

# The Effect of Insulin on In Vivo Cerebral Glucose Concentrations and Rates of Glucose Transport/Metabolism in Humans

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The continuous delivery of glucose to the brain is critically important to the maintenance of normal metabolic function. However, elucidation of the hormonal regulation of in vivo cerebral glucose metabolism in humans has been limited by the lack of direct, noninvasive methods with which to measure brain glucose. In this study, we sought to directly examine the effect of insulin on glucose concentrations and rates of glucose transport/metabolism in human brain using <sup>1</sup>H-magnetic resonance spectroscopy at 4 Tesla. Seven subjects participated in paired hyperglycemic ( $16.3 \pm 0.3$  mmol/l) clamp studies performed with and without insulin. Brain glucose remained constant throughout ( $5.3 \pm 0.3$   $\mu$ mol/g wet wt when serum insulin =  $16 \pm 7$  pmol/l vs.  $5.5 \pm 0.3$   $\mu$ mol/g wet wt when serum insulin =  $668 \pm 81$  pmol/l, *P* = NS). Glucose concentrations in gray matter-rich occipital cortex and white matter-rich periventricular tissue were then simultaneously measured in clamps, where plasma glucose ranged from 4.4 to 24.5 mmol/l and insulin was infused at  $0.5$  mU  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>. The relationship between plasma and brain glucose was linear in both regions. Reversible Michaelis-Menten kinetics fit these data best, and no differences were found in the kinetic constants calculated for each region. These data support the hypothesis that the majority of cerebral glucose uptake/metabolism is an insulin-independent process in humans. *Diabetes* 50:2203–2209, 2001

The brain relies on the continuous delivery of glucose via the blood to maintain normal metabolic function. How glucose delivery into the central nervous system is regulated and how that delivery is altered by different metabolic conditions in the living human have been difficult to ascertain. In particular, the role of insulin in the regulation of cerebral glucose metabolism has been difficult to directly assess. Although evidence acquired using both in vitro and in vivo approaches support and refute the hypothesis that insulin regulates the entry of glucose into brain tissue (1–9), studies performed in living animals have been limited by

their inability to directly measure cerebral glucose concentrations.

The brain is composed of both gray and white matter. Both rely on glucose for the maintenance of normal function, but the rates at which they metabolize glucose have been found to be different (9,10). Whether these differences in glucose metabolism equate to differences in cerebral glucose concentrations and whether insulin differentially regulates regional cerebral glucose metabolism have not been directly examined in humans because, until recently, direct methods to measure brain glucose concentrations in healthy subjects have not been feasible. Invasive methods to measure intracerebral glucose concentrations, such as the use of an implanted equilibrium dialysis probe, have been reported in abstract form (11), but such a technique is not acceptable in the study of normal human physiology. Indirect methods to quantitate brain glucose concentrations, such as those based on measuring the difference between glucose concentrations in the arterial circulation and the venous effluent of the brain (12), have provided important insights into understanding the regulation of brain-glucose uptake but are limited by the model assumptions used for data analysis (13). Positron emission tomography (PET) has also been used to effectively measure regional rates of cerebral glucose metabolism (14), but this method is limited by frequent reliance on a non-native glucose analog, an inability to directly measure metabolite concentration, and difficulty separating signals derived from different chemical species. PET also has difficulty obtaining serial studies from the same subject due to the radiation risks posed by the isotope used in the study (14).

Our group has recently developed magnetic spectroscopic methods that allow us to directly and noninvasively measure brain glucose concentrations in living humans during periods of tightly controlled plasma glucose (15,16). We used these methods to examine the hormonal regulation of glucose concentration in both the gray and white matter tissue of healthy humans. Our studies were designed to test the hypothesis that cerebral glucose transport/metabolism is largely an insulin-independent process. We also sought to determine whether gray and white matter glucose concentrations and metabolic rates differ under steady-state conditions.

## RESEARCH DESIGN AND METHODS

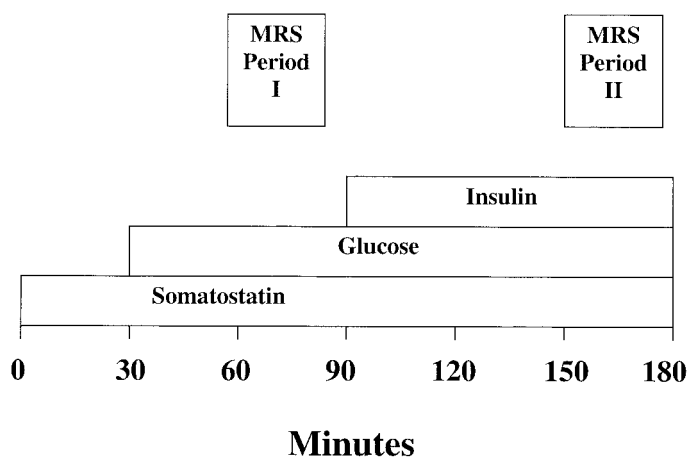
Healthy subjects were recruited from the students and staff at the University of Minnesota. Before participation, subjects provided informed consent as governed by the Institutional Review Board at the University of Minnesota. Magnetic resonance spectroscopy (MRS) was used to measure brain glucose

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MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance; PET, positron emission tomography.



**FIG. 1. Protocol for experiment 1.** Somatostatin infusion was begun at time zero and advanced to a rate of  $0.16 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 30 min and then maintained throughout the study. Glucose infusion was begun at minute 30 to maintain a constant plasma glucose concentration of 16.7 mmol/L.  $^1\text{H}$ -MRS was performed from minute 60 to 90 (period I) for later calculation of brain glucose concentration in the absence of insulin. Insulin was begun at  $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at minute 90.  $^1\text{H}$ -MRS was performed from minute 150 to 189 (period II) for later calculation of brain glucose concentration in the presence of insulin.

concentrations. During the spectroscopic studies, plasma concentrations of both glucose and insulin were controlled at a constant target level using the glucose/insulin clamp technique. We performed two sets of experiments to address our study hypothesis.

