi=k_3^*/k_3$ ) rate ratios for tracer and native glucose.

By substituting transfer constants of FDG for those of glucose, using  $\tau$  and  $\phi$ , the lumped constant was determined directly for each subject in each region of interest as described previously (29). In this study we used the value of  $\tau=1.48$  and  $\phi=0.39$  (30).

The net clearance of FDG was calculated as:

$$K^* = K_1^* \times k_3^* / k_2^* + k_3^*$$

The unidirectional glucose transfer was calculated as:

$$J_{\text{glc}} = K_1^* \times C_a / \tau,$$

where  $J_{\text{glc}}$  is the unidirectional transport of glucose from blood to brain (influx of glucose),  $C_a$  equals the arterial steady state plasma glucose concentration (300-360min), and symbols marked with \* indicate FDG.

The lumped constant was calculated as:

$$LC = \phi + (\tau - \phi) \times (k_3^* / k_2^* + k_3^*)$$

The net clearance of glucose was calculated as:

$$K_{\text{glc}} = K^* / LC$$

The cerebral metabolic rate of glucose was calculated as:

$$CMR_{\text{glc}} = K^* \times C_a / LC,$$

and the cerebral tissue glucose concentration  $C_t$  was calculated as:

$$C_t = \tau \times (J_{\text{glc}} - CMR_{\text{glc}}) / k_2^*.$$

**Calculations and statistics.** Primary endpoints were transfer coefficients of BBB glucose transport and unidirectional glucose transfer across the blood brain barrier at normal glycaemia. Secondary endpoints were cerebral metabolic rate for glucose, cerebral tissue glucose concentration and lumped constant.

The PET-data were analysed using a linear mixed effects model with subject and all interactions involving subject, that is the interaction between subject and area and the interaction between subject and treatment, as random effects. Treatment (GLP-1 / placebo), region, and the

interaction between the two were included in the analysis as fixed effects. Data were transformed when appropriate based on an inspection of the residuals and results are therefore presented as back-transformed estimated means with 95% confidence intervals. The statistical software used was PROC MIXED in SAS: SAS Institute Inc., Cary, NC, USA with a significance level of 5%. To compare the two treatment groups regarding circulating hormones and metabolites a similar statistical model was used with time instead of region. Data are presented as mean  $\pm$  SEM.

## RESULTS

The infusion of GLP-1 at a constant rate of 1.2 pmol/kg/min resulted in pharmacologically relevant plasma concentrations of the intact hormone (31) (**Figure 1**).

During PET the PG concentration was successfully maintained at  $4.6 \pm 0.04$  (GLP-1) and  $4.6 \pm 0.03$  (placebo) mM ( $P=0.74$ ) with similar glucose infusion rates ( $1.81 \pm 0.30$  vs.  $1.55 \pm 0.32$  mg/kg/min GLP1 vs. placebo,  $P=0.22$ ) (**Figure 2**). The plasma C-peptide level was suppressed ( $P=0.66$ ). As expected, there were no significant differences in circulating hormones and metabolites between sessions. Insulin, glucagon and GH infusions resulted in low, close to basal levels of these hormones ( $P>0.24$ ) (**Figure 3**). Ghrelin, norepinephrine, cortisol (data not shown) and epinephrine levels were also low and did not change between sessions ( $P>0.14$ ). Only plasma GLP-1 levels differed between sessions ( $P<0.001$ ).

There was an overall significant effect of GLP-1 on the unidirectional clearance of glucose  $K_{1\text{glc}}$  but not the same in all regions ( $P=0.015$  for the interaction between region and treatment) (**Table 1**).  $K_{1\text{glc}}$  was decreased by 20-27% ( $P= 0.02-0.08$ ) in the seven brain regions, the efflux coefficient for FDG ( $k_2^*$ , data not shown)

was decreased by 26-39% ( $P=0.03$ ) with GLP-1 infusion compared to placebo.

