The Role of Insulin in Human Brain Glucose Metabolism

An ^{18}Fluoro-Deoxyglucose Positron Emission Tomography Study

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The effect of basal insulin on global and regional brain glucose uptake and metabolism in humans was studied using 18-fluorodeoxyglucose and positron emission tomography (FDG-PET). Eight healthy male volunteers aged 49.3 ± 5.1 years were studied twice in random order. On each occasion, they received an infusion of 0.1 mg · kg^{-1} · min^{-1} somatostatin to suppress endogenous insulin production. In one study 0.3 mU · kg^{-1} · min^{-1} insulin was infused to replace basal circulating insulin levels, and in the other study a saline infusion was used as control. We sought stimulatory effects of basal insulin on brain glucose metabolism particularly in regions with deficiencies in the blood-brain barrier and high density of insulin receptors. Insulin levels were 27.07 ± 1.3 mU/l with insulin replacement and 3.51 ± 0.4 mU/l without (P = 0.001). Mean global rate of brain glucose utilization was 0.215 ± 0.030 mmol · kg^{-1} · min^{-1} without insulin and 0.245 ± 0.021 mmol · kg^{-1} · min^{-1} with insulin (P = 0.008, an average difference of 15.3 ± 12.5%). Regional analysis using statistical parametric mapping showed that the effect of basal insulin was significantly less in the cerebellum (Z = 5.53, corrected P = 0.031). We conclude that basal insulin has a role in regulating global brain glucose uptake in humans, mostly marked in cortical areas. *Diabetes* 51:3384–3390, 2002

The effect of insulin in peripheral tissues is the stimulation of glucose uptake, oxidation, and storage. The effect of insulin on the brain is less well defined. Elevations of circulating insulin can alter brain function, augmenting the counterregulatory response to hypoglycemia (1,2), altering feeding behavior (3,4), and modulating auditory evoked potentials (5). Whether these effects are mediated via an effect on brain glucose metabolism is unknown.

For insulin-stimulated glucose metabolism to occur in the brain, insulin, insulin receptors, and insulin-sensitive glucose transporters are required. Insulin receptors have been demonstrated throughout the human brain, with particularly high concentrations in the hypothalamus, cerebellum, and cortex (6). Insulin may be produced in the brain—rat neuronal cells in culture produce insulin in response to stimulation via potassium and calcium ions, in a biphasic manner akin to that shown by β-cells (7). However, most brain insulin is thought to originate from systemic circulation. Autoradiography has shown that insulin can cross the blood-brain barrier, penetrating to the circumventricular organs, including the arcuate and ventromedial nuclei of the hypothalamus (8). However, studies over a longer time course have shown that insulin does penetrate the cerebral spinal fluid, probably via receptor-mediated transport and does eventually reach the rest of the brain (9–11). More recently, Pardridge et al. (12) have shown insulin receptors on the endothelium of the blood-brain barrier, which allow receptor-mediated active transport of insulin into the brain. Finally, insulin-sensitive glucose transporters have been demonstrated at the blood-brain barrier and on glial cells in various studies of animal brain, both insulin-sensitive GLUT4 (13–16) and a partially insulin-sensitive GLUT1 (17).

Despite the evidence for appropriate mechanisms, most in vivo studies do not show an effect of incremental circulating insulin on glucose transport across the blood-brain barrier in either animal (18) or human (19) models. Some animal studies have found regional effects on brain glucose uptake and metabolism, both increases (in the area of the hypothalamic nuclei) (20) and decreases (in the hypothalamus, locus coerulus, and motor cortex) (21). Positron emission tomography (PET) studies in humans, however, have shown no effect of increasing insulin levels on global brain glucose uptake (22,19), and, based on the lack of effect of hyperinsulinemia, it has been concluded that human brain glucose metabolism is not insulin sensitive. This contrasts with the clear evidence for an effect of insulin on brain function mentioned above.

All studies to date have looked at the cerebral effects of hyperinsulinemia. To investigate the hypothesis that brain glucose uptake and metabolism is either partially insulin sensitive or operating at the top of the dose-response curve at basal (fasting) circulating insulin levels, we measured global and regional brain glucose uptake and metabolism using ^{18}fluoro-deoxyglucose (FDG)-PET during fasting and below fasting insulin levels.

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CSF, cerebrospinal fluid; CV, coefficient of variation; FDG, 18-fluorodeoxyglucose; LC, lumped constant; MRI, magnetic resonance image; PET, positron emission tomography; SPM, statistical parametric mapping; ROI, region of interest.

RESEARCH DESIGN AND METHODS

Eight male volunteers (aged 49.3 ± 5.1 years [range 42–58] and BMI <30 kg/m^2) were studied. Fully informed written consent was obtained before the study. The protocol was approved by the ethics committee of St. Thomas' NHS Trust and ARSAC (Administration of Radioactive Substances Advisory Committee) and conformed to the Helsinki Declaration. Volunteers were excluded if they had an abnormal fasting plasma glucose or HbA_{1c}, had significant cardiovascular disease, or were taking any regular medication, as detected during a screening examination.

Study protocol. Each subject was studied twice, between 2 and 8 weeks apart, with FDG-PET to measure global and regional brain glucose uptake. Subjects were studied at the same time on each occasion, after an overnight fast. Samples for later measurement of insulin were taken throughout the study. A T_1-weighted magnetic resonance image (MRI) of the brain was performed on a separate day to coregister with the PET images for anatomical definition.