**Experiment 1.** Subjects came to the Center for Magnetic Resonance Research in the morning after an overnight fast. In preparation for the study, an intravenous catheter was placed retrograde into one foot for the acquisition of blood samples and two additional intravenous catheters were placed into the upper extremities for the delivery of somatostatin, insulin, and glucose. The leg used for blood sampling was wrapped in heated towels and hot packs to arterialize the venous blood (17). After obtaining baseline samples for glucose and ketones, a somatostatin infusion was begun and advanced to a rate of  $0.16 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 30 min to suppress endogenous insulin secretion (18). Once the target somatostatin infusion rate had been achieved, glucose (50% dextrose) was infused as necessary to achieve and maintain the plasma concentration at 16.7 mmol/L. Beginning with the initiation of the somatostatin infusion, blood samples were obtained every 5 min for determination of plasma glucose concentration (using a Beckman Autoanalyzer). Additional samples were obtained every 30 min for later determination of serum insulin concentrations by radioimmunoassay (19). At the end of the first 60 min of somatostatin infusion, subjects were placed into the 4 Tesla magnet for examination by  $^1\text{H}$ -MRS in the absence of insulin (period I). When the collection of the spectral data was complete, subjects were removed from the magnet, given an intravenous infusion of insulin at a rate of  $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and maintained at a plasma glucose concentration of 16.7 mmol/L. The insulin infusion rate was selected to bring subjects to a serum insulin concentration in the high physiological range. After 1 h, subjects were placed back into the magnet and spectral data were collected in the presence of insulin (period II). Figure 1 details the protocol followed for experiment 1.

**Experiment 2.** For these experiments, subjects also reported to the Center for Magnetic Resonance Research in the morning after an overnight fast. The protocol as detailed for experiment 1 was followed, except that at time 0, insulin was begun at a rate of  $0.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . The insulin infusion rate was selected to bring subjects to a serum insulin concentration in the physiological range. After 45 min, subjects were either maintained at euglycemia or given a bolus injection of dextrose (50% in water) to bring them to target glycemia (5–30 mmol/L). Plasma glucose was then clamped at target glycemia by the infusion of dextrose (50% in water), and subjects were placed in the magnet for data acquisition. After an additional 45 min, some subjects were given an additional bolus injection of dextrose to bring them to a second hyperglycemic target.

**MRS.** Experiments were performed on a 4 Tesla Siemens/Oxford magnet interfaced to a Varian console as previously described (16,20). Subjects were placed supine on a bed above the surface coil and their heads were held in place by cushions. To minimize exposure to gradient noise, all subjects wore earplugs. A quadrature transmit/receive RF coil consisting of two 14-cm diameter single-turn coils was used (21). A 16–27 ml nominal volume of gray

matter-rich occipital cortex was selected for study for both experiments 1 and 2. For experiment 2, an additional 16 ml nominal volume of periventricular white matter-rich tissue was also selected for study. Localization of the signals was based on  $T_1$ -weighted MDEFT (22) or FSE (23) imaging. Localized shimming was performed using FASTMAP (24), which has shown consistent 7- to 8-Hz linewidths for the water resonance at 4 Tesla. For the measurements, we used  $TE = 4\text{--}20 \text{ ms}$ ,  $TM = 33 \text{ ms}$ ,  $TR = 4.5 \text{ (25)}$ . Water suppression was accomplished by the application of four 25-ms Gaussian pulses (26) or by applying a series of RF pulses according to the VAPOR scheme (27). In either case, we verified that water suppression did not affect the signal by  $>5\%$  outside  $\pm 0.4 \text{ ppm}$ . Outer volume saturation was achieved in slices adjacent to the volume of interest using hyperbolic secant pulses with variable RF power (28). Free induction decays were averaged over a time period of  $\sim 1 \text{ min}$ , stored separately in memory, and then corrected for small frequency changes and averaged over at least 10 min. In each case, the plasma glucose concentrations were maintained at steady state for at least 20 min before spectral data were acquired. For experiment 1, data were collected over a minimum of 10 min while glycemia was clamped at the target level. For experiment 2, data were acquired in alternating mode simultaneously from the occipital and the periventricular regions over a minimum of 20 min, during which glycemia was clamped at target level.

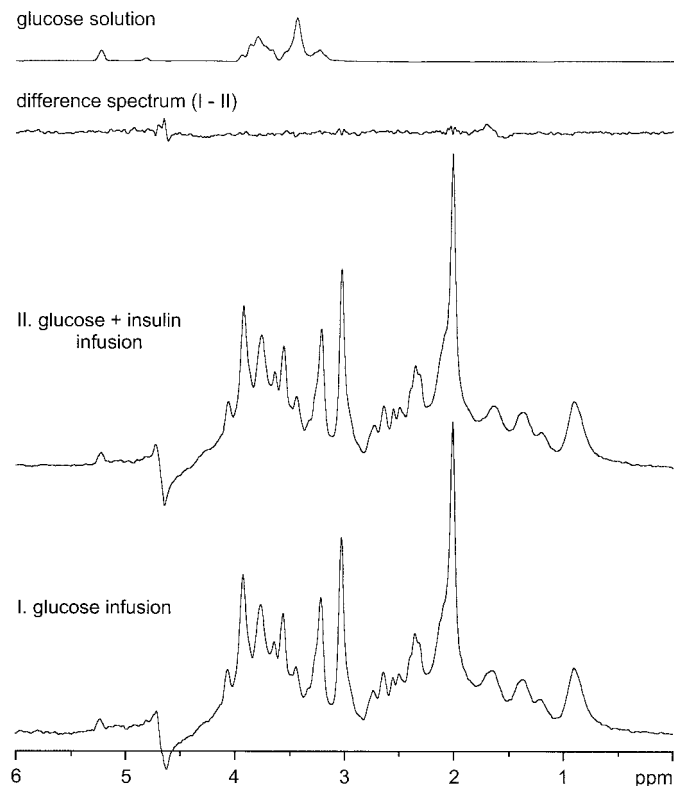
Localized proton nuclear magnetic resonance (NMR) signals, obtained by the methods described above, were zero filled and apodized with 2-Hz exponential linebroadening. Peak areas were quantified using peak-fitting software supplied by the spectrometer manufacturer as previously described (15,16). Quantification of the glucose peak at 5.23 ppm was performed as previously described and validated by comparison with measurements made using  $^{13}\text{C}$ -MRS in our laboratory (16). In brief, the area under the glucose peak at 5.23 ppm was calculated relative to the area under the creatine methyl resonance at 3.04 ppm. For gray matter-rich regions, the concentration of creatine was set to  $10 \mu\text{mol/g wet wt}$ , based on cortical concentrations of  $9.6 \mu\text{mol/g wet wt}$  (29) and on contributions of  $1 \mu\text{mol/g wet wt}$   $\gamma$ -aminobutyric acid and  $1\text{--}2 \mu\text{mol/g wet wt}$  glutathione in this region of the brain (30). The creatine concentration in the white matter-rich voxels was calculated to that in the gray matter using the relative requirements in RF power as a means to correct for the small differences in RF coil sensitivity. The half-volume RF coil design we used (21) resulted in the  $90^\circ$  RF power setting, varying by 1–2 dB, which corresponds to a signal correction between 12 and 25%. The creatine concentration was  $7.3 \pm 0.9 \mu\text{mol/g wet wt}$  (mean  $\pm$  SD) for the white matter-rich areas, which suggests that the majority of our voxel contained white matter according to Hetherington et al. (31); this conclusion was further supported by the approximately twofold higher choline to creatine ratio.

