A Ketogenic Diet Increases Brain Insulin-Like Growth Factor Receptor and Glucose Transporter Gene Expression

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A ketogenic diet suppresses seizure activity in children and in juvenile rats. To investigate whether alteration in brain IGF activity could be involved in the beneficial effects of the ketogenic diet, we examined the effects of this diet on IGF system gene expression in the rat brain. Juvenile rats were fed one of three different diets for 7 d: ad libitum standard rat chow (AL-Std), calorie-restricted standard chow (CR-Std), or a calorie-restricted ketogenic diet (CR-Ket). The calorie-restricted diets contained 90% of the rats’ calculated energy requirements. The AL-Std diet group increased in weight, whereas the two CR groups merely maintained their weight during the 7-d period. Glucose levels were significantly reduced in both CR groups compared with the AL-Std group, but only the CR-Ket group developed ketonemia. IGF1 mRNA levels were reduced by 30–50% in most brain regions in both CR groups. IGF1 receptor (IGF1R) mRNA levels were decreased in the CR-Std group but were increased in the CR-Ket diet group. Brain IGF binding protein (IGFBP)-2 and -5 mRNA levels were not altered by diet, but IGFBP-3 mRNA levels were markedly increased by the ketogenic diet while not altered by calorie restriction alone. Brain glucose transporter expression was also investigated in this study. Glucose transporter (GLUT) 4 mRNA levels were quite low and not appreciably altered by the different diets. Parenchymal GLUT1 mRNA levels were increased by the CR-Ket diet, but endothelial GLUT1 mRNA levels were not affected. Neuronal GLUT3 expression was decreased with the CR-Std diet and increased with the CR-Ket diet, in parallel with the IGF1R pattern. These observations reveal divergent effects of dietary caloric content and macronutrient composition on brain IGF system and GLUT expression. In addition, the data may be consistent with a role for enhanced IGF1R and GLUT expression in ketogenic diet-induced seizure suppression. (Endocrinology 144: 2676–2682, 2003)

THE KETOGENIC DIET is a high-fat, low-carbohydrate diet that is used for treating refractory epilepsy in children (1). Despite its long history of clinical use, it is still not entirely clear how the ketogenic diet affects the brain and what mechanism(s) underlie its seizure-suppressive action. Because both the ketogenic diet and fasting have beneficial effects on epilepsy, it has been assumed that they share a common mechanism in alleviating seizures. Because both the ketogenic diet and fasting produce elevated blood levels of β-hydroxybutyrate (β-OHB) and acetacacetate, it has been speculated that ketosis may have a beneficial effect upon brain seizure resistance. In addition to ketosis, other changes associated with the ketogenic diet might affect seizure activity. For example, changes in energy metabolism, in lipid composition of cell membranes, in the level of brain water content, and in brain pH have all been suggested to play a role in seizure suppression (2, 3).

Reduction in brain energy supply, e.g. from systemic hypoglycemia or locally from reduced brain glucose transporter (GLUT) 1 expression (4), induces seizure activity by impairing the ability of neurons to stabilize membrane potential. We have previously shown that IGF1 is a key regulator of glucose transport and utilization in the developing murine brain (5) and therefore considered the possibility that the ketogenic diet may enhance IGF1 activity, thereby improving energy utilization and protection from seizures. Supporting this possibility, expression of IGF system components is regulated by nutritional factors in many different species and in many different tissues (6–8) including the brain (9, 10). To investigate this hypothesis, we used an animal model to evaluate the effects of the ketogenic diet on brain IGF system expression. Bough et al. (11) demonstrated that rats fed a ketogenic diet had significant increases in levels of β-OHB and seizure resistance compared with rats fed either a calorie-restricted normal diet or a normal diet, ad libitum, with the greatest efficacy found in juvenile rats. The ketogenic diet was most effective when administered with a modest (10%) calorie restriction.

Three different diets were used for our study: unrestricted standard rat chow (Ad lib-Std), calorie-restricted standard rat chow (CR-Std), and calorie-restricted ketogenic diet (CR-Ket). The CR-Std group was included to account for any effects resulting from simply restricting calories. Expression of IGF1 system mRNAs, including IGF1, IGF receptor, IGF binding protein (IGFBP)-2, -3, and -5, and GLUTs 1, 3, and 4 were examined by in situ hybridization in brains of the three diet groups after 7 d on the experimental diets. IGFBP-2, -3, and -5 (12–15) and GLUTs 1, 3, and 4...
were investigated because these are all relatively abundant in brain.