No significant change in the phosphorylation coefficient for FDG ( $k_3^*$ , data not shown) was observed ( $P=0.39$ ) and the net clearance of FDG ( $K^*$ , data not shown) ( $P=0.58$ ) and of glucose ( $K_{glc}$ ) ( $P=0.25$ ) was unchanged with GLP-1 infusion. A tendency towards increased lumped constant with GLP-1 infusion was seen in all regions ( $P=0.08$ ).

The unidirectional glucose transfer ( $J_{glc}$ ) across the BBB was reduced by GLP-1 infusion in all brain regions but not by the same amount ( $P=0.01$  for the interaction between region and treatment) **Figure 4A**. In specific brain regions  $J_{glc}$  was reduced by 23-30% ( $P=0.02-0.06$ ) with GLP-1 infusion. The cerebral metabolic rate for glucose ( $CMR_{glc}$ ) was reduced by 12-18% with GLP-1 infusion, but the difference was not significant ( $P=0.28$ ) **Figure 4B**. The brain tissue glucose concentration remained the same in all regions with and without GLP-1 infusion ( $P=0.89$ ) **Figure 4C**.

Two subjects experienced transient nausea starting after approximately 1 and 3 hours and with a duration of 5 and 1 hour, respectively.

## DISCUSSION

In general, the search for a regulatory mechanism of BBB glucose transfer operating in the brain vasculature has failed (32). The only known factor affecting BBB glucose transfer remains therefore the plasma glucose level itself. In vitro, animal studies have shown that GLP-1 signalling is involved in neuronal plasticity and neuroprotection (13) and GLP-1R activation has effects in the nervous system comparable to those of nerve growth factor (NGF)(14). No human studies have linked GLP-1, cerebral glucose transport, and neuroprotection. Yet, maintaining a constant glucose concentration in brain tissue is important to

the preservation of neuronal functioning. Several studies have shown characteristic EEG changes during hypoglycaemia ( $PG < 2.0$  mM) in both type 1 diabetics and healthy subjects (33,34). Hyperglycaemia with concomitantly increased glucose concentration in brain tissue, accelerates ischaemic damage and worsens the outcome after ischaemic stroke (35). Although, physiologically, the BBB glucose clearance is lower in acute hyperglycaemia, the absolute flux is of course elevated (17), and it still remains unclear whether the reduction of plasma glucose during stroke is neuroprotective as trials are ongoing (36).

To our knowledge the current study for the first time reveals that GLP-1 potentially regulates BBB glucose transfer. Independently of the insulin and glucagon levels, GLP-1 reduces unidirectional glucose delivery across the intact BBB at normoglycaemia. The cerebral glucose metabolism tended also to decline, but the tissue glucose concentration remained constant. Together the data implies that GLP-1 maintains a constant glucose concentration in the brain at normal glycaemia by limiting the glucose delivery across the BBB. We anticipate that if the effect is due to a decline of the maximal transport ( $T_{max}$ ) it would be even more pronounced during hyperglycaemia.

However, the cerebral glucose metabolism does not depend strongly on the glucose concentration in the brain intracellular and interstitial fluids because brain hexokinase is saturated at the prevailing glucose concentration. Since the tissue glucose concentration depends on the glucose phosphorylation rate, the BBB transfer assumes a very important role in the maintenance of glucose concentration in the brain (37). In the brain, the unidirectional glucose transport rate increases concurrently with increasing glycaemia. Because the transport is only half-saturated at approximately 4 mM (38),

the fraction of glucose which passes from blood to brain continues to decrease with increasing glycaemia.

The net clearance of FDG(  $K^*$ ) in the current study was 4.4 (3.5-5.6) ml/100cm<sup>3</sup>/min which is comparable to the values found in the literature (4.2 ml/100g/min) (1g of brain tissue approximates a volume of 1 cm<sup>3</sup>). However, in the current study the unidirectional glucose transport in total cerebral grey matter was 93 (77-114)  $\mu$ mol/ 100cm<sup>3</sup>/min which is approximately twice as high as previously reported values for whole brain, mostly explained by the focus on grey matter in the present study and the still imperfect methods of computing whole-brain glucose transport rates from regional values with PET. Values of the lumped constant for humans, ranges from 0.50 to 0.80 and in the present study we obtained a lumped constant for grey matter of 0.55(0.52-0.58) (30). The cerebral metabolic rate for glucose we obtained in total cerebral grey matter was 37 (30-46)  $\mu$ mol/ 100 cm<sup>3</sup>/min, which is somewhat higher than the whole brain value of 25-30  $\mu$ mol/100g/min (39).