Infusion protocol. On the morning of the study, an intravenous catheter was inserted into a vein in the antecubital fossa of the dominant arm using aseptic technique and intradermal lidocaine 1% for skin anesthesia. This catheter was used for the infusion of fluids and drugs. After checking for a collateral circulation, a second catheter was placed in the radial artery of the nondominant hand, again with aseptic method and skin anesthesia. This was for continuous sampling of arterial blood sampling throughout the study. At time 0 min, infusions were started. For all PET studies, somatostatin was infused at 0.1mg · kg^{-1} · min^{-1} to suppress basal insulin secretion (23). Subjects were randomized to receive either 0.3 mU · kg^{-1} · min^{-1} regular human insulin (human Actrapid; NovoNordisk, Copenhagen, Denmark) diluted in a 4% saline solution of autologous blood or a control saline infusion first, with the alternative study second. Arterial plasma glucose was monitored every 5 min using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH) and maintained at euglycemia using 20% glucose i.v. (Baxter Healthcare, Norfolk, U.K.) if necessary. At the end of the scanning period, somatostatin and insulin (if used) were stopped and glucose infusion continued. Subjects were removed from the scanner and given lunch. The glucose infusion was stopped and the intravenous lines removed once plasma glucose was >5.5 mmol/l without glucose infusion.

Scanning protocol. After insertion of the intravascular lines, the subject was made comfortable in the PET scanner (CTI ECAT 951R scanner, axial field view 10.8 cm, in-plane spatial resolution 6.5 mm; CTI/Siemens, Knoxville, TN). The subject's head was aligned axially to the orbito-meatal line, secured by a restraining strap, and the position was monitored using a laser grid. Sensory disturbance was standardized by studying subjects with their eyes shut and with background scanner noise. Before injection of tracer, a 10-min transmission scan used to correct for photon attenuation was performed. After 90 min, when the glucose kinetics for somatostatin with or without insulin were in steady state, a single dose of ~185 MBq of 18 FDG, made up to 10 ml with normal saline, was injected peripherally over 10 s, and the injection line was then flushed with 10 ml normal saline. Dynamic scanning was started, taking 26 frames of variable duration over 90 min. Continuous on-line blood sampling to detect tracer levels was started 1 min before tracer injection at a rate of 5 ml/min for 15 min and then 2 ml/min for 15 min (IVAC 572 peristaltic pump; Eli Lilly, San Diego, CA). This blood was drawn through a Bismuth Germinate Oxalate Crystal Fluid Analyzer (Allogg, Stockholm, Sweden). Timed 2-ml hand-drawn arterial blood samples were taken at 5-min intervals throughout the scan for cross calibration of the fluid analyzer data with a well counter (calibrated to the PET scanner) and measurement of plasma glucose. The fluid analyzer data and timed samples were used to derive an arterial plasma input function for each study. PET images were reconstructed by filtered back-projection and smoothed with a Hanning 0.5 filter. This gave a spatial resolution of 8.5 mm full-width at half maximum transaxially and axially. Reconstructed images were displayed in a 128 × 128 × 31–voxel format, each voxel measuring 2.0 × 2.0 × 3.43 mm. The FDG uptake images were created by summation of the images acquired 30–90 min postinjection.

Image analysis

Whole-brain rate of glucose uptake with and without insulin. For each PET study, the dynamic PET image data and the arterial input function were combined to produce a parametric image of the rate of glucose utilization using the generic method described by Patlak et al. (24). This method requires that concentrations of unbound FDG in plasma and tissue rapidly reach equilibrium, and that phosphorylated FDG remains trapped in tissue for the duration of the study and has been validated for FDG in the brain (25). The method gives a value for the rate of glucose uptake in absolute units, where the rate of uptake is the product of the plasma glucose concentration and the rate constant for glucose uptake. It therefore provides a measure of the rate of glucose utilization that is independent of changes in the arterial tracer and glucose concentration between subjects and between studies. To provide a quantitative value for the rate of glucose uptake, it is necessary to apply a "lumped constant" (LC), which accounts for the difference in behavior between FDG and native glucose. We have used the standard value of 0.52 for LC (26). The average whole-brain rate of glucose uptake was calculated for the two different conditions, i.e., with or without basal insulin replacement, by the addition of all brain image slices after application of a 40% threshold to exclude noncerebral tissue activity.

Regional brain FDG uptake with and without insulin. To identify regional differences in brain glucose uptake in the presence or absence of insulin, a statistical package specifically designed for comparing PET brain images was used. Statistical parametric mapping (SPM) was performed using the SPM99 program (Wellcome Department of Cognitive Neurology, London, U.K., implemented in Matlab 4.2c, Mathworks, Sherborn, MA). In SPM, images are transformed into a common anatomical space, and a statistical analysis identifies significant differences between groups of data (in our case, with or without basal insulin replacement) on a voxel-by-voxel basis. Brain regions where significant differences are found are displayed as a new parametric image, where the image intensity at any point is related to the statistical significance. The SPM map can be displayed superimposed on a standard MRI anatomical image to identify the anatomical locations of statistically significantly different regions. SPM analysis was performed on the summed raw FDG uptake images, as this has been demonstrated to be most sensitive to small regional change (27). The effect of variation in whole-brain FDG uptake from variations in injected activities, plasma glucose concentrations, and plasma FDG concentrations was removed using a voxel-by-voxel ANCOVA, with values for mean whole-brain uptake as the confounding covariate. Clusters of voxels surviving a threshold Z score >3.0 (omnibus threshold $P <$ 0.001) were considered to show significant differences (28,29). To enhance anatomical localization, the locations of volumes of change were displayed by rendering the maximum intensity map onto orthogonal planes of a high-resolution T_1-weighted MRI brain scan in Talairach space provided with the SPM99 software. Voxel clusters with a highly significant P value after correction for multiple comparisons were identified to anatomical regions using the Talairach coordinates.

