Effect of Acute Hypoglycemia on Human Cerebral Glucose Metabolism Measured by $^{13}$C Magnetic Resonance Spectroscopy

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OBJECTIVE—To investigate the effect of acute insulin-induced hypoglycemia on cerebral glucose metabolism in healthy humans, measured by $^{13}$C magnetic resonance spectroscopy (MRS).

RESEARCH DESIGN AND METHODS—Hyperinsulinemic glucose clamps were performed at plasma glucose levels of 5 mmol/L (euglycemia) or 3 mmol/L (hypoglycemia) in random order in eight healthy subjects (four women) on two occasions, separated by at least 3 weeks. Enriched [1-$^{13}$C]glucose 20% w/w was used for the clamps to maintain stable plasma glucose labeling. The levels of the $^{13}$C-labeled glucose metabolites glutamate C4 and C3 were measured over time in the occipital cortex during the clamp by continuous $^{13}$C MRS in a 3T magnetic resonance scanner. Time courses of glutamate C4 and C3 labeling were fitted using a one-compartment model to calculate metabolic rates in the brain.

RESULTS—Plasma glucose $^{13}$C isotopic enrichment was stable at 35.1 $\pm$ 1.8% during euglycemia and at 30.2 $\pm$ 5.5% during hypoglycemia. Hypoglycemia stimulated release of counterregulatory hormones (all $P < 0.05$) and tended to increase plasma lactate levels ($P = 0.07$). After correction for the ambient $^{13}$C enrichment values, label incorporation into glucose metabolites was virtually identical under both glycemic conditions. Calculated tricarboxylic acid cycle rates ($V_{TCA}$) were 0.48 $\pm$ 0.03 μmol/g/min during euglycemia and 0.43 $\pm$ 0.08 μmol/g/min during hypoglycemia ($P = 0.42$).

CONCLUSIONS—These results indicate that acute moderate hypoglycemia does not affect fluxes through the main pathways of glucose metabolism in the brain of healthy nondiabetic subjects. Diabetes 60:1467–1473, 2011

Hypoglycemia is a major threat for brain function because the brain depends on a continuous glucose supply as principal source of energy. Thus, glucose counterregulatory responses are usually initiated when glucose levels fall below ~3.8 mmol/L to quickly restore euglycemia and maintain sufficient glucose delivery to the brain (1). The glucose level at which cognitive function declines is not fixed but depends on the complexity of the cognitive task and the cognitive domain that is tested. Nevertheless, although simple motor functions may be sustained despite even quite severe degrees of hypoglycemia, many aspects of cognitive performance become impaired at glucose levels between 3.1 and 3.4 mmol/L (2). During complex cognitive tasks, such as with motor vehicle driving, deterioration can already be observed at glucose levels as high as ~3.8 mmol/L (3).

Although the importance of maintaining sufficient glucose supply to the brain has been known for long, it is still unclear how hypoglycemia affects subsequent cerebral glucose metabolism. Various studies have indicated altered cerebral glucose handling during even mild symptomatic hypoglycemia. When the brain is supplied with an alternative energy source during hypoglycemia, such as lactate, the threshold level for initiation of glucose counterregulation shifts to lower glucose levels (4) and performance on cognitive function tests is maintained better (5). In accordance, upregulation of lactate transport into the brain during hypoglycemia has been associated with glucose counterregulatory defects (6). Using $^1$H magnetic resonance spectroscopy (MRS), Bischof et al. (7) reported discrete effects of moderate hypoglycemia (~3.1 mmol/L glucose) on cerebral glucose-derived metabolite levels in healthy volunteers. Finally, positron emission tomography (PET) studies with fluor-18-fluorodeoxyglucose (FDG) and $^{[1}]$C-O-methyl-D-glucose (CMG) have demonstrated regional, but not global, changes in cerebral glucose metabolism based on tracer uptake in the brain during hypoglycemia in patients with diabetes (8,9). Thus, many reports suggest that human brain glucose metabolism changes under hypoglycemic conditions, but the exact changes are unclear (10). In addition, neither with $^1$H MRS nor with PET can the cerebral metabolic rate of glucose conversion into its metabolites be determined.