**Data analysis.** Data are means  $\pm$  SE. To minimize the potential effects of intersubject variation and modifications to magnetic resonance methodology, all data acquired during experiment 1 were evaluated based on intrasubject paired comparison, using observations obtained during the same study session. The data acquired during experiment 2 were analyzed using the reversible Michaelis-Menten model as previously described (16). This model assumes that *trans* membrane glucose influences the affinity for *cis* membrane glucose transport (product inhibition). Differences between groups were detected using Student's *t* test.

## RESULTS

**Experiment 1.** Seven healthy subjects (four women and three men) were recruited for participation in our investigation. Their mean age was  $30 \pm 2$  years, and their mean BMI was  $21.8 \pm 0.2 \text{ kg/m}^2$ . During period I, when somatostatin and glucose were infused, the serum insulin concentration was  $16 \pm 7 \text{ pmol/L}$  and the plasma glucose concentration was maintained at  $16.5 \pm 0.5 \text{ mmol/L}$ . During period II, when somatostatin, glucose, and insulin were infused, serum insulin rose to  $668 \pm 81 \text{ pmol/L}$  and plasma glucose remained stable at  $16.0 \pm 0.4 \text{ mmol/L}$ .

The glucose signal at 5.23 ppm in the  $^1\text{H}$  spectrum was easily resolved under the hyperglycemic conditions of our study (Fig. 2). No difference was found between the brain glucose concentrations measured in the absence and presence of insulin. During period I, when insulin secretion was suppressed, brain glucose concentration was  $5.3 \pm 0.3 \mu\text{mol/g wet wt}$ , whereas during period II, when insulin was infused at  $2.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , brain glucose was  $5.5 \pm 0.3 \mu\text{mol/g wet wt}$  ( $P = \text{NS}$ ) (Fig. 3). The 95%

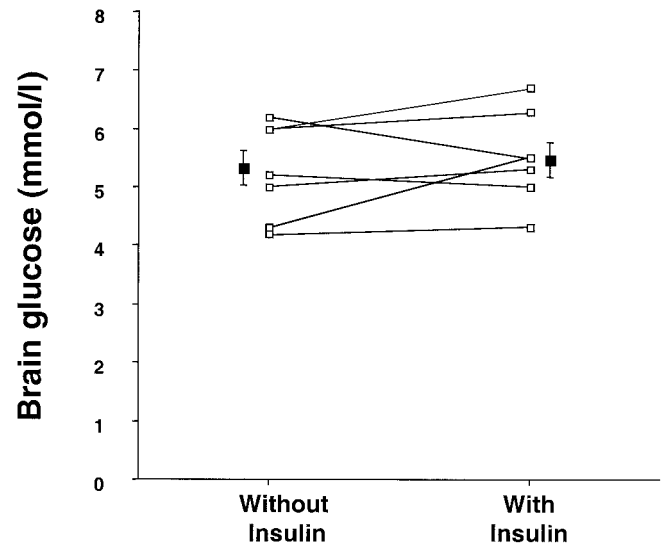


**FIG. 2.** Effect of insulin infusion on cerebral metabolites as measured by  $^1\text{H}$ -MRS. During a hyperglycemic clamp study, a subject was infused with somatostatin ( $0.16 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), and plasma glucose was held constant at  $15 \text{ mmol/l}$ . After 60 min of stable glycemia, a  $^1\text{H}$  spectrum was obtained from the occipital cortex (I. glucose infusion). The subject was then removed from the magnet and given an infusion of insulin ( $2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and a second  $^1\text{H}$  spectrum was obtained at the end of 90 min (II. glucose + insulin infusion). An in vivo difference spectrum was obtained by subtracting spectrum I from spectrum II. The glucose peaks at 5.23 and 3.44 ppm are readily seen in the spectrum of standard glucose solution and in spectrums I and II, but were found to be absent in the in vivo difference spectrum.

confidence interval for the difference in brain glucose concentration measured during periods I and II was  $0.1$ – $0.9 \mu\text{mol/g}$  wet wt, and the standard deviation of this difference was  $0.2 \mu\text{mol/g}$  wet wt. With this variability around the mean, our sample size of seven provided an 80% likelihood of identifying a difference of  $>0.3 \mu\text{mol/g}$  wet wt between the brain glucose concentration measured in the absence and presence of insulin and a 90% likelihood of detecting a difference  $>0.4 \mu\text{mol/g}$  wet wt.

Although the analysis was based on paired statistics and was therefore considered to be independent of interindividual differences, we sought to further evaluate our findings by generating in vivo difference spectra for each subject. These difference spectra were created by subtracting the  $^1\text{H}$  spectrum acquired in the absence of insulin from that acquired in the presence of insulin. In every subject, the resulting spectrum was a flat line, as shown in Fig. 2, suggesting that any difference in brain glucose signal acquired under the two experimental conditions was below the noise level of the instrument.

