Journal of Neuroscience Research 62:279–285 (2000)

Effect of Acute Hyperglycemia on Visual Cortical Activation as Measured by Functional MRI

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To determine whether acute hyperglycemia changes the hyperemic response to functional activation of brain, the area and magnitude of the activation were measured in healthy volunteers maintained at euglycemia and then at hyperglycemia using the hyperglycemic clamp technique. Activation of the visual cortex (8–16 Hz) was assessed by functional MRI with blood oxygenation level dependent (BOLD) contrast using a 4 Tesla magnet and a multi-slice echo-planar imaging sequence (TE = 30 msec, TR = 1.5 sec). At euglycemia (4.8 ± 0.2 mM, mean ± SEM, n = 6), the number of activated pixels in the occipital lobe was 79 ± 10 and the intensity of activation was 4.5 ± 0.5%. During hyperglycemia (plasma glucose 300% of control), the number of activated pixels was 90 ± 20% of control and the BOLD activation was 3.5 ± 0.3%, respectively. The change in BOLD signal was below 0.2%/mM plasma glucose. This study demonstrates that acute hyperglycemia is without substantial effect on the size and intensity of activation of the occipital cortex. The results further suggest that fluctuations in blood glucose within the physiologic range are without effect on the functional activation of the cerebral cortex measured by BOLD fMRI. J. Neurosci. Res. 62: 279–285, 2000. © 2000 Wiley-Liss, Inc.

Key words: magnetic resonance imaging; hyperglycemia; visual cortex; central neuropathy

Changes in serum glucose concentrations have long been recognized to have cerebral effects. Acute changes in glycemia, like that seen in patients with hyperosmolar coma, can lead to hemiparesis and confusion (Arieff and Carroll, 1972) whereas chronic hyperglycemia has been linked to impairments in cognitive function, particularly with respect to memory and visual/spatial reasoning in children with type 1 diabetes (Haumont et al., 1979; Rovet and Alvarez, 1997; Ryan et al., 1992) and in older adults with type 2 diabetes (Perlmutter et al., 1984; Reaven et al., 1990; Langen et al., 1991). Measures of electrical potentials evoked in response to auditory or visual stimuli have also been found to be decreased in patients with diabetes mellitus (Khardori et al., 1986; Pozzessere et al., 1988; Nakamura et al., 1991; Uccioli et al., 1995; Comi, 1997; Parisi et al., 1997). In addition, acute elevations in serum glucose concentration have also been reported to decrease cerebral blood flow in experimental animals (Duckrow et al., 1987; Harik and LaManna, 1988). Whether acute increases in glycemia have an effect on the hyperemic response to functional activation is uncertain. As more in vivo investigation of the cerebral cortex relies on the injection of labeled glucose to monitor cerebral metabolism (Gruetter et al., 1999; Gruetter et al., 1998; Shen et al., 1999), however, understanding the effect of acute hyperglycemia on functional activation will be essential for accurate data interpretation.

Brain activation has been studied in vivo using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Although PET has been successful in mapping brain function to certain areas, it requires exposure to radioactivity (Watson, 1997). Functional magnetic resonance imaging presents an alternative method with less risk to the subject. With this technique, individuals are provided a stimulus of the central nervous system and the activated neural tissue is detected through the use of BOLD (blood oxygenation level dependent) MRI signals (Ogawa and Lee 1990; Ogawa et al., 1990, 1992, 1993; Bandettini et al., 1992; Kwong et al., 1992). It seems to be a general phenomenon that during focal brain activation, blood flow increases disproportionately relative to the increase in the amount of oxygen extracted, resulting in a net reduction of venous deoxyhemoglobin. Deoxyhemoglobin is a paramagnetic molecule that has been shown to act as an endogenous contrast agent on the bulk water signal and thus a change in deoxyhemoglobin content can change MRI signal intensity by several percent. Typically, areas of activation are identified by comparing pixel intensities in the MRI measured during a control and a task state.

Contract grant sponsor: US Public Health System (NIH); Contract grant number: RR00400, RR08079, NS35561.