With $^{13}$C MRS, it is possible to study the dynamics of glucose metabolism in vivo in the human brain. Because the natural abundance of $^{13}$C is only 1.1%, it can be applied as a nonradioactive magnetic resonance tracer. For this purpose, often $^{13}$C enriched glucose labeled at the C-1 position is used (11). With this method, the uptake of glucose in brain tissue, as well as its conversion into several downstream metabolites, can be followed over time. To optimize the intensity of the $^{13}$C signals of these metabolites, which occur at rather low concentration, most studies applying dynamic $^{13}$C MRS to the human brain have been performed under hyperglycemic conditions. We previously developed a specific protocol that has enabled us to apply $^{13}$C MRS with infusion of $^{13}$C-labeled glucose under both euglycemic and hypoglycemic conditions in human volunteers (12). This allows for mathematical

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modeling and calculation of metabolic fluxes of glucose metabolism (13,14). The aim of the current study was to compare human in vivo brain glucose metabolism under euglycemic and hypoglycemic conditions using 13C MRS.

RESEARCH DESIGN AND METHODS

Eight healthy nondiabetic volunteers (four men and four women aged 23.2 ± 2.5 years with BMI 23.9 ± 4.5 kg/m2) were enrolled for this study. The study was approved by the institutional review board of the Radboud University Nijmegen Medical Centre, and all volunteers gave written informed consent before participation. All participants were examined on two occasions: once under euglycemic conditions and once under hypoglycemic conditions, scheduled in random order and separated by at least 3 weeks. Female subjects were tested at 4- or 8-week intervals to ensure that experiments took place during corresponding phases of the menstrual cycle.

Hyperinsulinemic glucose clamps. Subjects came to the magnetic resonance research facility at 8:00 a.m. after an overnight fast and after having abstained from alcohol and caffeine-containing substances for 24 h. The brachial artery of the nondominant arm was cannulated under local anesthesia for frequent blood sampling. An intravenous catheter was inserted in the antecubital vein of the contralateral arm for administration of [1-13C]glucose and insulin. After a 30-min equilibration period, subjects were placed on the scanner bed in supine position with their heads positioned in the magnetic resonance coil. Arterial blood was sampled to obtain baseline variables, and reference spectra were obtained without administration of exogenous 13C-labeled material during the next 30 min. Subsequently, a hyperinsulinemic (60 mU/min/m2, equating approximately 1.5 mU/min/kg) euglycemic (5.0 mmol/L) or hypoglycemic (3.0 mmol/L) glucose clamp was initiated with [1-13C]glucose 20% w/w, as described previously (12). Briefly, a bolus of 30 mL of 100% enriched [1-13C]glucose 20% w/w was infused over 10 min at the initiation of the clamp to rapidly increase plasma 13C enrichment. For the remainder of the experiments, variable infusions of 40 or 50% enriched [1-13C]glucose 20% w/w were infused during the euglycemic and hypoglycemic experiments, respectively, in order to maintain plasma glucose levels at predetermined target values. Blood was sampled every 5 min for immediate determination of plasma glucose by the glucose oxidation method (Beckman Glucose Analyzer II; Beckman Coulter, Fullerton, CA) and later determination of plasma 13C isotopic enrichment of glucose and lactate by high-resolution proton nuclear magnetic resonance (1H NMR) at 500 MHz (15,16). Plasma lactate concentration was also determined from 1H NMR spectra. Every 30 min, additional blood was sampled for measurement of insulin and counterregulatory hormones as previously described (12).

MRS. All studies were performed on a 3T magnetic resonance system (Magnetom Trio; Siemens, Erlangen, Germany) with a 1H volume coil and a circularly polarized 13C surface coil inserted into the 1H coil (17). A distortionless enhanced polarization transfer (DEPT) sequence for 1H to 13C polarization transfer combined with proton image-selected in vivo spectroscopy (1H ISIS) localization (18) was used for acquisition of 13C magnetic resonance spectra. Adiabatic 13C radiofrequency pulses were used in all sequences to ensure homogeneous excitation using the 13C surface coil, as well as wideband alternating phase low-power technique for zero residue splitting (WALTZ-16) proton decoupling (19) to simplify the spectra and enhance signal-to-noise ratio (SNR). A voxel of ~125 mL was placed in occipital brain tissue. In all experiments, one spectrum consisted of 72 repetitions of 2 s, allowing for a time resolution of 2.5 min. Eight reference spectra were obtained before the start of [1-13C]glucose infusion, during which 13C MR spectra were acquired continuously. Visual stimulation was avoided by dimming the lights in the magnet room during the experiments.