FDG uptake in specific areas. The SPM analysis does not provide values for the magnitude of the regional differences it identifies. To quantify any regional changes, regions of interest (ROIs) were placed manually on each subject's MRI image in anatomical areas corresponding to those suggested by published work on insulin receptor location and those identified by the SPM analysis as exhibiting significant differences. These ROIs were then transferred from the anatomical MR images onto the PET images following coregistration of the MRI and PET images (30). Raw images of FDG uptake, while not providing quantitative values in absolute units, have a much higher signal-to-noise ratio than the parametric described above. The rate of glucose utilization for the cerebral cortex was measured by transferring ROIs directly onto the Patlak images, but for smaller regions. Rates of glucose utilization were obtained by measuring the ROI–to–whole-brain ratio from the FDG uptake image and multiplying this by the whole-brain rate of glucose utilization derived above.

Biochemical analysis. Plasma insulin was measured by radioimmunoassay (Diagnostic Systems Laboratories, London, U.K.) with a sensitivity of 1.3 mU/l. The intra-assay coefficient of variation (CV) was 8.3% at 4.8 mU/l, 4.5% at 17.6 mU/l, and 6.4% at 54.6 mU/l. The interassay CV was 12.2% at 4.9 mU/l, 9.9% at 16.2 mU/l, and 4.7% at 52.9 mU/l, with 50% cross-reactivity for proinsulin and split products.

C-peptide was measured by radioimmunoassay (Diagnostic Systems Laboratories) with a sensitivity of 0.01 ng/ml. The intra-assay CV was 3.3% at 1.8 ng/ml, 4.3% at 4.3 ng/ml, and 7.9 at 9.7 ng/ml. The interassay CV was 5.3% at 1.6 ng/ml, 2.4% at 1 ng/ml, and 5.1% at 9.7 ng/ml.

Statistical analysis. Data are presented as means ± SD. Data were compared using paired Student's t tests, using SSPS for Windows 10.5 (SPSS, Woking, U.K.), and differences were regarded as statistically significant if P values were <0.05. Raw FDG-PET data were compared by SPM as described above.

RESULTS

Mean plasma glucose was 5.6 ± 0.6 mmol/l during somatostatin alone and 5.2 ± 0.4 mmol/l during somatostatin plus insulin infusion (P = 0.038). The increment during somatostatin alone was confined to the last 30 min of scanning (Fig. 1) and is adjusted for in the PET analysis (see RESEARCH DESIGN AND METHODS).

Somatostatin alone suppressed insulin levels to 3.51 ± 0.4 mU/l. Levels were eightfold higher during somatostatin plus insulin infusion at 27.07 ± 1.3 mU/ml, P = 0.001 (Fig.

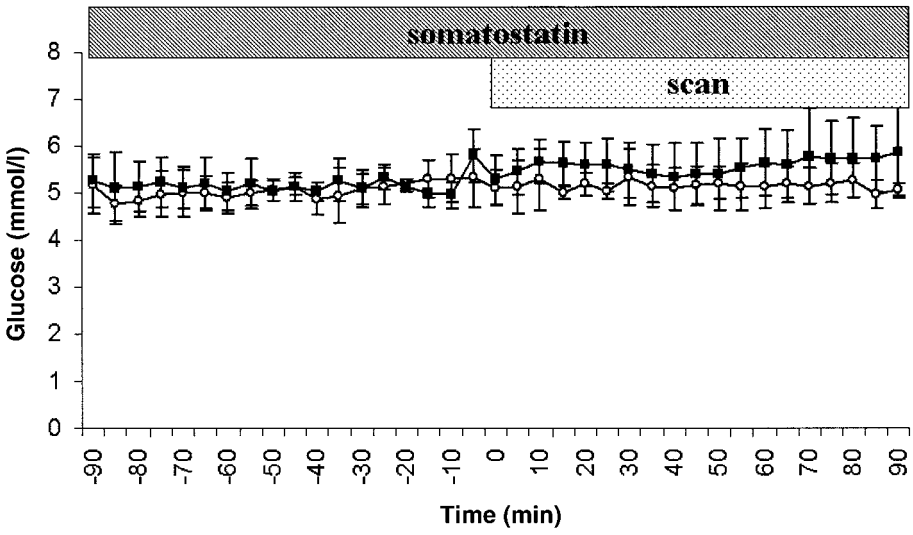

FIG. 1. Mean plasma glucose levels ± 1 SD with insulin (–○–) and without insulin (–■–) infusion.

2), not significantly different from the basal presomatostatin levels (14.9 ± 15.3 mU/ml, $P = 0.1$). C-peptide—a measure of endogenous insulin production—was suppressed to near zero during the period of the scan for both studies (Fig. 3). Absolute glucose infusion during the scanning period was more when insulin was infused than when no insulin was infused (292.45 vs. 13.35 mg/kg, $P = 0.0005$).