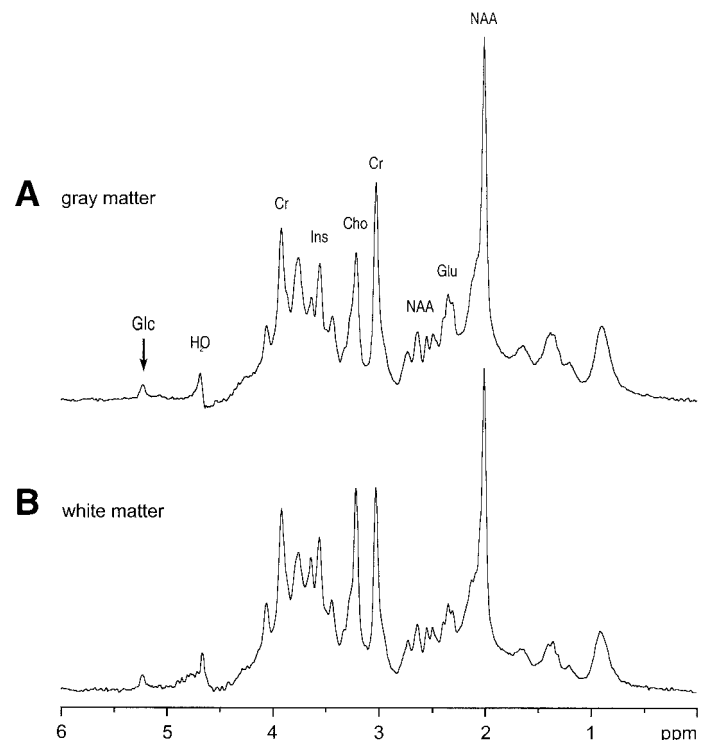
**Experiment 2.** Twenty healthy subjects were recruited for participation in this experiment. Their mean age was  $35 \pm 2$  years, and their mean BMI was  $26.1 \text{ kg/m}^2$ . Brain glucose concentrations were measured at serum glucose concentrations ranging from  $4.4$  to  $24.5 \text{ mmol/l}$ . Serum



**FIG. 3.** Effect of insulin infusion on brain glucose concentrations. Seven subjects were studied using the protocol described in the text. Brain glucose concentrations measured without insulin were not different from concentrations measured with insulin.  $\square$ , Individual subjects;  $\blacksquare$ , means  $\pm$  SE.

insulin concentrations averaged  $108 \pm 6 \text{ pmol/l}$  during these experiments. Figure 4 shows representative spectra acquired simultaneously from both gray and white matter-rich regions in a single subject.

The relationship between plasma glucose concentration and brain glucose concentration in both the gray and white matter-rich areas was observed to be linear (Fig. 5).



**FIG. 4.**  $^1\text{H}$ -MRS of gray and white matter. A single subject was studied at stable hyperglycemia during the infusion of somatostatin ( $0.16 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and insulin ( $0.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Spectra were acquired simultaneously from a 27-ml volume of gray matter-rich cortex (A) and a 16-ml volume of white matter-rich periventricular tissue (B). Cho, choline; Cr, creatine; Glc, glucose; Glu, glutamate; Ins = inositol; NAA, N-acetyl-aspartate.



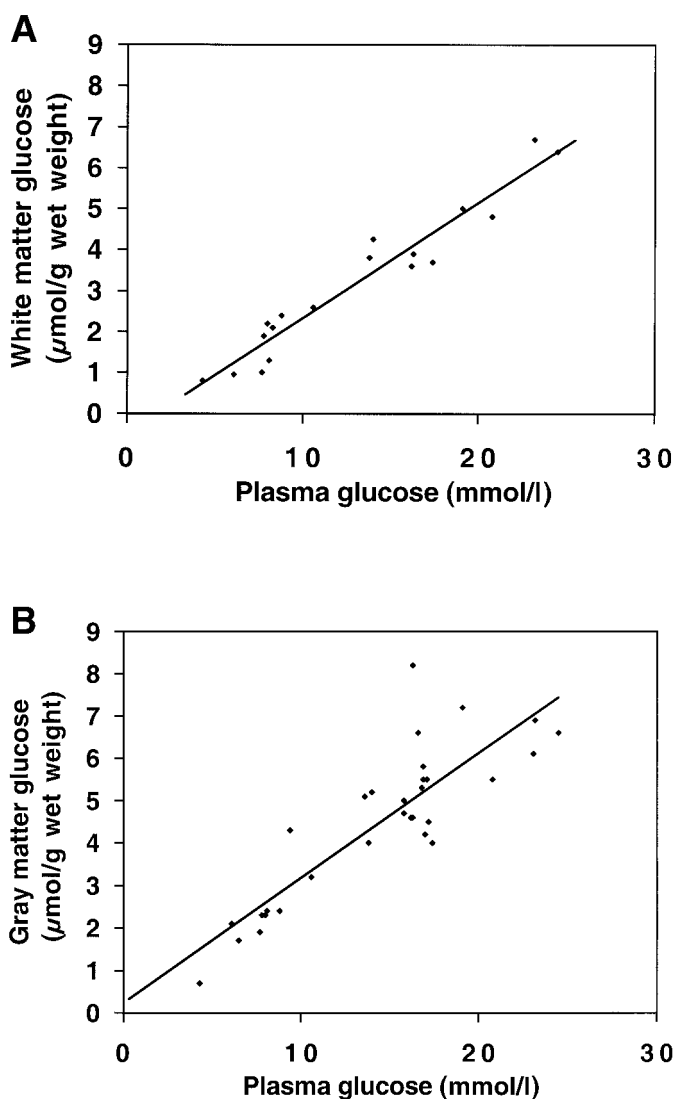


FIG. 5. Relationships between glucose concentrations in plasma and brain at physiologic insulin levels. Healthy subjects participated in clamp experiments as described in RESEARCH DESIGN AND METHODS. Each ■ represents a single experiment. **A:** Data acquired from the periventricular white matter of 10 subjects during a total of 18 experiments. **B:** Data acquired from the gray matter-rich occipital cortex of 23 subjects during a total of 31 experiments. A linear relationship was found between plasma glucose concentrations and the concentrations of glucose measured in each brain region.