**Materials and Methods**

**Animals and diets**

The use of the rats in these experiments was approved by the Georgetown University Animal Care and Use Committee. Eighteen male Sprague Dawley rats at postnatal day 20 (P20) were randomly divided into three groups of six each. Groups were weight-matched at the beginning of the experiment. The control group received standard Purina 5001 rat chow (Purina Mills, St. Louis, MO) ad libitum (Ad lib-Std). Another group was also given standard Purina 5001 chow but was calorie restricted (CR-Std), and the ketogenic group was also calorie restricted (CR-Ket). The CR-Std and CR-Ket diets were isocaloric. The calorie restriction was modified to be approximately 40% of the calculated energy requirement. The CR-Std and CR-Ket groups were hybridized to sense probes and processed together with antisense probes (Sigma, St. Louis, MO). Samples were immediately frozen in dry ice. Trunk blood was collected for simultaneous glucose and ketone measurement. The brains were weighed and immediately frozen in dry ice. Trunk blood was collected for simultaneous glucose and ketone measurement. The brains were weighed and stored at −70 °C. Blood ketones were assayed by measuring the levels of β-OHB present in blood plasma using a diagnostic kit (Sigma, St. Louis, MO). Samples were immediately transferred to 3-ml Li+-heparin vacutainers (Becton Dickinson and Co., Franklin Lakes, NJ) and centrifuged at 2000 × g for 5–8 min. β-OHB levels were determined spectrophotometrically using 20 μl of plasma (GDS Technology, Elkhart, IN). Blood glucose was measured by placing a drop of trunk blood on the test strip and inserting it into the One Touch Profile glucose meter (LifeScan Inc., Johnson & Johnson, Milpitas, CA).

**In situ hybridization**

Sagittal sections of 10-μm thickness were cut at −15 °C and thaw-mounted onto poly-t-lysine-coated slides for histochemical analysis. The in situ hybridization protocol has been previously described in detail (19). The generation of cRNA probes for GLUT1, 3, and 4 (20), IGF1 and the IGF1 receptor (IGFIR) (21) and IGBP2, -3, and 5 (22) have been detailed elsewhere. After hybridization, sections were exposed to film and later dipped in Kodak NTB emulsion for 7–21 d. Parallel sections were hybridized to sense probes and processed together with antisense hybridized sections. The quantitation of hybridization signal was carried out in a blinded fashion. Hybridization signal was captured at ×200 using a high-power light microscopy was used to count grains within a constant area defined by an eyepiece reticule under direct visualization. Background signal from a sense probe was subtracted from these counts before further analysis. The signal for GLUT1 in capillary endothelial cells was scored at ×400 under oil. Two sections from each brain were scored, and four measurements were made in each section; thus, eight measurements were obtained and averaged for each animal. Hybridization signals overlying Purkinje cells were counted manually on individual cell under ×1000 magnification. Ten randomly selected Purkinje cells were analyzed on each section, and two sections from each brain were analyzed.

**Statistics**

Differences between groups were compared by ANOVA followed by Fisher’s least significant difference tests.

**Results**

**Diet effects on weight and systemic metabolism**

At the beginning of the experiment, all of the rats weighed approximately 40 g, and the groups average weights were not different. Table 1 shows the effects of the diets on body and brain weights. After the 7 d on their respective diets, the control (AL-Std) rats doubled in weight (85.8 g ± 3.0), whereas the calorie-restricted groups (CR-Std) and CR-Ket both remained approximately the same weight (41.7 ± 2.1 g and 42.5 ± 3.8 g). The differences in brain weight were much less pronounced. Mean AL-Std brain weight was approximately 15% greater than mean brain weight in the CR-Ket and CR-Std groups (P < 0.01), whereas brain weights were not significantly different in CR-Ket vs. CR-Std groups.