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Received 16 March 2000; Revised 26 June 2000; Accepted 28 June 2000

We and others have recently begun to determine the effect of functional activation on rates of cerebral energy metabolism (Hyder et al., 1996; Chen et al., 2000). In such studies, an infusion of 1-^{13}C-glucose is used to monitor rates of glucose metabolism in an area responding to a neural stimulus. Whether the acute elevation of serum glucose that typically follows the infusion of ^{13}C-labeled glucose affects fMRI of functional activation is uncertain. In the current investigation we sought to address this question. We used fMRI with BOLD contrast to determine whether acute hyperglycemia alters the size of the brain area or the intensity of activation during visual stimulation in healthy humans. We found that the functional activation of the visual cortex was not altered by experimental hyperglycemia. Based on this study, we propose that interpretation of fMRI data obtained during moderate hyperglycemia after the infusion of labeled glucose does not depend in a substantial way on the plasma glucose concentration at euglycemia or above.

MATERIALS AND METHODS

Subjects were recruited from the University of Minnesota community and were studied in the morning after an 8–10 hr fast using procedures approved by the Institutional Review Board. Subjects were prepared for the study by placing intravenous catheters into veins in each arm and in the distal leg. The catheters in the arms were used for the infusion of somatostatin and glucose and the catheter in the leg was used for venous sampling. Venous blood in the extremities was arterialized throughout the study by the continuous administration of Temp-Aid Hot Packs (Baxter Healthcare Corporation, Valencia, CA) and heated towels (Seaquist, 1997). At Time 0, subjects were infused with somatostatin (0.16 μg/kg/min) to suppress endogenous insulin secretion and euglycemia was maintained through the intravenous infusion of 20% glucose as needed. Subjects were then placed into the magnet, and after adjusting the MR scanner, presented with a visual task. When the task was completed, plasma glucose was increased by a bolus infusion of 20% or 50% glucose in water. After plasma glucose had been maintained at a stable hyperglycemic level that was at least 50% above euglycemia for 20 or more minutes, the visual stimulation task was again presented and the magnetic resonance measures were repeated identically. Throughout the study, blood samples for glucose measurement were obtained every 5 min and samples for insulin were drawn every 20 min. Plasma glucose concentrations were measured using a Beckman Autoanalyzer (Fullerton, CA) and insulin concentrations were determined by radioimmunoassay (Morgan and Lazarow, 1963).

Functional MRI was performed using a 4Tesla/125 cm magnet equipped with an unshielded gradient coil insert (30 mT/m in 200 μs, 31 cm inner diameter). Images were acquired using echo-planar imaging (EPI; 64 × 64 image size, 20 cm field of view) with an echo time TE = 30 msec and a repetition time TR = 1.5 sec and a 450 flip angle for the slice selective excitation pulse. Five coronal slices (5 mm thickness) with 7.5 mm inter-slice distance were acquired in an interleaved fashion that covered the extent of the calcarine fissure. Shimming of a 30 ml large region of interest was done using all first and second order shim coils with FASTMAP (Gruetter 1993), that resulted in a 7–9 Hz full width at half height for the water signal in 27 ml vol in the occipital lobe. Visual stimulation was performed using a standard GRASS stimulator set at 8–16 Hz using home-built LED goggles (Ogawa et al., 1993). The goggles were alternated starting with currents turned off, according to the following pattern: OFF (45 sec)–ON (30 sec)–OFF (30 sec)–ON (30 sec)–OFF (45 sec) with a total duration of 180 sec.

One hundred-twenty images per slice were thus collected. Data analysis was performed on images 6–115, that omitted the first and last 7.5 seconds of the trial period.

Image analysis was performed using STIMULATE software (Ugurbil). Activated pixels were identified by comparing the fMRI signal before activation (e.g., average of images 6–15) to that during activation (e.g., average of images 18–25) by accounting for a small delay in hemodynamic response. Likewise, signal change was tested by comparing activated signal to the subsequent period where goggles were turned off. Given that two stimulation periods were used, this resulted in 4 statistical tests, namely one each for a signal increase at the onset of the first and second stimulus, respectively, and one each for a signal decrease at the end of the first and second stimulus, respectively. Pixels were considered activated only if motion was determined to be insignificant based on center-of-mass time courses and verification of lack of negative signal changes at the tissue edges for each of the 4 tests performed. Pixels that displayed a significant ($P < 0.01$, two-tailed t-test) difference in intensity between basal and stimulated conditions were included in the statistical analysis. To determine the size of the cortex responsive to visual stimulation, the number of significantly activated pixels identified in the region of interest were counted within a 30 ml region of interest that was identified relative to anatomic landmarks to encompass the calcarine fissure. The position and size of this VOI was identical for the hyperglycemic and euglycemic trial in any given subject. The intensity of the activation was determined by calculating the percentage change in MR signal (the % BOLD effect) noted between basal and activated conditions in those pixels identified by statistical t-testing. The identical analysis was performed on data collected during eu- and hyperglycemia.