Reversible Michaelis-Menten kinetics fit these data best, as we previously found in experiments in which serum insulin concentrations were suppressed by the infusion of somatostatin (16). The kinetics constants calculated for the occipital cortex from these experiments with insulin were indistinguishable from the constants calculated from the data previously obtained during the experiments without insulin (Table 1).

In 10 experiments, data were simultaneously acquired from both the gray and white matter-rich regions during stable glycemia. As shown in Table 2, the values calculated for the white matter-rich periventricular tissue were significantly lower than the values calculated for the gray matter-rich occipital cortex ( $P < 0.01$ ). However, when the data were corrected for the small difference in water content between gray (0.83 ml/g) and white (0.75 ml/g) matter (32), the average ratio between gray and white

TABLE 1

Kinetic constants calculated using reversible Michaelis-Menten model

	$K_t$ (mmol/l)	$T_{max}/CMR_{glc}$
White matter-rich region in the presence of insulin	$1.96 \pm 2.45$	$2.15 \pm 0.25$
Gray matter-rich cortex in the presence of insulin	$-0.98 \pm 2.13$	$2.24 \pm 0.23$
Gray matter-rich cortex in the absence of insulin*	$0.6 \pm 2.0$	$2.3 \pm 0.2$

Data are means  $\pm$  SE. \*Data previously published (16).

matter glucose concentration was  $1.04 \pm 0.04$ , which is no longer significantly different from 1.0, as judged from fitting  $y = ax$ . No statistically significant differences were found between kinetic constants calculated for these separate regions of cerebral tissue (Table 1).

## DISCUSSION

The purpose of these experiments was to determine whether insulin plays a significant role in the regulation of cerebral glucose transport/metabolism in healthy human subjects. To address this question, we used the novel technique of high-field  $^1H$ -MRS and, for the first time, directly and noninvasively examined the effect of this hormone on the concentration of native glucose in the living human brain. We found that the infusion of insulin was without significant effect on the in vivo glucose concentration measured in the gray matter-rich occipital cortex of normal human volunteers. We further observed that the glucose transport kinetics calculated from data acquired from the occipital cortex in the presence of insulin were not different from those calculated previously in the absence of insulin. Taken together, these findings support the hypothesis that cerebral glucose transport/metabolism are largely an insulin-independent process.

The role of insulin in the regulation of brain-glucose metabolism has long been an area of controversy. Although most investigators have concluded that insulin was without effect on glucose transport across the blood-brain barrier (3–6,9), Hertz et al. (1) reported that insulin increased glucose transport in humans and Namba et al. (8) made similar observations in rats. However, a recent re-evaluation by Knudsen et al. (33) of the data from Hertz et al. (1) suggested that insulin may not truly have an effect on glucose transport/metabolism in humans, a conclusion that is also supported by the report of Cranston et al. (7). Interestingly, both insulin receptors (34–36) and insulin-sensitive GLUT4 (37–41) have been found at the blood-brain barrier, and insulin has been shown to be an important modulator of the autonomic response to hypoglycemia (42) and of feeding behavior (43). Insulin crosses the blood-brain barrier via receptor-mediated transcytosis. Despite the obvious importance of the hormone in regulating some cerebral functions, our observations, particularly the finding that the in vivo difference spectrum created by subtracting the data collected in the presence of insulin from that collected in its absence is a flat line, provide support for the hypothesis that insulin does not alter glucose content or any other brain metabolites detectable by  $^1H$ -NMR spectroscopy. Hence, insulin ap-

TABLE 2  
Simultaneously measured gray and white matter glucose concentrations during stable glycemia

Subject	Plasma glucose (mmol/l)	Gray matter glucose ( $\mu\text{mol/g}$ wet wt)	White matter glucose ( $\mu\text{mol/g}$ wet wt)	Gray matter glucose: white matter glucose ( $\mu\text{mol/g}$ wet wt)	Gray matter glucose ( $\mu\text{mol/ml}$ brain water)*	White matter glucose ( $\mu\text{mol/ml}$ brain water)†	Gray matter glucose: white matter glucose ( $\mu\text{mol/ml}$ brain water)
1	8.4 $\pm$ 0.1	2.4	2.4	1.0	2.9	3.2	0.9
2	5.9 $\pm$ 0.1	2.1	1.0	2.1	2.5	1.3	1.9
3	13.8 $\pm$ 0.1	4.0	3.8	1.1	4.8	5.1	0.9
4	7.8 $\pm$ 0.1	2.3	1.9	1.2	2.8	2.5	1.1
5	20.5 $\pm$ 0.3	5.5	4.8	1.1	6.6	6.4	1.0
6	8.1 $\pm$ 0.1	2.4	1.3	1.8	2.9	1.7	1.7
7	23.2 $\pm$ 0.3	6.9	6.7	1.0	8.3	8.9	0.9
8	13.8 $\pm$ 0.1	5.2	4.3	1.2	6.3	5.7	1.1
9	19.1 $\pm$ 0.1	7.2	5	1.4	8.7	6.7	1.3
10	8.0 $\pm$ 0.1	2.3	2.2	1.0	2.8	2.9	1.0
11	24.2 $\pm$ 0.5	6.6	6.4	1.0	8.0	8.5	0.9
12	16.3 $\pm$ 0.2	4.6	3.9	1.2	5.5	5.2	1.1
13	7.7 $\pm$ 0.1	1.9	1	1.9	2.3	1.3	1.8
14	17.9 $\pm$ 0.4	4.0	3.7	1.1	4.8	4.9	1.0
15	10.6 $\pm$ 0.1	3.2	2.6	1.2	3.9	3.5	1.1
16	16.3 $\pm$ 0.1	4.6	3.6	1.3	5.5	4.8	1.1
17	4.7 $\pm$ 0.1	0.7	0.8	0.9	0.8	1.1	0.7
Mean $\pm$ SE	—	—	—	1.4 $\pm$ 0.2	—	—	1.1 $\pm$ 0.1

Data are means  $\pm$  SE. \*Assumed gray matter water content = 0.83 ml/g (31); †assumed white matter content = 0.75 ml/g (31).

pears to be without effect on glucose transport across the blood-brain barrier when metabolism remains unchanged.