13C MRS data processing and quantification. The eight 13C MR reference spectra were averaged and subtracted from the dynamic spectra acquired during the 13C glucose clamp to correct for natural abundance 13C magnetic resonance signals. To enhance the SNR, the corrected spectra were added in running averages of 15 min. The resulting spectra were fitted with the advance magnetic resonance (AMARES) algorithm (20) in the java-based MR user interface (JMRUI) software package (21). The natural abundance signal of myo-inositol (ml) was used to quantify glutamate C4 and glutamine C3 in the spectra based on the premise that ml has a stable concentration of 6 mmol/L and is not labeled with 13C in the time frame of the experiment (22). The natural abundance signal of ml was quantified in JMRUI from the spectra obtained by adding all dynamic spectra before correction with reference spectra. DEPT 13C magnetic resonance spectra measured from a phantom were used to eliminate effects of the pulse sequence profile on the experimental spectra.

Metabolic modeling. Rates of metabolic fluxes were determined using a standard one-compartment metabolic model (14,23,24) as depicted in Fig. 1. These rates (V) were assessed from the time courses of incorporation of 13C isotopes into different metabolites. Time courses of glutamate C4 and glutamine C3 concentrations and plasma values and isotopic enrichments of glucose were used as input factors for the model. In this specific situation, it was also decided to take plasma lactate values and isotopic enrichment into account as input variables for the modeling process. The model was implemented in Matlab R2008b (MathWorks, Natick, MA) and consisted of differential equations describing the inflow and outflow of label from each metabolite pool. These equations were solved numerically, and a nonlinear least-squares approach was used to fit the model to the experimental data by varying free flux parameters representing VTCa, the loss of label through exchange with unlabeled glucose (Vex), and the exchange of intracellular lactate with plasma lactate (Vlact). Vlact represented the exchange between α-ketoglutarate and glutamate over the mitochondrial membrane and was assumed to be 5 mmol/min. Furthermore, the following assumptions were made for total pool concentrations: [Glu]: 10 mmol/L; [Gln]: 2.5 mmol/L; [OAA]: 0.3 mmol/L; [αKG]: 0.25 mmol/L; [Asp]: 1.5 mmol/L; and [Pyruvate]: 0.15 mmol/L.

FIG. 1. One-compartment model for uptake of [1-13C]glucose and its conversion into labeled metabolites. Brain glucose uptake from the blood was modeled by reversible Michaelis-Menten kinetics (40). V bypass represents the glycolysis rate and has a value of 0.5 × VTCa. The cycling of glutamate and glutamine between neurons and astroglia is approached by the parameter Vgin and assumed equal to VTCa. VTCa, Vgin, and Vefflux are the free parameters of the model, representing TCA cycle flux, exchange of plasma, and brain lactate and efflux of labeled glutamine, respectively. Vlact represents lactate dehydrogenase and was assumed to be 3 mmol/min; Vr represents the exchange between α-ketoglutarate and glutamate over the mitochondrial membrane and was assumed to be 5 mmol/min. Furthermore, the following assumptions were made for total pool concentrations: [Glu]: 10 mmol/L; [Gln]: 2.5 mmol/L; [OAA]: 0.3 mmol/L; [αKG]: 0.25 mmol/L; [Asp]: 1.5 mmol/L; and [Pyruvate]: 0.15 mmol/L.

RESULTS

As a consequence of the [1,13C]glucose bolus, plasma glucose levels transiently increased to maximally 7.1 ± 0.4 mmol/L during the euglycemic experiment and to 6.6 ± 1.0 mmol/L during the hypoglycemic experiment. Thereafter,
plasma glucose levels were allowed to fall and were maintained stable at 5.1 ± 0.3 mmol/L with a coefficient of variation (CV) of 4.1 ± 1.0% during the euglycemic clamp and at 3.0 ± 0.4 mmol/L (CV 5.9 ± 2.2%) during the hypoglycemic clamp (Fig. 2A). In response to hypoglycemia, plasma levels of glucagon, adrenaline, noradrenaline, cortisol, and growth hormone all significantly increased compared with baseline and compared with similar time points during the euglycemic clamp (Table 1). As expected, glucose infusion rates were approximately threefold lower during hypoglycemia than during euglycemia (2.2 ± 0.4 vs. 6.6 ± 0.9 μmol/kg/min; P < 0.001).