**IGF1 and IGF gene expression**

IGF system mRNA levels were determined by in situ hybridization on serial brain sections from each animal. IGF1 mRNA levels were reduced in both CR-Std and CR-Ket groups by 30% or more in fore- and mid-brain regions including frontal cortex, temporal cortex, thalamus, striatum, and inferior colliculus (Fig. 1). There was no difference, however, in IGF1 mRNA levels in cerebellar Purkinje cells among different diet groups. The two calorie-restricted diets had opposite effects on IGFIR gene expression. The CR-Std diet resulted in reduced IGFIR mRNA levels similar to the effect on IGF1 (Fig. 2). In contrast, the CR-Ket diet increased IGFIR mRNA levels in virtually all regions of the brain compared with both AL-Std and CR-Std diet groups (Fig. 2). IGFIR mRNA levels were not appreciably altered affected by either

| Table 1. Effects of diets on body and brain weight, serum glucose, and ketone levels |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | AL-Std          | CR-Std          | CR-Ket          |
| Body weight (g)                | 85.8 ± 3.0      | 41.7 ± 2.1<sup>a</sup> | 42.5 ± 3.8<sup>b</sup> |
| Brain weight (mg)              | 1.4 ± 0.05      | 1.25 ± 0.03<sup>a</sup> | 1.27 ± 0.02<sup>b</sup> |
| Glucose (mg/dl)                | 179 ± 10.5<sup>a</sup> | 127 ± 15.9<sup>b</sup> | 57.5 ± 7.2<sup>b</sup> |
| Ketones (mM)                   | 0.27 ± 0.001    | 0.23 ± 0.03     | 4.4 ± 0.67<sup>b</sup> |

<sup>a</sup> P < 0.01 compared with AL-Std.
<sup>b</sup> P < 0.0001 compared with AL-Std and CR-Std.
diet treatment in the Purkinje and granule cell layers of cerebellum.

**IGFBPs**

Brain IGFBP-2, -3, and -5 mRNA levels were compared in all diet groups. No changes were observed for IGFBP-2 or -5 (data not shown), but there was a marked increase in IGFBP-3 mRNA levels in the brains of the CR-Ket group (Fig. 3). IGFBP-3 mRNA was increased in Purkinje cells of the CR-Ket brains compared with AL-Std controls. IGFBP-3 mRNA was also elevated in other brain regions, such as frontal cortex and striatum on CR-Ket diet (data not shown).

Because the expression levels in these regions were very low in both AL-Std and CR-Std rats, essentially equivalent to hybridization background levels, the fold difference of increase between CR-Ket and the other two groups cannot be expressed. IGFBP-3 mRNA levels were not significantly affected by the CR-Std diet (Fig. 3).

**GLUTs**

GLUT1 and GLUT3 are two major facilitative GLUTs expressed in murine brains with GLUT1 expressed by vascular endothelium and glial cells, whereas GLUT3 is widely expressed in neurons (16). Animals on the ketogenic diet

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**Fig. 1.** Effects of dietary manipulation on IGF1 mRNA expression in the rat temporal cortex. A–C, Representative dark-field micrographs of IGF1 mRNA hybrid signals in brains from AL-Std (A), CR-Std (B), and CR-Ket (C) groups. D, Bright-field view of the section shown in C. The inset dark-field micrograph in D shows background signal produced by sense probe hybridization. IGF1 mRNA is concentrated in neurons that have relatively large nuclei (arrows). E, Quantitation of IGF1 mRNA levels in different brain regions. $P < 0.05$ (a) and $P < 0.01$ (b) compared with AL-Std. FC, Frontal cortex; TC, temporal cortex; Th, thalamus; St, striatum; Inf, inferior colliculus; Pk, Purkinje cells.
showed small but significant increases in GLUT1 levels in the brain parenchyma (by 26% compared with control, $P < 0.01$), with the CR-Std diet having modest effect on GLUT1 (Fig. 4A). GLUT1 mRNA was not, however, appreciably altered in brain blood vessels by the different diets (Fig. 4A). GLUT3 mRNA levels were decreased in the CR-Std group and increased in the CR-Ket group, similar to the pattern for IGF1R expression (Fig. 4B). GLUT4 is also expressed in neurons, but its expression levels are much lower and did not change appreciably in response to the different diets (data not shown).

The effects of the different diets on brain gene expression are summarized in Table 2.

**Discussion**

This study has shown that diet has important and complex effects on brain IGF system and GLUT gene expression. Calorie restriction reduces brain IGF1 and IGF1R mRNA levels in rats on a standard, carbohydrate-dominant diet, with no appreciable effect of this dietary manipulation on brain IGFBP-3 expression. A diet with the same calorie content composed primarily of lipid, however, increases brain IGF1R, IGFBP-3, and GLUT mRNA levels. These novel findings demonstrate that the caloric content and macronutrient composition of the diet exert independent effects on brain gene expression and may provide insight into the mechanisms of ketogenic diet-induced seizure suppression.