In our first experiment, we used a paired comparison to determine whether insulin altered intracerebral glucose concentrations. With a sample size of seven, this study had sufficient power to detect a difference as small as 0.36  $\mu\text{mol/g}$  wet wt, with 90% likelihood. Such a value represents 7% of the mean brain glucose concentration measured during our experiments and is unlikely to be of major physiological significance. Thus, although a small effect of insulin on glucose transport across the blood-brain barrier cannot be completely eliminated with our data, such as an effect on the slow glycogen metabolism in brain (44), we feel confident that this hormone does not play a clinically relevant role in the regulation of brain-glucose uptake.

Glucose concentrations in the brain are achieved through a careful balance between glucose entering the brain across the blood-brain barrier and glucose leaving the brain through metabolism. For glucose concentration to remain constant under steady-state conditions, the rate of glucose influx must equal the rate of glucose efflux. Recent investigations using PET have demonstrated that insulin was without effect on the metabolic rate of glucose in the cerebral cortex of humans (7,9). These observations, coupled with the data presented in the present study, provide strong evidence that insulin is without effect on the kinetics of glucose transport across the blood-brain barrier. Future investigation into the role of insulin in the regulation of intracerebral-glucose metabolism will therefore be of interest. Particularly intriguing are the observations of Nelson et al. (45), who reported that insulin administration before death was shown to increase the amount of glycogen present in the post-mortem rat brain. However, the micromolar glycogen

concentrations that have recently been measured in anesthetized rats in vivo (44) suggest that the contribution of glycogen to overall cerebral glucose metabolism must be small.

In the second experiment, we measured the kinetic constants for glucose transport across the blood-brain barrier for both gray and white matter-rich regions. Using the reversible Michaelis-Menten model, the resulting kinetic constants were not statistically significantly different, implying that insulin has similar effects on glucose uptake/metabolism in both gray and white matter glucose uptake/metabolism. Interestingly, we observed that the steady-state concentrations of the white matter-rich periventricular tissue were significantly lower than simultaneously measured concentrations in the gray matter-rich occipital cortex. This quantification ( $\mu\text{mol/g}$  wet wt) was performed on a per gram of wet weight basis, and the ratio between gray and white matter was  $1.4 \pm 0.2$ . However, the results do not imply that a significant chemical glucose concentration gradient exists between white and gray matter. When expressing the glucose concentrations on a per milliliter of brain water basis, i.e., when correcting for the small difference in water content between gray (0.83 ml/g) and white (0.75 ml/g) matter (32), the average ratio between gray and white matter glucose concentration ( $\mu\text{mol/ml}$  brain water) was  $1.1 \pm 0.01$ , which is no longer significantly different from 1.0. Therefore, we conclude that the chemical concentration gradient between gray and white matter is not significant. Because glucose transport in the brain occurs by facilitated diffusion, this implies that there is no net glucose mass transfer between gray and white matter. Other investigators have consistently measured the metabolic rate of glucose in white matter to be significantly lower than that in gray matter (9,10), which implies that there are

marked differences in  $T_{\max}$  between these tissues. This difference can be considered to also reflect the density of the transporter at the blood-brain barrier (the permeability surface area product); therefore, it is related to the blood volume in gray and white matter. Thus, it appears that brain glucose content is heavily regulated in normal brain tissue, underscoring the important role it plays in normal brain function.

The study of in vivo cerebral glucose metabolism in humans has been limited by the inability to directly and noninvasively measure brain glucose concentrations. Techniques such as the indicator dilution method (12) and PET have provided important insights into the regulation of cerebral glucose transport and metabolism in humans but are by their very nature indirect and based on assumptions that are difficult to experimentally confirm. The microdialysis method allows direct measurement of extracellular brain glucose, which appears to be equal to intracellular glucose concentrations (46), but may become inaccurate over time because of the scarring that occurs in the tissue surrounding the probe. In addition, the invasiveness of the technique precludes its application to general investigation of healthy volunteers.  $^{13}\text{C}$ -MRS at low field has offered a solution to the limitations presented by these indirect or invasive techniques, but it requires the infusion of  $^{13}\text{C}$ -glucose to achieve sufficient signal-to-noise to allow quantification of brain glucose content (47).  $^1\text{H}$ -MRS does not require the infusion of substrate and uniquely allows for the direct study of native glucose in brain tissue. However, at lower field strengths, the resolution of the glucose signal in the  $^1\text{H}$  spectrum has been difficult. Most investigators working at lower field strengths have relied on a glucose signal of 3.43 ppm for quantification purposes (48–50). However, this peak overlaps with *myo*-inositol and does not easily allow for precise quantification (15,51). The peak at 5.23 ppm is free of spectral overlap with other metabolic species (15) and can be well resolved for quantification using a field strength of 4 Tesla and the methods of shimming used in the current study.

One limitation of  $^1\text{H}$ -MRS is the difficulty that can be encountered in measuring brain glucose content under hypoglycemic conditions. However, if long acquisition times are used so that the signal-to-noise ratio can be optimized, data sufficient for accurate quantification of glucose can be acquired. Another limitation is that the glucose content measured in a region reflects the glucose present in both cerebral tissue and the intravascular space. However, since cerebral blood volume is minimal (on the order of 2–4% of total volume) (52–54), the contribution of blood glucose to the measurement of brain glucose is small and without effect on the calculation of glucose transport kinetics across the blood-brain barrier. At a plasma concentration of 15 mmol/l, the contribution of blood to the glucose concentration measured in gray matter would be  $\sim 0.6$  mmol/l or  $\sim 10\%$  of the total glucose concentration measured in a volume of cerebral tissue. When the data are adjusted for the contribution of intravascular glucose to the overall glucose concentration in the brain, the conclusions drawn from the data are unchanged. Consequently,  $^1\text{H}$ -MRS at high field strengths represents the best way to directly measure the concentrations of native glucose in the brain of human volun-

teers. This is supported by the observation of resolved glucose resonances in the rat brain at 9.4 Tesla (27).