Cerebral blood flow was not measured in this study, however clearance (K) in principle is a measure that includes any blood flow effect, as it is the product of an extraction fraction (unidirectional or net extraction) and the plasma flow. Therefore any blood flow effect is included in the clearance, which in turn is the measure that describes the flux when multiplied with the concentration. This description does not of course exclude the possibility that the change in clearance is the result of a blood flow change but the effect is the effect measured as the clearance.

The effect of GLP-1 on the unidirectional clearance of glucose was not the same in all regions. But indeed a trend was observed in all regions indicating that the effect potentially is uniformly seen in all brain regions with an intact BBB.

In the brain, facilitated glucose diffusion across the BBB maintains a glucose concentration in brain tissue that is lower than in plasma and similar to cerebrospinal fluid (40). Calculated levels in the present brain regions are consistent with the highest levels in the most active part of grey matter and the lowest levels in white matter.

Individual lumped constants were calculated for each brain region. The lumped constant tended to increase during GLP-1 infusion in all brain regions. Because of the Michaelis-Menten kinetic competition for blood-brain transfer of glucose and glucose analogues, the lumped constant is in fact a variable that depends on the plasma glucose concentration and the relation between brain and plasma glucose.

In a single FDG-PET study, GLP-1 receptor activation reduced glucose metabolism in areas of the hypothalamus and brainstem involved in feeding and glucose sensing where receptors are abundant (41). However, it was not apparent whether the effect was due to reduced glucose transport across the BBB and/or reduced phosphorylation of glucose by hexokinase. No effect was observed in other brain areas. Caveats of the study noted are that circulating levels of glucose, insulin and glucagon were uncontrolled during and after the short (30 min) GLP-1 exposure and that PET commenced 10 min after the end of GLP-1 infusion. As the half-life of GLP-1 is less than 2 min due to enzymatic degradation by ubiquitously represented DPP-IV (42), the true plasma GLP-1 concentrations are uncertain and it is unknown whether pharmacologically relevant levels were reached during the PET examination.

In the present study, we demonstrate that GLP-1 reduces BBB transport of glucose in general in the brain. The interaction of GLP-1 with the normal human BBB is therefore a novel finding.

The glucose transport across the BBB is mediated by the transporter GLUT 1. We speculate that GLP-1 interacts with the GLUT1 transporter and the GLP-1 receptor in the BBB.

GLP-1 has a role in the maintenance of cerebral glucose balance and may have neuroprotective effects linked to both peripheral and cerebral glucose metabolism.

During hyperglycaemia GLP-1 is secreted from the gut into the blood stream, and may also be produced in the brain as central GLP-1 signalling appears to be linked to the control of blood glucose concentrations(15,43).

GLP-1 efficiently promotes disposal of glucose and circulating glucose is reduced by means of increased insulin secretion and decreased glucagon secretion, thereby limiting glucose transport across the BBB. Here we show that GLP-1 has an important effect which could be independent of insulin action.

Hence, both peripheral and central actions of

GLP-1 efficiently limits glucose fluctuations in the brain that may be of neuroprotective significance.

Thus under conditions with pharmacologically elevated levels of GLP-1 we propose a new extra-pancreatic effect of GLP-1 in the brain that provides a link between brain glucose transport and neuroprotection.

#### **ACKNOWLEDGMENTS**

We thank The Lundbeck Foundation, The Novo Nordisk Foundation, The Danish Diabetes Association, The Danish Medical Research Council, The Danish National Science Foundation, and the FOOD and the DIRAC groups for support of this study. Ms. Annette Mengel and the staff at the PET-Center, Aarhus University Hospital are thanked for their outstanding technical assistance.