Data are expressed as mean ± SEM. Statistical differences between euglycemic and hyperglycemic conditions were evaluated using Student's t-test for paired data as well as the nonparametric sign test. A P value <0.05 was considered statistically significant.

RESULTS

Five healthy female subjects participated in this study. One subject was studied on two separate occasions, resulting in six studies. Their mean age was 40 ± 3 years and their mean weight was 72.4 ± 3.9 kg. For each study, subjects were studied sequentially at euglycemia (plasma glucose ranging from 4.5–6.0 mM) and hyperglycemia (9.6–18.6 mM) within 2 hr. The hyperglycemic phase of the study was performed at least 20 min after the euglycemic part of the protocol, that is detailed in Figure 1. Plasma insulin concentrations were below 30 pM in all studies.

Activation images color-coded for statistical significance or percent BOLD signal change were constructed

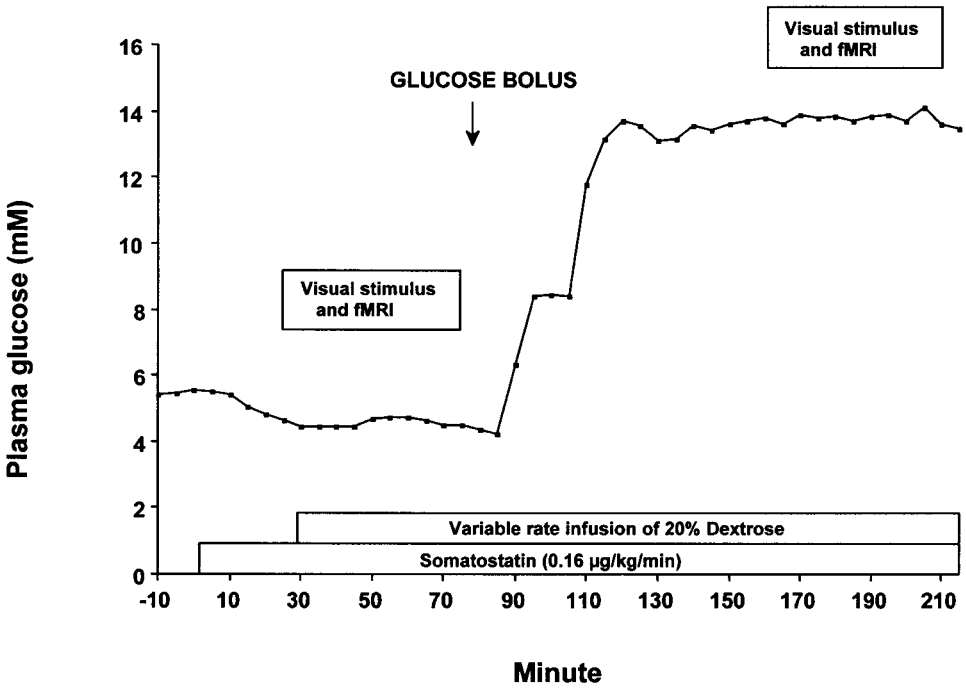

Fig. 1. Experimental protocol. Shown are the plasma glucose concentrations measured in a single subject during our study protocol. Subjects underwent a two step clamp procedure with fMRI performed during stable euglycemia and stable hyperglycemia. Plasma glucose was controlled by adjusting the rate of the glucose infusion based on plasma glucose concentrations measured every 5 min.