In summary, our experiments demonstrate that  $^1\text{H}$ -MRS at 4 Tesla is a powerful investigative tool with which to directly and noninvasively measure the concentration of glucose in cerebral tissue. Using this technique, we demonstrated that the infusion of insulin during constant hyperglycemia was without effect on brain glucose concentrations and that the kinetics of glucose transport calculated from data acquired in the presence of insulin are not different from those calculated from data acquired in the absence of insulin. In addition, we found that the kinetic constants calculated for both gray and white matter-rich regions in the presence of insulin are similar, indicating that the hormone has similar effects on glucose uptake/metabolism in both types of cerebral tissue. All together, this study demonstrated the power that MRS brings to the study of in vivo metabolism and illustrated how the method can be used to uniquely and directly address questions important in human physiology and disease.

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#### REFERENCES

- Hertz MM, Paulson OB, Barry DI, Christiansen JS, Svendsen PA: Insulin increases glucose transfer across the blood-brain barrier in man. *J Clin Invest* 67:5579–5604, 1981
- Gottstein U, Held K, Sebening H, Walpurger G: Der glucoseverbrauch des menschlichen gehirns unter dem einfluss intravenoser infusionen von glucose, glucagon und glocose-insulin. *Klinische Wochenschrift* 43:973–974, 1965
- Hofer RE, Lanier WL: Effects of insulin on blood, plasma, and brain glucose in hyperglycemic diabetic rats. *Stroke* 22:505–509, 1991
- Eastman RC, Carson RE, Gordon MR, Berg GW, Lillioja S, Larson SM, Roth J: Brain glucose metabolism in noninsulin-dependent diabetes mellitus: a study in Pima Indians using positron emission tomography during hyperinsulinemia with euglycemic glucose clamp. *J Clin Endocrinol Metab* 71:1602–1610, 1990
- Crone C: Facilitated transfer of glucose from blood into brain tissue. *J Physiol* 181:103–113, 1965
- Daniel PM, Love ER, Pratt OE: Insulin and the way the brain handles glucose. *J Neurochem* 25:471–476, 1975
- Cranston I, Marsden P, Matyka K, Evans M, Lomas J, Sonksen P, Maisey M, Amiel SA: Regional differences in cerebral blood flow and glucose utilization in diabetic man: the effect of insulin. *J Cereb Blood Flow Metab* 18:130–140, 1998
- Namba H, Lucignani G, Nehlig A, Patlak C, Pettigrew K, Kennedy C, Sokoloff L: Effects of insulin on hexose transport across blood-brain barrier in normoglycemia. *Am J Physiol* 252:E299–E303, 1987
- 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
- Jakobsen J, Nedergaard M, Aarslew-Jensen M, Diemer N: Regional brain glucose metabolism and blood flow in streptozocin-induced diabetic rats. *Diabetes* 39:437–440, 1990
- Wallace EM, During M, Sherwin RS: Direct measurement of interstitial glucose concentration in the human brain: effect of changing circulating glucose (Abstract). *Diabetes* 43 (Suppl. 1):47A, 1994