Part of the data were presented at the 67<sup>th</sup> ScientificSessions-ADA, Chicago 2007.

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**TABLE 1. Initial clearance of glucose ( $K_{1\text{glc}}$ ), net clearance of glucose ( $K_{\text{glc}}$ ) and lumped constant (LC) during GLP-1 and placebo infusion.**

	$K_{1\text{glc}}(\text{ml}/100\text{cm}^3/\text{min})$			$K_{\text{glc}}(\text{ml}/100\text{cm}^3/\text{min})$		LC	
	Placebo	GLP-1	P-value	Placebo	GLP-1	Placebo	GLP-1
GM	20 (16-25)	15 (13-18)	0.07	8.0(6.5-9.8)	6.8(5.5-8.3)	0.55 (0.52-0.58)	0.59 (0.56-0.62)
CC	20 (16-24)	15 (12-18)	0.08	8.2(6.6-10.0)	6.9(5.6-8.6)	0.56 (0.53-0.59)	0.60 (0.57-0.64)
TH	22 (18-28)	16 (14-19)	0.07	7.9(6.4-9.7)	6.8(5.5-8.4)	0.53 (0.50-0.55)	0.57 (0.54-0.60)
ST	20 (16-25)	15 (13-18)	0.08	8.5(6.9-10.5)	7.2(5.9-8.9)	0.57 (0.54-0.60)	0.61 (0.57-0.64)
CEC	23 (19-30)	17 (14-21)	0.08	7.1(5.7-8.7)	6.0(4.9-7.4)	0.50 (0.48-0.53)	0.53 (0.50-0.55)
BS	15 (13-18)	11 (9-13)	0.02	4.8(3.9-5.9)	3.9(3.2-4.8)	0.51 (0.48-0.53)	0.54 (0.51-0.57)
WM	10 (9-12)	8 (7-9)	0.04	3.7(3.0-4.6)	3.3(2.6-4.0)	0.54 (0.51-0.57)	0.56 (0.53-0.59)

P-value all regions = 0.25

P-value all regions = 0.08

P-value for overall GLP-1 effect on  $K_{1\text{glc}}$ =0.015. For calculation of  $K_{\text{glc}}$  and LC see methods. P-value for  $K_{\text{glc}}$  in all regions=0.25. P-value for LC in all regions=0.08. Data are medians with 95% confidence intervals. TGM = total cerebral grey matter, CC = cerebral cortex, TH = thalamus, ST = striatum, CEC = cerebellar cortex, BS = brain stem and WM = white matter.

## FIGURE LEGENDS

**Figure 1. Total and intact GLP-1 concentrations** during GLP-1(black dot) and placebo(white dot) infusion. GLP-1 infusion was initiated at  $t = 60$  min. PET was performed from 300-360 min. Data are mean  $\pm$  SEM.

**Figure 2. PG, insulin and C-peptide concentrations and glucose infusion rate** during GLP-1(black dot) and placebo(white dot) infusion. Data are mean  $\pm$  SEM.

**Figure 3. FFA, glucagon, GH and epinephrine concentrations** during GLP-1(black dot) and placebo(white dot) infusion. Data are mean  $\pm$  SEM.

**Figure 4. Unidirectional glucose transfer,  $J_{glc}$  (A), Cerebral metabolic rate for glucose,  $CMR_{glucose}$  (B) and Brain tissue glucose concentration,  $C_t$  (C)** during placebo or GLP-1 infusion.

The regions are total cerebral grey matter (TGM), cerebral cortex (CC), thalamus (TH), striatum (ST), cerebellar cortex (CEC), brain stem (BS) and white matter (WM). Dark blue bars and light blue bars represent placebo and GLP-1 respectively. Data are medians with 95% confidence intervals.

P-value for overall GLP-1 effect on  $J_{glc}$  = 0.011.

\*  $P \leq 0.05$ . †  $P = 0.06$

For  $CMR_{glucose}$ ,  $P = 0.28$  in all regions.

For  $C_t$ ,  $P = 0.89$  in all regions.