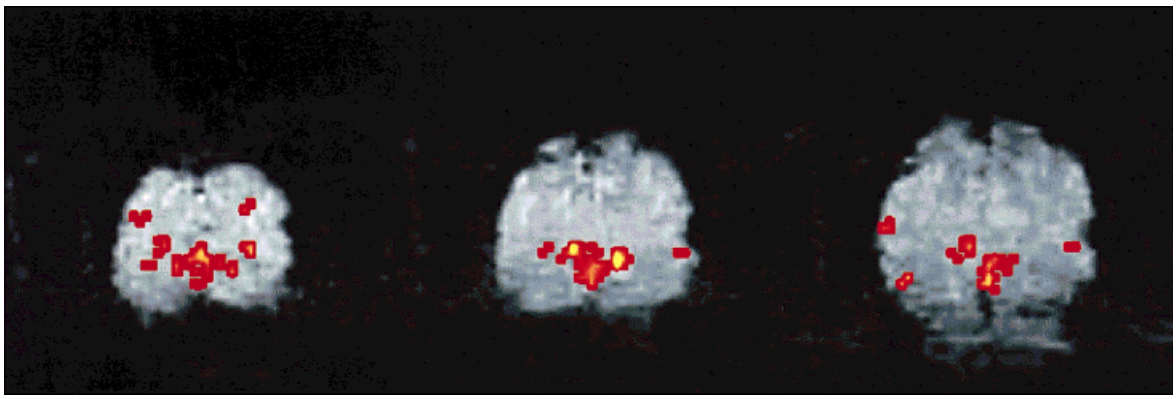

Fig. 2. Activation maps acquired during euglycemia in the same subject. The activation map (coronal orientation intersecting the calcarine fissure) is overlaid on an image generated with the same imaging sequence, EPI (TR = 1.5 sec, TE-30 msec, 45° flip angel, 64 phase encode steps).

for all subjects during eu- and hyperglycemic conditions by including only pixels considered activated. An example of such a map obtained from a single subject during euglycemia is shown in Figure 2. The number of pixels activated during euglycemia was not significantly different from the number of pixels activated during hyperglycemia (Table I). The number of pixels activated during hyperglycemia (70 ± 16) corresponds to 90 ± 20% of the number of pixels activated during euglycemia. Likewise, the BOLD effect was similar at both levels of glycemia (4.5 ± 0.5% vs. 3.5 ± 0.3%, P = NS, Table I). When examining the differences using paired t-test, however, a significant change was found.

To further examine the effect of increased plasma glucose concentration on the intensity of activation, we extracted the time courses for each activated pixel and calculated the average MRI signal change for each study. These data were then averaged over all 6 studies to yield the two fMRI time courses shown in Figure 3A. As shown in the figure, the image intensity varied under both glycemic conditions as the visual stimulus was turned on and off. When a difference curve was created by subtracting the average time course acquired at hyperglycemia (Fig. 3A top; left scale) from that acquired during euglycemia (Fig. 3A middle; left scale), a flat line within the background noise of the measurement was obtained (Fig. 3A bottom; right scale). No statistically significant difference was found between the data acquired during eu- and hyperglycemia. Although this difference time course is consistently above zero, this difference cannot be consid-

TABLE I. Summary of Data from Individual fMRI Studies

	Euglycemia			Hyperglycemia		
Study	Plasma glucose (mM)	No. of activated pixels ($P < 0.01$)	BOLD (%)	Plasma glucose (mM)	No. of activated pixels ($P < 0.01$)	BOLD (%)
1	4.6	82	5.1	17.4	79	4.5
2	5.8	59	5.3	18.6	54	3.2
3	4.6	68	5.9	11.2	80	4.1
4	4.6	52	3.8	9.6	137	3.0
5	4.5	121	2.9	16.1	30	2.8
6	4.5	91	4.1	13.8	38	3.5
Mean ± SEM	4.8 ± 0.2	79 ± 10	4.5 ± 0.5	14.5 ± 1.5	70 ± 16	3.5 ± 0.3

Data shown are obtained from six studies performed on five subjects. The size of the area of activation is indicated by the number of activated pixels and the intensity of activation is indicated by BOLD %.

ered significant, as one study showed a decrease in the difference activation curve ($P > 0.05$ using the sign test). To determine whether a trend in the BOLD effect was discernible, the BOLD effect was plotted as a function of plasma glucose, shown in Figure 3B. Regression analysis indicated that r^2 was 0.18 and the statistical test for correlation was not significant ($P > 0.05$). The slope was -0.00077 ± 0.0005/mM plasma glucose, that corresponds to a $0.08 \pm 0.05\%$ change per mM plasma glucose and thus was considered negligible ($P > 0.05$). When calculating the change in BOLD effect for each subject individually, a slope of $0.12 \pm 0.04\%$/mM change in plasma glucose was calculated.