Image analysis

Whole-brain rate of glucose utilization with and without insulin. Global brain rates of glucose uptake for all subjects increased in all eight subjects in the presence of basal insulin (Fig. 4). The average global cerebral metabolic rate of glucose was 0.215 ± 0.030 mmol · kg^{-1} · min^{-1} without insulin and 0.245 ± 0.021 mmol · kg^{-1} · min^{-1} with insulin replacement, $P < 0.008$. The rate of glucose utilization for the whole-brain was 15.3 ± 12.5% higher in the presence of basal insulin levels.

Regional brain FDG uptake with and without insulin. The SPM image in Fig. 5 indicates the areas where the relative FDG uptake (compared with the average whole-brain value) differed significantly between the two insulin levels. The highlighted areas include the cerebellum and areas of the brain stem. In the presence of basal insulin, compared with no insulin, there was a significantly reduced relative tracer uptake in both the right and left cerebellar lobes (corrected $P = 0.031$, $Z = 5.53$ and corrected $P = 0.052$, $Z = 5.37$), indicating that the increase identified by the global analysis was less in these regions than in the rest of the brain. The loss of difference in the inferior cerebellum when the SPM data are projected onto an anatomical MRI scan occurred because the PET scan did not include the lowest levels of the cerebellum. The change in relative tracer uptake in the brain stem did not reach statistical significance. For the rest of brain, there were no relative differences, and these nonhighlighted areas were therefore responsible for the increase in global glucose uptake with basal insulin identified above.

Quantitative rates of glucose utilization in specific brain areas. Values for the rate of glucose utilization in ROIs placed in the cerebellum, a brain stem region, and the cortex are given in absolute units in Table 1. In agreement with the SPM analysis, while the cerebellum shows a slightly higher rate in the presence of insulin, the increase was small and nonsignificant. The brain stem showed no significant change. The average increase in the cortex was similar to that for whole-brain.

DISCUSSION

Our study results challenge the accepted view that human brain glucose uptake is an entirely insulin-independent

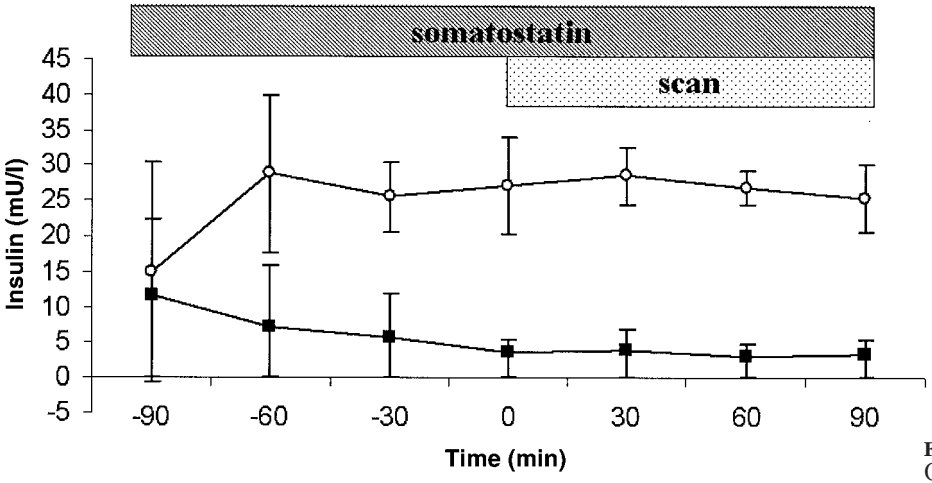

FIG. 2. Mean insulin levels ±1 SD with insulin (–○–) and without insulin (–■–) infusion.

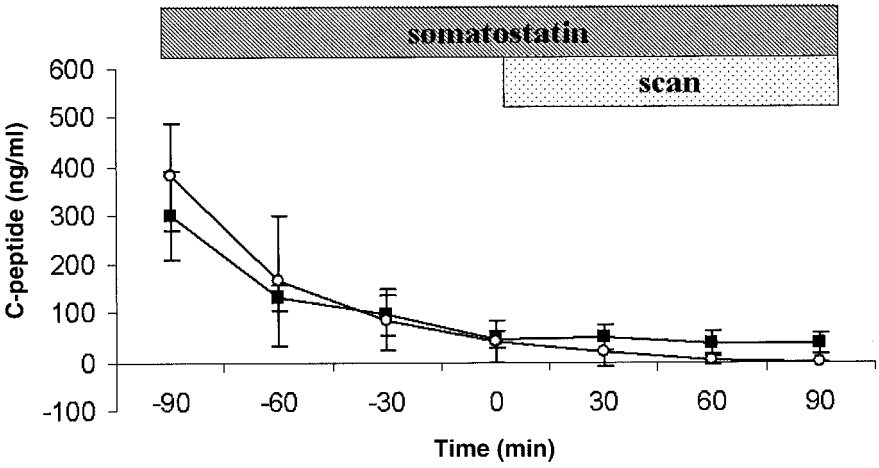

FIG. 3. Mean C-peptide levels ±1 SD with insulin (–○–) and without insulin (–■–) infusion.

process. Instead, our data suggest that there is a significant element of brain glucose uptake that is insulin sensitive, with a dose-response curve that is shifted well to the left of dose-response curves for other insulin-sensitive tissues, such as muscle or liver. Previous studies have demonstrated no change in rate of brain glucose metabolism in response to elevations of circulating insulin above the basal (fasting) range (19,22). Our data, examining the effect of basal insulin replacement during somatostatin infusion, show that brain glucose uptake is however partially insulin sensitive, as there was a significant reduction in global brain glucose uptake when circulating insulin levels were reduced below this. Coupled with the published data showing no effect of increasing circulating insulin above fasting levels, we can infer that brain glucose metabolism is maximally stimulated at these fasting insulin concentrations. The magnitude of the effect may not seem large, but this is because it is superimposed on a background of insulin-independent brain glucose uptake. A 15% increase in brain glucose uptake secondary to insulin stimulation may have clinical significance.