FIGURE 1

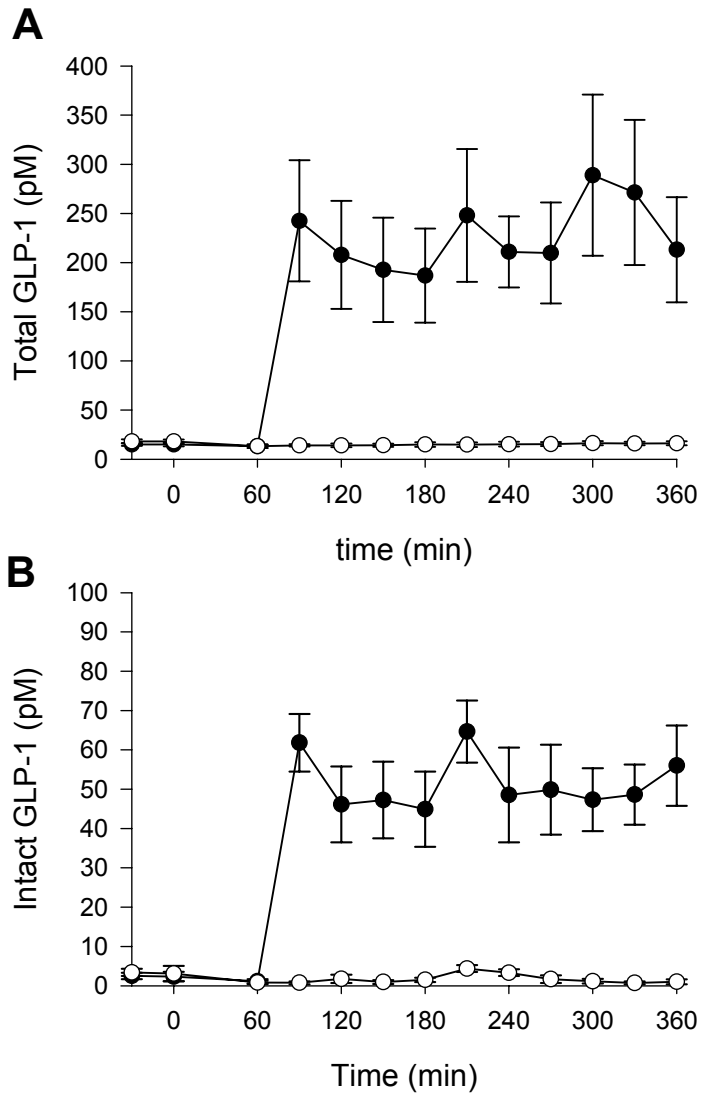
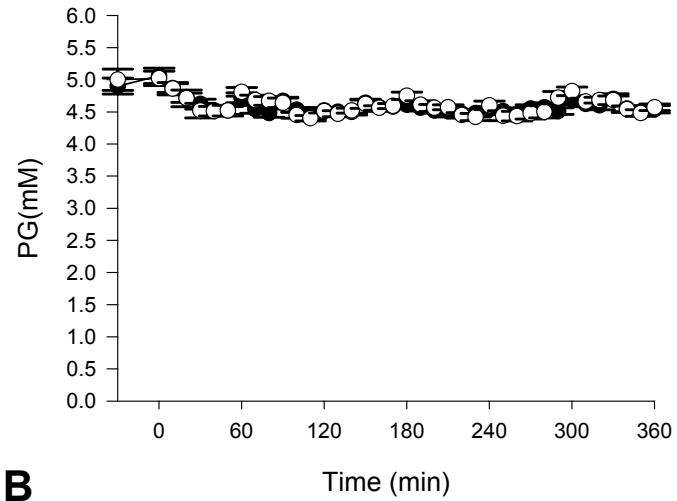
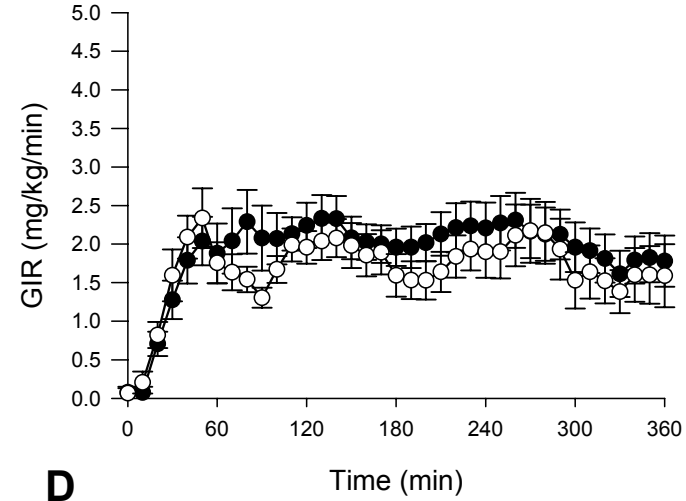