Isotopic enrichment of plasma glucose also peaked immediately after the bolus infusion. From t = 20 min onward, plasma 13C glucose labeling was stable during both the euglycemic and the hypoglycemic clamps, although the level was slightly lower during hypoglycemia (35.1 ± 1.8% [CV 3.6 ± 2.1] vs. 30.2 ± 5.5% [CV 6.8 ± 2.4]) (Fig. 2B).

After an initial rise, plasma lactate levels remained stable during euglycemia at 1.1 ± 0.3 mmol/L, but tended to increase further in response to hypoglycemia (from 1.1 ± 0.4 to 1.5 ± 0.6 mmol/L, P = 0.07 vs. euglycemia). Conversely, plasma lactate isotopic enrichment remained constant (after an initial increase) during hypoglycemia, whereas it increased further during euglycemia (from 6.1 ± 3.2 at t = 20 min to 8.9 ± 2.7% at t = 100 min), P < 0.0001, versus hypoglycemia (Fig. 2C and D).

All 13C MR spectra were of similar spectral quality with sufficient SNR to analyze the signals of the metabolites of interest under both euglycemic and hypoglycemic conditions (Fig. 3). Under either condition, the 13C label was progressively incorporated into glutamate C4, C3, and C2; glutamine C4, C3, and C2; aspartate C3 and C2; and lactate C3. Figure 4A shows the time courses of glutamate C4 labeling, which appears during the first turn of the TCA cycle, and of glutamate C3 labeling, which appears during the second turn of the TCA cycle. The isotopic enrichment of the glutamate pool was lower under hypoglycemic conditions than under euglycemic conditions. However, after correction for the lower plasma glucose 13C enrichment level during hypoglycemia, the time courses for both these metabolites were virtually superimposable for the two glycemic conditions (Fig. 4B).

The time courses of 13C label incorporation into glutamate C4 and C3 were fitted with a one-compartment metabolic model (Fig. 1) to compare metabolic fluxes under euglycemic and hypoglycemic conditions. The average of individually calculated TCA cycle rates (VTCA) under
hypoglycemic clamp conditions was similar to the average values obtained under euglycemic conditions (Table 2). When the TCA cycle rates were calculated from averaged datasets (Fig. 4C; individual datasets of all participants can be found in Supplementary Fig. 1), similar values were obtained (0.48 and 0.43 μmol/g/min for euglycemia and hypoglycemia, respectively). There were also no differences between the two glycemic conditions with regard to other flux parameters. The absence of an effect of hypoglycemia on metabolic fluxes was similar among men and women in the study (data not shown).

**DISCUSSION**

In this study, we used $^{13}$C MRS to investigate the effect of moderate hypoglycemia on glucose handling by the human brain in vivo. Despite the fact that hypoglycemia considerably stimulated glucose counterregulation, which is under control of the central nervous system, metabolism of glucose in the occipital tissue of the brain was remarkably similar under hypoglycemic and euglycemic conditions. Indeed, the time courses of $^{13}$C labeling of both glutamate C4 and glutamate C3 during hypoglycemia and during euglycemia were superimposable, when corrected for plasma isotopic enrichment, and the rate of TCA cycle flux was ~0.5 μmol/g/min on both occasions. These calculated fluxes are in line with previously reported values for $V_{TCA}$ under hyperglycemic conditions (27–29).

This is the first study to investigate and quantify cerebral glucose metabolism under hypoglycemic conditions in humans using $^{13}$C MRS. The lack of a difference in cerebral glucose metabolism between hypo- and euglycemia contrasts with a $^{1}$H MRS study, which suggested slowing of the TCA cycle rate during hypoglycemia based on a reduction of the glutamate-to-creatine ratio (7). However, snapshot $^{1}$H magnetic resonance spectra provide steady-state levels of metabolites and cannot be used to quantify cerebral glucose metabolic fluxes, as is possible with dynamic $^{13}$C MRS. Moreover, our findings are in line with a $^{13}$C MRS study in rats (30) in which overall cerebral metabolism in control animals was not affected by hypoglycemia.