One other study in the recent literature that has sought to examine the effects of basal insulin concentrations on brain glucose metabolism failed to find an effect (31). The study protocol was very different from ours, using 1H magnetic resonance spectroscopy. It was required to be performed at hyperglycemia and always with the insulin withdrawn state first. Hyperglycemia may have elevated glucose uptake by mass action, masking the effect of the change in insulin. Furthermore, in PET studies, first scans are associated with an ~6% increase in measured global brain glucose uptake, an order effect attributed to anxiety and slightly elevated catecholamine levels (22). It is possible that a similar effect might occur with MRI.

Our study used FDG-PET to trace brain glucose uptake. FDG traces glucose uptake into cells and its subsequent phosphorylation but then does not continue along the glucose metabolic pathway. Indeed this is integral to its performance in PET measurements of glucose uptake, which are based on the accumulation of the FDG in the cells. However, we cannot draw any conclusions about the fate of the insulin-stimulated glucose uptake we observed using FDG. It can be assumed that the increased uptake is brought about by recruitment of glucose transporters to the plasma membrane of cells within the brain. If this is a direct effect of the insulin, then the partially insulin-

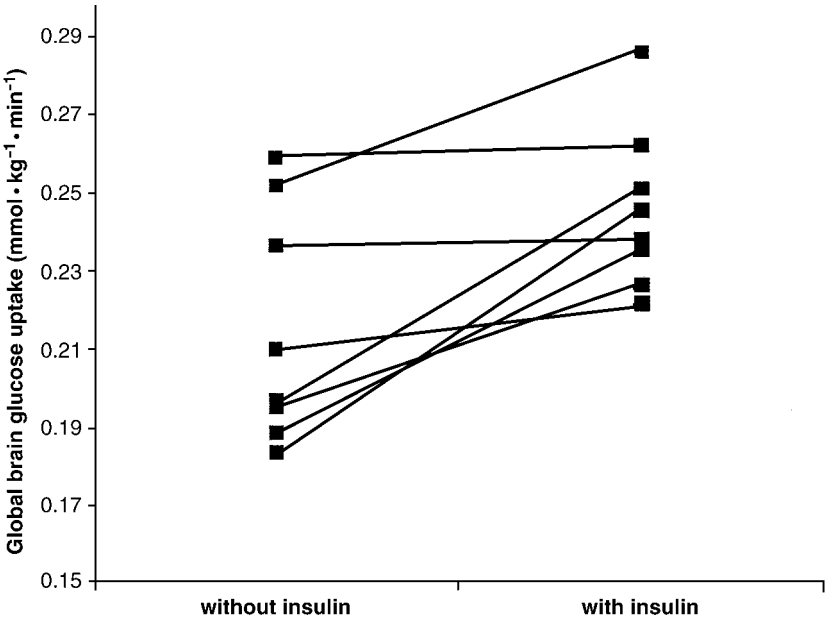

FIG. 4. Scatter plot of global brain glucose uptake with and without insulin infusion.

TABLE 1
Region of interest values for brain glucose uptake

	Glucose uptake (mmol · kg^{-1} · min^{-1})				
	No insulin	Insulin	Difference	Difference (%)	*P*
Whole-brain (from Patlak)	0.214963	0.245278	0.030315	15.28	0.008*
Cerebellum (from ratio)	0.231353	0.230212	−0.001141	−0.49	0.948
Brainstem (from ratio)	0.169826	0.185892	0.016066	9.46	0.178
Cortex (from Patlak)	0.278883	0.3158752	0.036992	14.71	0.014*

*Significant.

sensitive GLUT1, the major transporter of glial cells, may be largely responsible (32) because the principle neuronal transporter GLUT3 is considered insulin insensitive. The glucose may then be converted into lactate for neuronal use (33,34) or be converted into glycogen (35). The debate as to whether the neuronal energy requirements are fulfilled by glial-supplied lactate (33,34) or glucose (36,37) continues and cannot be determined from our study design. In contrast to the glucose transporters, insulin receptors in the brain are widely distributed on neurons and glial (38). While in culture, glial cells respond to insulin by stimulation of glucose uptake (39,40); the neuronal response to insulin is stimulation of neurotransmitter release (41), so it is possible that the increased glucose uptake we observe is secondary to neuronal activation by insulin. In any event, the net effect of insulin in our study remains an increase in brain glucose uptake, whatever the ultimate fate of the glucose.

Other possible explanations for the decrease in global brain glucose uptake with removal of basal insulin must be considered. An increase in nonglucose substrates for cerebral metabolism during somatostatin infusion, suppressed by insulin replacement, might explain our data, as the brain can use lactate or 3-OH-hydroxybutyrate as a metabolic fuel. However, published data suggest that somatostatin infusion, as used here, is unlikely to have resulted in sufficient plasma concentrations of either, as lactate does not increase significantly and the increase in plasma ketone concentration is much lower than any used to show physiological effects on brain function (42). Any alteration in insulin antagonist hormones induced by somatostatin would be equal in both groups and, as such, unlikely to account for our findings. There is also no reason to suppose that somatostatin would alter the lumped constant used to calculate glucose uptake rates from FDG data, but in any event, the effect would have been the same in both groups.