FIGURE 2

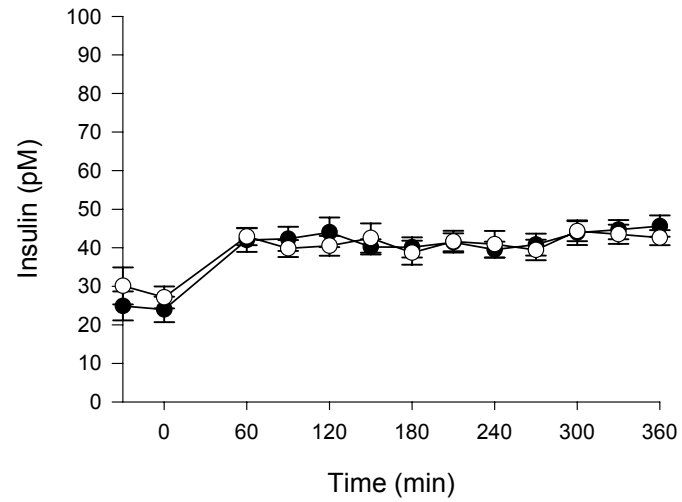
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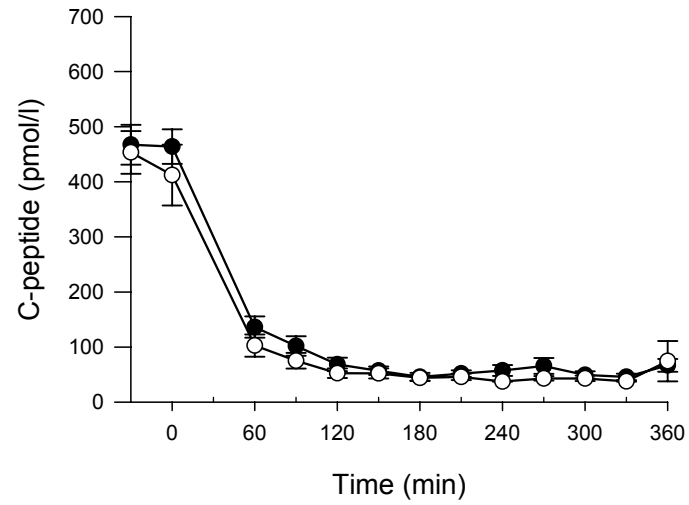
C