DISCUSSION

The effect of acute hyperglycemia on the cerebral response to a visual stimulus was studied in volunteers using functional magnetic resonance imaging with BOLD contrast. Both the size of the region activated by a visual stimulus as well as the intensity of that activation was equivalent and within the experimental error of the method during euglycemia as compared to a 3-fold elevation in plasma glucose concentration. These data provide support for the hypothesis that acute hyperglycemia, at least within the range studied in these experiments, is without substantial effect on the perception of a visual stimulus in humans.

When comparing the BOLD effect within each subject, however, a significant effect due to the supraphysiologic hyperglycemia was noted. The change in BOLD effect was 0.12%/mM plasma glucose, that implies that the magnitude of the BOLD signal decreases at most by 0.5% for the typical physiological range of plasma glucose between 5 and 10 mM. Because the area of activation was unchanged, such a small change in BOLD signal implies that physiologic changes in plasma glucose concentration are without concern to brain activation studies of the human visual cortex, as further emphasized by Figure 3A. The fact that these changes only reached statistical significance when using the paired (intra-individual) test, implies that inter-individual differences are larger and that they effectively mask a small change in BOLD signal during hyperglycemia, that is quantitatively of minor importance.

The effect of hyperglycemia on cerebral function in humans has been difficult to define. Although abnormalities in evoked responses (Pozzessere et al., 1988) and neuropsychological functioning (Reaven et al., 1990) have been linked to poor glycemic control in patients with diabetes, distinguishing between the effects of acute and chronic hyperglycemia has been problematic in humans because meticulous control of the glycemia experienced by the subject before or during the study has not always been achieved. In this investigation, normal subjects were studied during euglycemia and a subsequent acute 2- to 3-fold elevation of their plasma glucose concentration using the glucose/insulin clamp procedure. Because BOLD contrast relies on the interplay between cerebral blood flow, cerebral blood volume, and cerebral oxygen consumption (Ogawa et al., 1998), our observations suggest that acute hyperglycemia such as that experienced in response to high carbohydrate feeding after a nocturnal fast is without effect on these variables. Our experiments focused on functional activation in the visual cortex, that is a highly reproducible activation paradigm. Acute hyperglycemia might alter functional activation in other brain regions, but given the universal detection of the BOLD effect, this may occur only in highly specialized brain regions.

In the present study, endogenous insulin secretion was minimized by an infusion of somatostatin, leading to undetectable concentrations of insulin in the blood. Although this protocol effectively controlled for differences in insulin concentrations among study subjects, it does not allow us to determine whether insulin itself has an effect on brain activation. Insulin receptors have been identified in the brain (Hill et al., 1986; Werther et al., 1992) and the vasculature of other tissues has been shown to be responsive to the vasodilatory effects of insulin (Laakso et al., 1990; Baron et al., 1995; Yki-Jarvinen and Utriainen, 1998). A recent study by Cranston et al. (1998) in which diabetic subjects were studied by PET scanning in the absence and presence of insulin, however, demonstrated that cerebral blood flow was the same under both exper-