Might the insulin replacement or the somatostatin infusion be altering cerebral blood flow? Previous PET studies using labeled water (19,22) have shown no effect of increased insulin levels on this. In any event, even moderate changes in blood flow have not been shown to affect

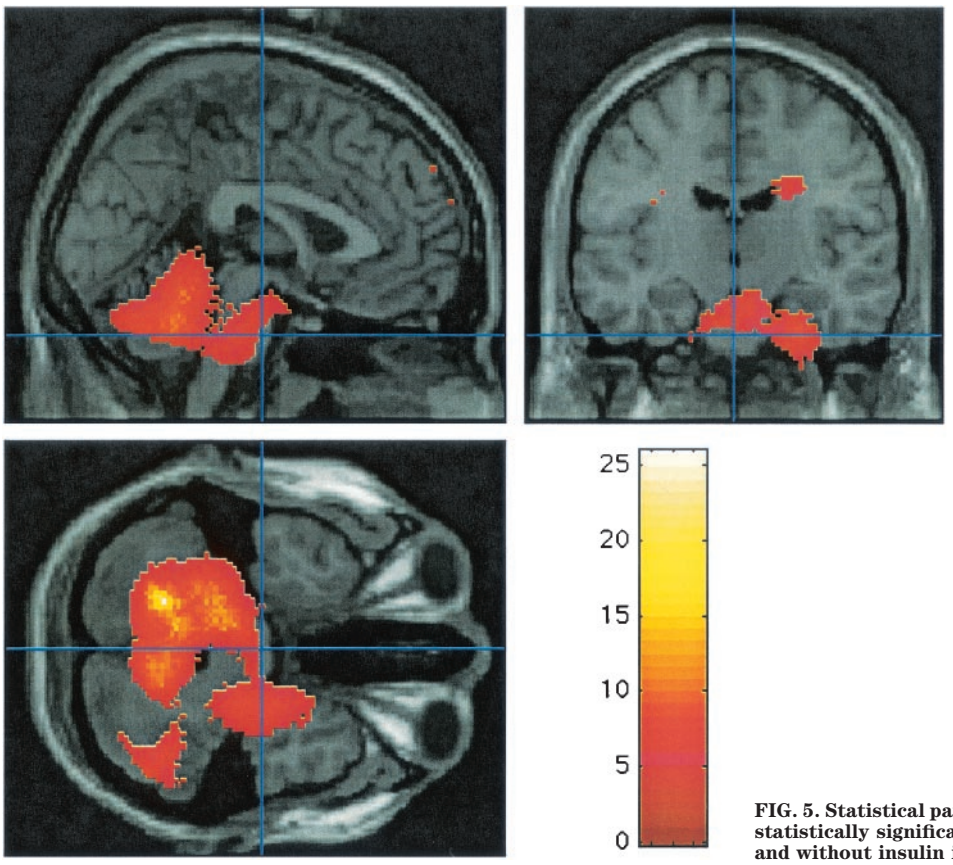

FIG. 5. Statistical parametric map of raw brain image data showing statistically significant differences in regional tracer uptake with and without insulin infusion.

tracer uptake (43). Somatostatin was present for both studies and it has a very limited ability to cross the blood-brain barrier. Animal data shows that the direct effect of somatostatin on cerebral vasculature is vasoconstriction (44) and thus it is unlikely to be the explanation for the difference in brain glucose uptake.

Parenthetically, it should be noted that the difference in plasma glucose levels during the somatostatin alone infusion could not account for our findings. The lumped constant used to describe the ratio between transport and phosphorylation of glucose and its tracer is insensitive to small changes in plasma glucose and the analysis of the PET data accounts for the ambient plasma glucose. To be certain, we reanalyzed our data using only the first 60 min, when plasma glucose was not different between the studies (data not shown) and found exactly the same effect. The amount of glucose infused to maintain plasma glucose at 5 mmol/l was significantly higher when insulin was infused. There is no evidence that this would have an effect on brain glucose uptake (19,22).

The effects of peripheral insulin in brain in vivo may be expected to be modest because of limited access to the intracerebral insulin receptors. In vivo, insulin can access the brain cells both via the cerebrospinal fluid reached through regions of deficiency in the blood-brain barrier (8), and directly through the blood-brain barrier elsewhere via specific insulin receptors that can act as transporters (12). These have recently been demonstrated to be saturable at modest insulin levels (10) that may limit the effect of increased insulin levels on brain glucose utilization. In addition, studies of insulin kinetics from plasma to cerebrospinal fluid (CSF) (11) have shown that increments in plasma insulin are only followed by increases in CSF insulin 60–150 min later, so a longer run-in period may have given us more dramatic effects. Any regional differences in insulin uptake (45) are unlikely to explain the regional differences in glucose uptake seen.

Our regional data are at first glance surprising, showing a lesser effect of basal insulin replacement in brain regions with the highest densities of insulin receptors. Either glucose metabolism in these, phylogenetically older, brain regions is relatively insulin independent or, as we did not remove insulin entirely from the circulation, glucose uptake was already maximally stimulated, even at the very low insulin levels of our somatostatin and saline infusions. The possible importance of an effect of insulin on brain glucose uptake, which is maximal at normal fasting insulin levels but which is reduced as insulin levels decrease to near zero as might be seen during starvation, are potentially logical. In normal health with adequate nutrition, glucose supply to the brain is the first priority. If, during extreme fasting, glucose supply is becoming critical, it may be that even the brain has to accept a reduced glucose uptake. The regional differences in insulin sensitivity would support maintained glucose supply to areas of the brain critical to homeostasis and immediate survival, i.e., brain stem and cerebellum (46), while permitting reduced glucose supply to the cortex. Additionally it may be that some areas of the brain are more able to use alternative substrates or glucose stores during periods of fasting, explaining the regional differences seen.