12. Knudsen GM, Pettigrew KD, Paulson OB, Hertz MM, Patlak CS: Kinetic analysis of blood-brain barrier transport of D-glucose in man: quantitative evaluation in the presence of tracer backflux and capillary heterogeneity. *Microvasc Res* 39:28–49, 1990
13. Pelligrino DA, LaManna JC, Duckrow RB, Bryan Jr RM, Harik SI: Hyperglycemia and blood-brain barrier glucose transport. *J Cereb Blood Flow Metab* 12:887–899, 1992
14. Huang SC, Phelps ME, Hoffman EJ, Sideris K, Selin CJ, Kuhl DE: Noninvasive determination of local cerebral metabolic rate of glucose in man. *Am J Physiol* 238:E69–E82, 1980
15. Gruetter R, Garwood M, Ugurbil K, Seaquist ER: Resolved observation of glucose signals in  $^1\text{H}$  NMR spectra of the human brain at 4 Tesla. *Magn Reson Med* 36:1–6, 1996
16. Gruetter R, Ugurbil K, Seaquist ER: Steady-state cerebral glucose concentration and transport in the human brain. *J Neurochem* 70:397–408, 1998
17. Seaquist ER: Comparison of arterialized venous sampling from the hand and foot in the assessment of *in vivo* glucose metabolism. *Metabolism* 46:1364–1366, 1997
18. Yen SSC, Siler TM, DeVane GW: Effect of somatostatin in patients with acromegaly. *N Engl J Med* 290:935–938, 1974
19. Morgan C, Lazarow A: Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic, and diabetic rats. *Diabetes* 12:115–126, 1963
20. Seaquist ER, Gruetter R: Identification of a high concentration of scyllo-inositol in the brain of a healthy human subject using  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR. *Magn Reson Med* 39:313–316, 1998
21. Adriany G, Gruetter R: A half-volume coil for efficient proton decoupling in humans at 4 tesla. *J Magn Reson* 125:178–184, 1997
22. Lee JH, Garwood M, Menon R, Adriany G, Andersen P, Truwit CK, Ugurbil K: High contrast and fast three dimensional imaging at high fields. *Magn Reson Med* 34:308–312, 1995
23. Hennig J, Nauerth A, Friedburg H: RARE imaging: a fast imaging method for clinical MR. *Magn Reson Med* 3:823–833, 1986
24. Gruetter R: Automatic, localized *in vivo* adjustment of all first- and second-order shim coils. *Magn Reson Med* 29:804–811, 1993
25. Tkac I, Seaquist ER, Gruetter R: A comparison of several methods for quantification of resolved glucose signals in  $^1\text{H}$  NMR of the human brain (Abstract). In *Proceedings of the Annual Meeting of the International Society of Magnetic Resonance in Medicine*. Berkeley, CA, SMR, 2000, p. 1938
26. Moonen CTW, van Zijl PCM: Highly effective water suppression for *in vivo* proton NMR spectroscopy (DRYSTREAM). *J Magn Reson* 88:28–41, 1990
27. Tkac I, Starcuk Z, Choi I-Y, Gruetter R: *In vivo*  $^1\text{H}$  NMR spectroscopy of rat brain at 1 ms echo time. *Magn Reson Med* 41:649–656, 1999
28. De Graaf RA, Luo Y, Terpstra M, Garwood M: Spectral editing with adiabatic pulses. *J Magn Reson B* 109:184–193, 1995
29. Petroff OA, Spencer DD, Alger JR, Prichard JW: High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. *Neurology* 39:1197–1202, 1989
30. Rothman D, Petroff O, Behar K, Mattson R: Localized  $^1\text{H}$  NMR measurements of GABA levels in human brain *in vivo*. *Proc Natl Acad Sci U S A* 90:5662–5666, 1993
31. Hetherington HP, Pan JW, Mason GF, Adams D, Vaughn MJ, Twieg DB, Pohost GM: Quantitative  $^1\text{H}$  spectroscopic imaging of human brain at 4.1 T using image segmentation. *Magn Reson Med* 36:21–29, 1996
32. Siegel G, Agranof B: *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. Vol. 21. Philadelphia, PA, Lippincott-Raven, 1999.
33. Knudsen GM, Hasselbalch SG, Hertz MM, Paulson OB: High dose insulin does not increase glucose transfer across the blood-brain barrier in humans: a re-evaluation. *Eur J Clin Invest* 29:687–691, 1999
34. Hill JM, Lesniak MA, Pert CB, Roth J: Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* 17:1127–1138, 1986
35. Unger J, McNeill TH, Moxley RT, White M, Moss A, Livingston JN: Distribution of insulin receptor-like immunoreactivity in the rat forebrain. *Neuroscience* 31:143–157, 1989
36. Werther G, Hogg A, Oldfield B, McKinley M, Figdor R, Allen F, Mendelsohn A: Localization and characterization of insulin receptor in rat brain and pituitary gland using *in vitro* radiography and computerized densitometry. *Endocrinology* 121:1562–1570, 1992
37. Brant AM, Jess TJ, Milligan G, Brown CM, Gould GW: Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochem Biophys Res Commun* 192:1297–1302, 1993
38. 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
39. Vannucci SJ, Koehler-Stec EM, Li K, Reynolds TH, Clark R, Simpson IA: GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res* 797:1–11, 1998
40. El Messari S, Leloup C, Quignon M, Brisorgueil MJ, Penicaud L, Arluison M: Immunocytochemical localization of the insulin-responsive glucose transporter 4 (Glut4) in the rat central nervous system. *J Comp Neurol* 399:492–512, 1998
41. Apelt J, Mehlhorn G, Schliebs R: Insulin-sensitive GLUT4 glucose transporters are colocalized with GLUT3-expressing cells and demonstrate a chemically distinct neuron-specific localization in rat brain. *J Neurosci Res* 57:693–705, 1999
42. Davis SN, Colburn C, Dobbins R, Nadeau S, Neal D, Williams P, Cherrington AD: Evidence that the brain of the conscious dog is insulin sensitive. *J Clin Invest* 95:593–602, 1995
43. Woods SC, Porte D, Jr., Bobbioni E, Ionescu E, Sauter JF, Rohner-Jeanrenaud F, Jeanrenaud B: Insulin: its relationship to the central nervous system and to the control of food intake and body weight. *Am J Clin Nutr* 42 (Suppl. 5):1063–1071, 1985
44. Choi IY, Tkac I, Ugurbil K, Gruetter R: Noninvasive measurements of  $[1-^{13}\text{C}]$ glycogen concentrations and metabolism in rat brain *in vivo*. *J Neurochem* 73:1300–1308, 1999
45. Nelson SR, Schulz DW, Passonneau JV, Lowry OH: Control of glycogen levels in brain. *J Neurochem* 15:1271–1279, 1968
46. Gruetter R, Novotny EJ, Boulware SD, Rothman DL, Shulman RG:  $^1\text{H}$  NMR studies of glucose transport in the human brain. *J Cereb Blood Flow Metab* 16:427–438, 1996
47. Gruetter R, Novotny EJ, Boulware SD, Rothman DL, Mason GF, Shulman GI, Shulman RG, Tamborlane WV: Direct measurement of brain glucose concentrations in humans by  $^{13}\text{C}$  NMR. *Proc Natl Acad Sci U S A* 89:1109–1112, 1992
48. Merboldt KD, Bruhn H, Hanicke W, Michaelis T, Frahm J: Decrease of glucose in the human visual cortex during photic stimulation. *Magn Reson Med* 25:187–194, 1992
49. Kreis R, Ross BD: Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy. *Radiology* 184:123–130, 1992
50. Gruetter R, Rothman DL, Novotny EJ, Shulman GI, Prichard JW, Shulman RG: Detection and assignment of the glucose signal in  $^1\text{H}$  NMR difference spectra of the human brain. *Magn Reson Med* 27:183–188, 1992
51. Kreis R, Ernst T, Ross BD: Development of the human brain: *in vivo* quantification of metabolite and water content with proton magnetic resonance spectroscopy. *Magn Reson Med* 30:424–437, 1993
52. Marchal G, Rioux P, Petit-Taboue MC, Sette G, Traverso JM, Le Poec C, Courtheoux P, Derlon JM, Baron JC: Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Arch Neurol* 49:1013–1020, 1992
53. Hino A, Ueda S, Mizukawa N, Imahori Y, Tenjin H: Effect of hemodilution on cerebral hemodynamics and oxygen metabolism. *Stroke* 23:423–426, 1992
54. Sabatini U, Celsi P, Viallard G, Rascol A, Marc-Vergnes JP: Quantitative assessment of cerebral blood volume by single-photon emission computed tomography. *Stroke* 22:324–330, 1991