The proximate causes of the diet-induced changes in brain gene expression remain open to conjecture. Caloric restriction is well known to suppress IGF1 expression in peripheral tissues (6–8), but the mechanism of this effect is unknown. Observations on the effects of caloric restriction on brain IGF1 expression are less consistent, with results apparently dependent on developmental age and experimental protocol (9, 10). It is possible that systemic glucose levels regulate IGF1 expression in brain, given that blood glucose levels were reduced in both CR groups. However, brain IGF1 levels were equally reduced or reduced to a greater degree in the CR-Std group compared with the CR-Ket group (Fig. 1E), whereas glucose levels were more profoundly reduced in the latter group. This lack of correlation between systemic glucose levels and brain IGF1 expression may indicate that another, unidentified factor related to nutritional status influences brain IGF1 expression. Alternatively, the hypoglycemic effect may be maximal in the CR-Std group.

The effects of diet on brain IGF1R and IGFBP expression have not been previously investigated, to the best of our knowledge. IGF1R mRNA levels were decreased with calorie restriction in a carbohydrate-enriched diet, but increased with an isocaloric fat-based diet. These effects do not seem obviously related to blood glucose levels that were reduced in both the CR-Std and CR-Ket diets (Table 1), but it is possible that qualitatively different effects may occur at very low glucose levels. Supporting these observations on the opposite effects of the ketogenic diet on IGF1 and IGF1R expression, we have obtained similar results in a related model system. The suckling rat ingests a high-fat diet from maternal milk, in what is viewed as a natural model of the ketogenic diet (23). The pups develop a marked ketosis shortly after birth that persists during the whole suckling period. We compared IGF1 and IGF1R mRNA and polypeptide levels in pups that were weaned early (P16) to regular rat chow with littermates that continued nursing until P19 and found that IGF1 levels were lower and IGF1R levels higher in the suckling group (our unpublished data).

The present study has also found that brain IGFBP-3 expression is markedly increased in the context of the ketogenic diet. There is little information on the nutritional regulation of IGFBP-3 expression (24). It is possible that ketone bodies or fatty acids, both elevated in the ketogenic diet, augment both IGF1R and IGFBP-3 gene expression. IGFBP-2 and -5 were both considerably more abundant than IGFBP-3 in the rat chow diet. There is little information on the nutritional regulation of IGFBP-3 expression (24). It is possible that ketone bodies or fatty acids, both elevated in the ketogenic diet, augment both IGF1R and IGFBP-3 gene expression. IGFBP-2 and -5 were both considerably more abundant than IGFBP-3 in the rat chow diet. However, brain IGF1R and IGFBP-3 expression may be due to...
the marked reduction in systemic glucose levels seen in the CR-Ket diet group because hypoglycemia promotes GLUT expression (27–30). Neither GLUT1 nor GLUT3 mRNAs were altered by the modest 25% reduction in blood glucose produced by the CR-Std diet, but both were increased by the CR-Ket diet, in which the reduction of blood glucose was more pronounced. We found GLUT1 to be increased in brain parenchymal cells, but not in cortical microvasculature, in contrast to a previous study that found hypoglycemia-induced increases in capillary but not parenchymal GLUT1 (30). The different observations are likely explained by major methodological differences in the two studies. It is possible that GLUT3 expression was increased due to enhanced IGF activity in brain, as we have previously shown an association between increased IGF1R and increased GLUT3 expression in the monkey brain (31). Although IGF1 mRNA levels were reduced, IGF1R and IGFBP-3 were both increased. The role of locally produced IGFBPs with respect to IGF action is not
known, but IGFBP-3 protects IGF1 from proteolysis and clearance in the bloodstream, and it thus seems likely that brain IGFBP-3 production could augment local IGF1 effect by protecting it from proteolysis. Thus, particularly in the context of increased IGFR1 expression, there could be enhanced IGF1 effect, despite a reduction in IGFR1 production suggested by decreased IGF1 mRNA levels.

The ketogenic diet’s ability to suppress seizure activity may be related to the increased IGFR1 and GLUT1 and 3 gene expression described for the first time in the present study, although further studies are required to establish that these changes in IGFR1 and GLUT mRNA levels are reflected by enhanced IGFR1 and GLUT expression. GLUT1 deficiency results in a seizure disorder that is highly responsive to a ketogenic diet (32), and overexpression of GLUT1 protects against seizure-induced neuron loss (33). Further study is required to elucidate the specific signaling pathways whereby diet composition regulates brain IGF system expression and to evaluate the functional consequences of these changes on seizure susceptibility.

Acknowledgments

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