**FIG. 3.** Representative $^{13}$C magnetic resonance spectra of the human brain measured at the end of a euglycemic experiment and a hypoglycemic experiment. PPM, parts per million.

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**TABLE 1**

Counterregulatory hormone and insulin values during the euglycemic and hypoglycemic clamps

|                        | Baseline | 30 | 90 | 120 | $P$   |
|------------------------|----------|----|----|-----|-------|
| Glucagon (pmol/L)      | 35.5 ± 6.9 | 30.5 ± 7.2 | 28.4 ± 7.3 | 41.4 ± 11.1 | 0.0080 |
|                        | 27.6 ± 5.1 | 41.9 ± 19.8 | 53.6 ± 16.4 | 77.0 ± 41.9 |       |
| Adrenaline (nmol/L)    | 0.22 ± 0.09 | 0.24 ± 0.08 | 0.42 ± 0.20 | 0.41 ± 0.12 | <0.0001 |
|                        | 0.20 ± 0.10 | 0.82 ± 1.22 | 3.96 ± 2.10  | 4.84 ± 2.51  |       |
| Noradrenaline (nmol/L) | 0.91 ± 0.22 | 0.97 ± 0.21 | 1.07 ± 0.26 | 1.14 ± 0.31 |       |
|                        | 0.94 ± 0.4  | 1.15 ± 0.39 | 1.98 ± 1.06 | 1.96 ± 0.64 | 0.0316 |
| Cortisol (μmol/L)      | 0.44 ± 0.29 | 0.53 ± 0.13 | 0.42 ± 0.15 | 0.42 ± 0.13 |       |
|                        | 0.47 ± 0.18 | 0.47 ± 0.17 | 0.64 ± 0.33 | 0.89 ± 0.21 | 0.0048 |
| Growth hormone (mU/L)  | 5.91 ± 7.45 | 6.14 ± 7.84 | 14.86 ± 14.43 | 19.30 ± 18.34 |       |
|                        | 6.17 ± 7.16 | 14.44 ± 33.19 | 46.38 ± 47.96 | 74.09 ± 55.55 | 0.0462 |
| Insulin (pmol/L)       | 76.6 ± 44.1 | 777.8 ± 190.7 | 685.9 ± 114.9 | 694.1 ± 114.9 | 0.6824 |

Data are means ± SD. Statistical tests performed with two-way ANOVA.

Mathematical modeling of the data was performed to quantify and compare metabolic processes under the two glycemic conditions. Our model showed that the rates for free flux parameters ($V_{TCA}$, $V_{dil}$, and $V_{efflux}$) were similar during hypoglycemia and euglycemia. The $V_{TCA}$ values were within the published range (27–29), but $V_{dil}$ and $V_{efflux}$ were higher than previously reported (31,32). Because several assumptions for $V_{Gln}$, $V_{LDH}$, and $V_X$ had to be made to derive the rates for free flux parameters, these data need to be interpreted with caution. There is ongoing debate as to which estimated values are appropriate. In the literature, for $V_X$ values below 1 and as high as 57 μmol/g/min have been used (23,25). In the current study, the data were fitted assuming $V_X = 5$ μmol/g/min to optimize the fitting. Refitting the data with $V_X = 1$ μmol/g/min resulted in slightly lower values for $V_{TCA}$ of 0.43 μmol/g/min during euglycemia and 0.38 μmol/g/min during hypoglycemia, but the difference between the two groups remained non-significant. We cannot completely exclude the possibility...
that $V_{\text{glu}}$, $V_{\text{LDH}}$, and $V_{\text{X}}$ are modulated by the level of glycemia itself, but there are no data available to support such a notion. Thus, identical assumptions for the fixed model parameters were used for both glycemic conditions.