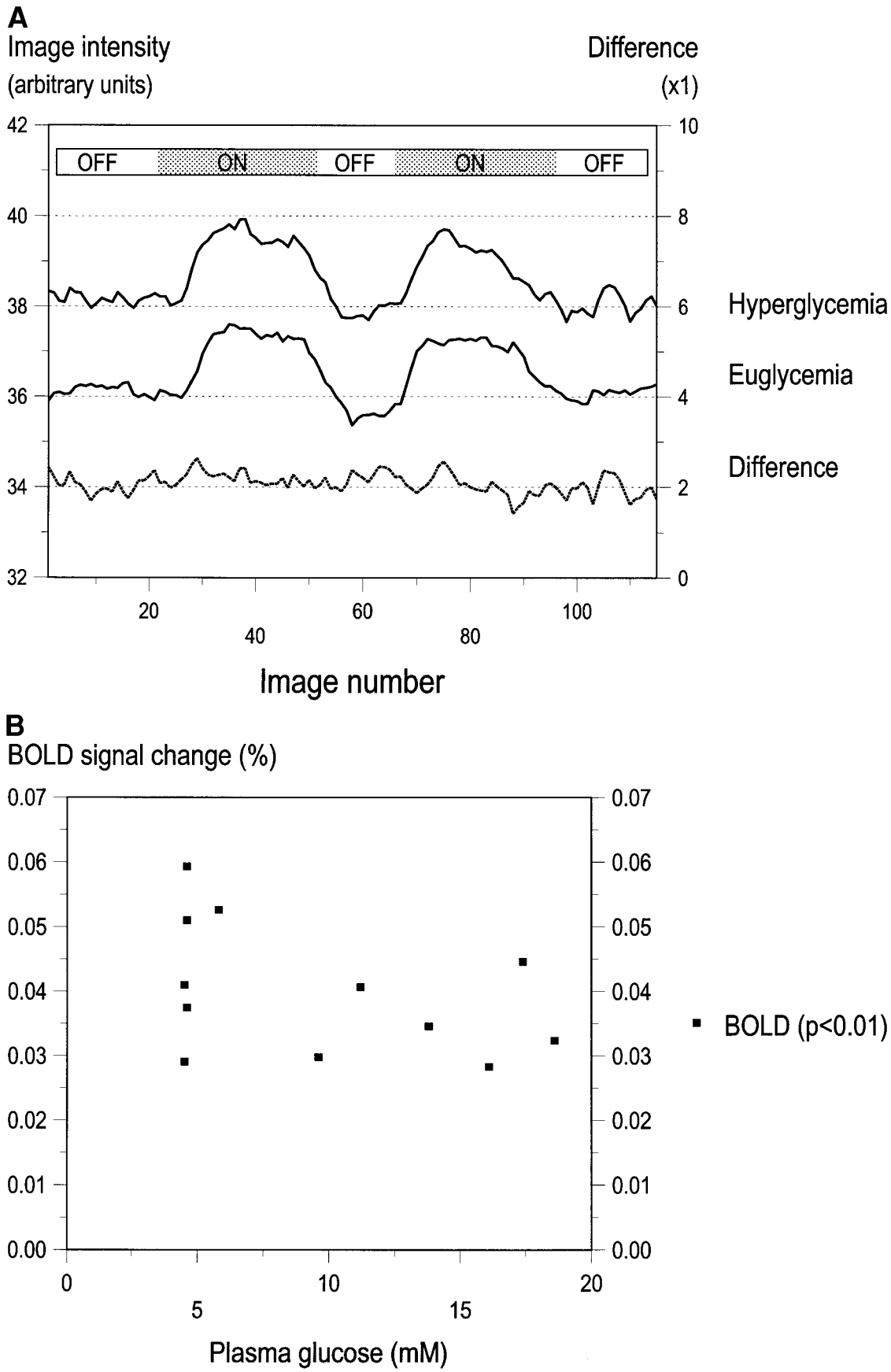

Fig. 3. Average time courses at eu- and at hyperglycemia. **A:** Shown are the time courses averaged over all six studies at euglycemia and at hyperglycemia during the task shown above (left scale; boxes above [on/off] indicate when visual stimulus was on or off, respectively). The average relative difference between the two is shown with the same scaling (right scale). **B:** Shown is the BOLD effect in fractional signal change for each of the six studies as a function of plasma glucose.

imental conditions. Consequently, we think it is unlikely that our experimental approach of suppressing insulin secretion by the infusion of somatostatin masked a physiologically relevant effect of insulin on blood flow that could confound our efforts to determine the effects of hyperglycemia on brain activation.

In summary, severe acute hyperglycemia does not seem to have a substantial effect on BOLD imaging during visual stimulation in healthy humans. Under supraphysiologic concentrations of blood glucose we found the size of the area of brain activated by the GRASS stimulator comparable to that measured during euglycemia. Although the intensity of the activation was significantly reduced, the correlation with plasma glucose was not significant. Our observations have relevance to studies of functional activation where cellular metabolism is monitored with labeled glucose. In such work investigators may now be certain that any functional changes noted are the result of their experimental manipulation and are not due to the acute hyperglycemia often necessary to perform the study. Our experiments also show that variation in blood glucose does not affect the BOLD signal substantially, providing further evidence that fMRI with BOLD is a robust modality for imaging brain function during physiologic variations in plasma glucose concentrations.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the secretarial assistance of Tanya Doble.

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