Of further interest is the possible existence of cerebral brain insulin resistance. Whole-body insulin resistance has recently been linked in epidemiological studies with increased risk of cortical dysfunction (47) and the rising prominence of insulin-resistance syndromes in disease (48) makes this an important area for further study.

In conclusion, we have shown that insulin does have a significant effect on global brain glucose metabolism and that this effect is mainly in the cerebral cortex. This may be either a direct effect of insulin stimulating glucose uptake and metabolism, as in peripheral tissues, or an indirect effect achieved via insulin-stimulated neuronal activation with secondary increment in cell glucose metabolism. Either way, the data show that insulin can access the insulin receptors of the brain and have a metabolic effect on the brain, which may be maximal at basal (fasting) circulating insulin concentrations. These data give us a tool for the further investigation of brain glucose metabolism in health and disease.

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REFERENCES

1. Fruewald-Schultes B, Kern W, Deininger E, Wellhoener P, Kerner W, Born J, Fehm HL, Peters A: Protective effect of insulin against hypoglycaemia associated counterregulatory failure. *J Clin Endo Metab* 84:1551–1557, 1999
2. Davis M, Mellman M, Shamoon H: Physiologic hyperinsulinemia enhances counterregulatory hormone responses to hypoglycaemia in IDDM. *J Clin Endo Metab* 76:1383–1385, 1993
3. Rodin J, Wack J, Ferrennini E, DeFronzo RA: Effect of insulin and glucose on feeding behaviour. *Metabolism* 34:826–831, 1985
4. Debons AF, Krimsky I, From A: A direct action of insulin on the hypothalamic satiety center. *Am J Physiol* 219:938–943, 1970
5. Kern W, Kerner W, Pietrowsky R, Fehm HL, Born J: Effects of insulin and hypoglycaemia on the auditory brain stem response in humans. *J Neurophysiol* 72:678–683, 1994
6. Hopkins DF, Williams G: Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity. *Diabet Med* 14:1044–1050, 1997
7. Clarke DW, Mudd L, Boyd FT, Fields M, Raizada MK: Insulin is released from rat brain neuronal cells in culture. *J Neurochem* 47:831–836
8. Van Houten M, Posner BI, Kopriwa BM, Brawer JR: Insulin binding sites in the rat brain: in vivo localisation to the circumventricular organs by quantitative autoradiography. *Endocrinology* 105:666–673, 1979
9. Steffens AB, Scheurink AJW, Porte D, Woods SC: Penetration of peripheral glucose and insulin into the cerebrospinal fluid in rats. *Am J Physiol* 255:R200–R204, 1988
10. Banks WA, Jaspan JB, Huang W, Kastin AJ: Transport of insulin across the blood brain barrier: saturability at euglycaemic doses of insulin. *Peptides* 18:1423–1429, 1997
11. Schwartz MW, Sipols A, Kahn SE, Lattemann DF, Taborsky GJ, Bergamn RN, Woods SC, Porte D: Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am J Physiol* 259:E378–E383, 1990
12. Pardridge WM, Eisenberg J, Yang J: Human blood brain barrier insulin receptor. *J Neurochem* 44:1771–1778, 1985
13. Brant AM, Jess TJ, Milligan G, Brown CM, Gould GW: Immunlogical

analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochem Biophys Res Commun* 192: 1297–1302, 1993
14. McCall AL, van Bueren AM, Huang L, Stenbit A, Celnik E, Charron MJ: Forebrain endothelium expresses GLUT4, the insulin responsive glucose transporter. *Brain Res* 744:318–326, 1997
15. Rayner DV, Thomas MEA, Trayhurn P: Glucose transporters (GLUTS1–4) and their mRNAs in the regions of the rat brain: insulin-sensitive transporter expression in the cerebellum. *Can J Physiol Pharmacol* 72:476–479, 1994
16. Ngarmukos C, Baur EL, Kumagai AK: Co-localisation of GLUT1 and GLUT4 in the blood brain barrier of the ventromedial hypothalamus. *Brain Res* 900:1–8, 2001
17. Mueckler M: Facilitative glucose transporters. *Eur J Biochem* 219:713–725, 1994
18. Hom FG, Goodner CJ, Berrie MA: A (^{3}H)2-deoxyglucose method for comparing rates of glucose metabolism and insulin responses among rat tissues in vivo: validation of the model and the absence of an insulin effect on brain. *Diabetes* 33:141–152, 1984
19. Hasselbalch SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, Paulson OB: No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes* 48:1915–1921, 1999
20. Lucignani G, Namba H, Nehlig A, Porrino LJ, Kennedy C, Sokoloff L: Effects of insulin on local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab* 7:309–314, 1987
21. Grunstein HS, James DE, Storlien LH, Smythe GA, Kraegen EW: Hyperinsulinemia suppresses glucose utilization in specific brain regions: in vivo studies using the euglycaemic clamp in the rat. *Endocrinology* 116:604–610, 1985
22. Cranston I, Marsden PK, Matyka K, Evans ML, Lomas J, Sonksen P, Maisey M, Amiel SA: Regional differences in cerebral blood flow and glucose utilisation in diabetic man: the effect of insulin. *J Cereb Blood Flow Metab*18:130–140, 1998
23. DeFeo P, Perriello G, Torlone E, Ventra MM, Santeusanio F, Brunetti P, Gerich JE, Bolli GB: Demonstration of a role for growth hormone in glucose counterregulation. *Am J Physiol* 256:E835–E843, 1989
24. Patlak CS, Blasberg RG, Fenstermacher JD: Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 3:1–7, 1983
25. Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE: Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)-2-fluoro-2-deoxy-D-glucose: validation of method. *Ann Neurol* 6:371–388, 1979
26. Reivich M, Alavi A, Wolf A, Fowler J, Russell J, Arnett C, et al: Glucose metabolic rate kinetic model parameter determination in humans: the lumped constant and rate constants for (18f) fluoro-deoxyglucose and (c11) deoxyglucose. *J Cereb Blood Flow Metab* 5:179–192, 1985
27. Reed LJ, Marsden P, Lasserson D, Sheldon N, Lewsi P, Stanhope N, Guinan E, Kopelman MD: FDG-PET analysis and findings in amnesia resulting from anoxia. *Memory* 7:599–612, 1999
28. Friston KJ, Holmes AP, Worsley KJ, Poline J-B, Frith CD, Frackowiak RSJ: Statistical parametric maps in functional imaging: a general linear approach. *Human Brain Mapping* 2:189–210, 1995
29. Friston KJ, Holmes A, Poline J-B, Frith CD: Detecting activations in PET and fMRI: levels of inference and power. *Neuorimage* 40:223–235, 1996
30. Studholme C, Hill DLG, Hawkes DJ: Automated 3-D registration of magnetic resonance and positron emission tomography brain slices by multi-resolution optimisation of voxel similarity measures. *Med Physics* 24:24–25, 1997
31. Seaquist ER, Damberg GS, Tkac I, Gruetter R: The effect of insulin on in vivo cerebral glucose concentrations and rates of glucose transport/metabolism in humans. *Diabetes* 50:2203–2209, 2001
32. Boyd FTJ, Clarke DW, Muther TF, Raizada MK: Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J Biol Chem* 260:15880–15884, 1983
33. Choi I-Y, Tkac I, Ugurbil K, Gruetter R: Noninvasive measurements of [1–13C] glycogen concentrations and metabolism in rat brain in vivo. *J Neurochem* 73:1300–1308, 1999
34. Chih CP, Lipton P, Roberts EL: Do active cerebral neurons really use lactate rather than glucose? *Trends in Neurosciences* 24:573–578, 2001
35. Dienel GA, Hertz L: Glucose and lactate metabolism during brain activation. *J Neuroscience Res* 66:824–38, 2001
36. Magistretti PJ: Cellular bases of functional brain imaging: insights from neuron-glia metabolic coupling. *Brain Research* 886:108–112, 2000
37. Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ, Sherwin RS: Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. *J Cereb Bl Fl Metab* 22:271–279, 2002
38. Klip A, Paquet MR: Glucose transport and glucose transporters in muscle and their metabolic regulation. *Diabetes Care* 13:228–243, 1990
39. Clarke DW, Boyd FT, Kappy MS, Raizada MK: Insulin binds to specific receptors and stimulates 2-deoxy-D-glucose uptake in cultured glial cells from rat brain. *J Biol Chem* 259:11672–11675, 1984
40. Walker PS, Donovan BG, Van Ness RG, Fellows RE, Pessin JE: Glucose dependent regulation of glucose transport activity, protein and mRNA in primary cultures of rat brain glial cells. *J Biol Chem* 263:15594–15601, 1988
41. Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R, Allen AM, Mendelsohn FA: Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry *Endocrinology* 121:1562–1570, 1987
42. MetcalfeP, Johnston DG, Nosadini R, Orksov H, Alberti KGMM: Metabolic effects of acute and prolonged growth hormone excess in normal and insulin deficient man. *Diabetalogia* 20:123–128, 1981
43. Huang SC, Phelps ME: Principles of tracer kinetic modelling in positron emission tomography and autoradiography. In *Positron Emission Tomography and Autoradiography: Principles and Applications in the Brain and Heart*. Phelps ME, Mazziotta JC, Schelbert HR, Eds. New York, Raven Press, 1996, p. 328–329
44. Long JB, Rigamonti DD, Dosaka K, Kraimer JM, Martinez-Arizala A: Somatostatin causes vasoconstriction, reduces blood flow and increases vascular permeability in the rat central nervous system. *J Pharm and Exp Ther* 260:1425–1432, 1992
45. Schulingkamp RJ, Pagano TC, Hung D, Raffa RB: Insulin receptors and insulin action in the brain: review and clinical implications. *Neuroscience Biobehav Rev* 24:855–872, 2000
46. Wozniak M, Rydzewski B, Baker P, Raizada MK: The cellular and physiological actions of insulin in the central nervous system. *Neurochem Int* 22:1–40, 1993
47. Stolk RP, Breteler MMB, Ott A, Pols HAP, Lamberts SWJ, Grobbee DE, Hofman A: Insulin and cognitive function in an elderly population. *Diabetes Care* 20:792–795, 1997
48. Kuusisto J, Koivisto K, Mykkanen L, Helkala EL, Vanhanen M, Hanninen T, Kesaniemi A, Riekkinen PJ, Laakso M: Association between features of the insulin resistance syndrome and Alzheimer's: cross sectional population based study. *BMJ* 315:1045–1050, 1997