There are several potential mechanisms that could maintain normal cerebral glucose metabolism during hypoglycemia. A plausible explanation would be a compensatory increase in cerebral uptake of lactate. In agreement with previous observations (33), plasma lactate increased by approximately 50% in response to hypoglycemia. Lactate can be used by the brain as an alternative energy source and may reduce the cerebral need for glucose (34–36). After conversion to pyruvate, lactate carbons may enter the TCA cycle as the carbons of glucose do (37). A study by Mason et al. (6) has indicated that brain transporter activity for monocarboxylic substrates such as lactate can be increased twofold during hypoglycemia, which would support the hypothesis of increased lactate consumption during hypoglycemia. Moreover, even under resting conditions, increases in lactate availability stimulate consumption of lactate by the brain at the cost of reductions in glucose utilization (34). Our model does not include net lactate uptake because this flux is small and can be neglected under physiological conditions. However, it is possible that the increased plasma lactate level during hypoglycemia could result in increased net lactate uptake in the brain. Therefore, to assess the potential contribution of net lactate uptake, we performed additional modeling incorporating net lactate uptake (0.2 μmol/g/min, estimated from lactate Michaelis-Menten kinetics through the blood-brain barrier) into the

**TABLE 2**

| Metabolic flux values during euglycemia and hypoglycemia |
|-----------------|-----------------|-------|
|                | Euglycemia      | Hypoglycemia | $P^*$ |
| $V_{\text{TCA}}$ (μmol/g/min) | 0.48 ± 0.03     | 0.43 ± 0.08   | 0.42 |
| $V_{\text{dil}}$ (μmol/g/min)  | 0.61 ± 0.25     | 0.61 ± 0.39   | 0.89 |
| $V_{\text{eflux}}$ (μmol/g/min) | 0.41 ± 0.26     | 0.41 ± 0.20   | 0.83 |

Data are means ± SD. *Student t test.
model during hypoglycemia and found that it did not affect the values of \( V_{rCA} \) significantly (<5% change). Thus, this is unlikely to explain our findings.

Alternatively, we cannot exclude the entrance of unlabeled carbons into the TCA cycle via glycogen breakdown as was suggested by an in vivo study by Oz et al. (38). Breakdown of glycogen located in astrocytes can then provide neurons with lactate to maintain energy metabolism (39).

This study provides no evidence for a link between a decline in cognitive function and moderate hypoglycemia. As \(^{13}\)C MRS of the brain was usually performed in a limited area of the occipital cortex, we cannot exclude the possibility that regional variation in cerebral glucose metabolism occurs. It is also possible that increased cortical activation in response to hypoglycemia stimulated glucose uptake so that any fall in glucose metabolism was sufficiently compensated for. PET studies have demonstrated increased cortical activation during hypoglycemia (~2.6 mmol/L) in various regions of the brain in diabetic men (8,9) but not in the occipital cortex, where reduced rather than increased activation was observed.

It is possible that the relatively mild hypoglycemic condition imposed during this study was only strong enough to stimulate hormonal counterregulation but that deeper hypoglycemia is required to induce impairments in cerebral glucose metabolism. However, a clamp at much lower glucose levels may be hard to achieve while maintaining stable glucose infusion at a sufficient rate, which is required for administration of the \(^{13}\)C isotope. In addition, more severe hypoglycemia would expose participants to significantly more discomfort and risk.

In conclusion, our results indicate that acute moderate hypoglycemia does not affect cerebral glucose metabolism in healthy volunteers. Thus, the healthy human brain appears sufficiently resilient to withstand moderate drops in plasma glucose, potentially as a consequence of increased lactate availability. Our study provides new insights into mechanisms that protect the brain from substrate deprivation, which may have important clinical implications if this finding can be reproduced in patients with type 1 diabetes, especially those suffering from repeated hypoglycemic episodes.

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K.C.C.v.d.V. analyzed data, drafted the manuscript, contributed to interpreting data and to editing the content, and approved the final version of the paper. B.E.d.G. analyzed data, drafted the manuscript, designed the study, contributed to interpreting data and to editing the content, and approved the final version of the paper. M.v.d.G. analyzed data, designed the study, contributed to interpreting data and to editing the content, and approved the final version of the paper. A.A.S. and P.-G.H. contributed to interpreting data and to editing the content and approved the final version of the paper. C.J.J.T. and A.H. designed the study, contributed to interpreting data and to editing the content, and approved the final version of the paper.

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