B

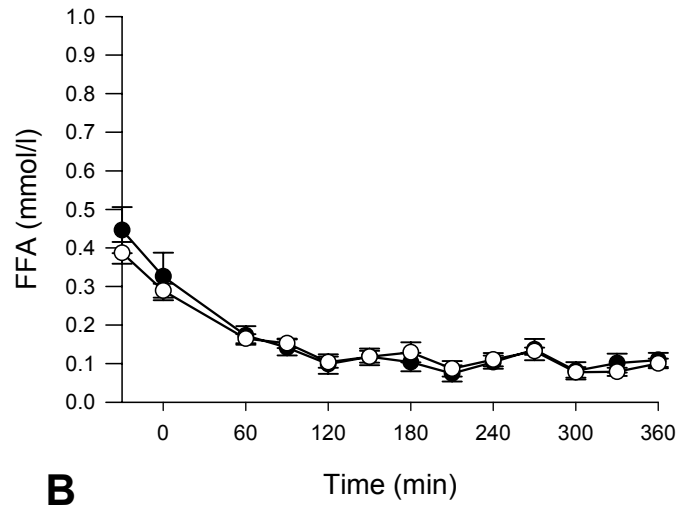


D

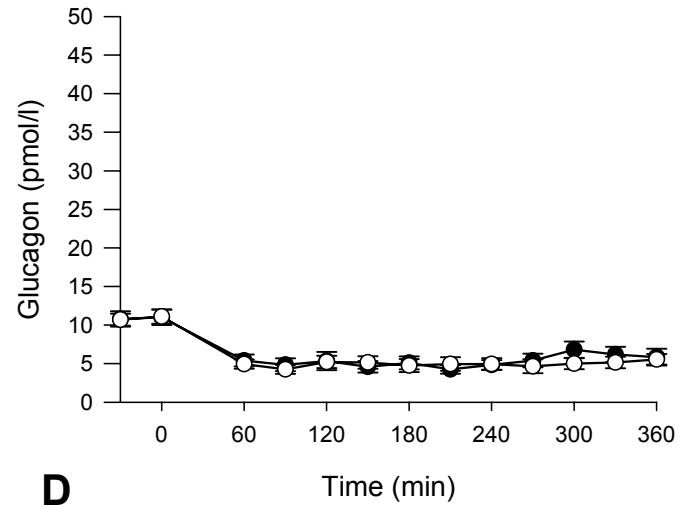


**FIGURE 3**

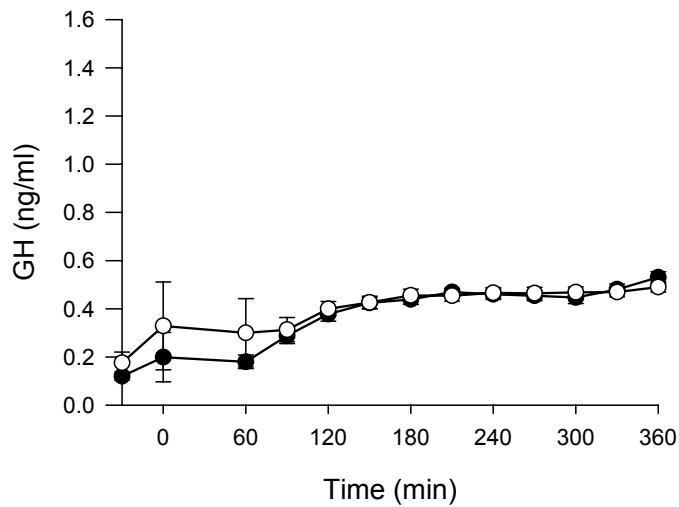
**A**



**C**



**B**



**D**

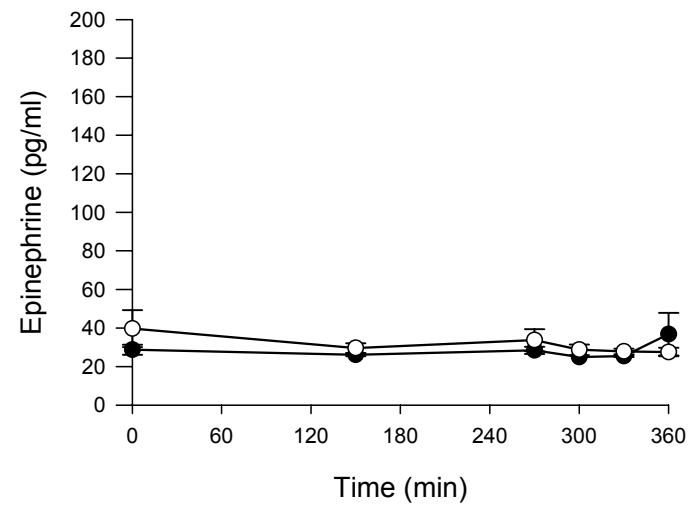


FIGURE 4

