Ketogenic diet exhibits anti-inflammatory properties

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SUMMARY

The ketogenic diet (KD) is an established treatment for refractory epilepsy, including some inflammation-induced epileptic encephalopathies. In a lipopolysaccharide (LPS)–induced fever model in rats, we found that animals given the KD for 14 days showed less fever and lower proinflammatory cytokine levels than control animals. However, KD rats exhibited a decrease in circulating levels of arachidonic acid and long-chain n-3 polyunsaturated fatty acids (PUFAs), suggesting that the anti-inflammatory effect of KD was probably not due to an increase in anti-inflammatory n-3 PUFAs derivatives. These properties might be of interest in some conditions such as fever-induced refractory epileptic encephalopathy in school-aged children.

KEY WORDS: Epilepsy, FIRES, IL-1β, Ketogenic diet, Polyunsaturated fatty acids.

Ketogenic diet (KD) is a high-fat low-carbohydrate diet used as an effective treatment in patients with refractory epilepsy, including some inflammation-induced epileptic encephalopathies such as fever-induced refractory epileptic encephalopathy in school-aged children (FIRES).

In experimental settings, KD provides significant neuroprotection in status epilepticus models, although its anticonvulsant profile differs between models. Although its anticonvulsant mechanisms are currently unknown, there are several hypotheses under investigation including the role of the “high-fat” component of the KD.

More than 10 experimental studies have suggested anticonvulsant properties of polyunsaturated fatty acid (PUFA). It has been suggested that the increase of PUFA intake provided by the KD may play a role in the effect of KD. Moreover, N-3 PUFA, particularly eicosapentaenoic acid (EPA) (20:5 n-3) and docosahexaenoic acid (DHA) (22:6 n-3), also have anti-inflammatory actions, mediated primarily by their hydroxylated metabolites.

Recently, it has been shown that KD attenuates thermal nociception and decreases peripheral edema, suggesting an anti-inflammatory effect. We decided to determine whether KD indeed exerts anti-inflammatory properties by using a rat model of fever induced by lipopolysaccharide (LPS) injections, and examining the effects of KD on body temperature, peripheral and cerebral inflammation, and blood PUFA levels.

METHODS

Animals and treatments

All experiments were performed on adult (P75) male Wistar rats (200–260 g; Charles River, L’Arbresle, France). Animals were housed three per cage with a 12 h/12 h light/dark cycle. All procedures were approved by the animal research committee of Bichat-Debré University and were performed...
in agreement with local, institutional, national, and international laws and regulations. Two groups were studied: a KD group (n = 8) that received KD for 14 days ad libitum without any weaned period (Ketocal; Nutricia-SHS, Liverpool, United Kingdom) and a control group (n = 8) that received a standard diet for 14 days (Table S1) without any difference in mean weight between the group (Fig. S1).

After 14 days of diet, both groups received an intraperitoneal injection of 50 μg/kg of LPS (Sigma; Escherichia coli serotype 055:B5; at 10:00 a.m.) after a blood sample taken by tail vein catheterization for white blood cell counts and PUFA measurements.

**Body temperature recordings**

Body temperature was assessed by rectal measurement every 15 min for 4 h after LPS injection. Room temperature was maintained at 20–22°C throughout the experiment.

**Cytokine and prostaglandin measurements**

Blood was collected by tail vein catheterization immediately before and 1, 2, and 4 h after LPS injection. Blood for cell count was collected on ethylenediaminetetraacetic acid (EDTA). Automated blood counts using flow cytometer were used. Blood samples were centrifuged for 20 min at 1,000 × g at 4°C and plasma was collected. Measurements of the cytokines tumor necrosis factor α (TNFα) and IL-1β and of prostaglandin E2 (PGE2) were carried out using ELISA kits according to the manufacturer’s instructions (R&D Systems, Abingdon, United Kingdom).

Blood fatty acid measurements were performed as described previously. The results are reported as quantity of fatty acid (mg/ml).

**RNA extraction and qRT-PCR**

Total RNA from both hippocampi was extracted with the RNeasy mini kit according to the manufacturer’s instructions (Qiagen, Courtaboeuf, France). Equal amounts of total RNA (1 μg) were subjected to reverse transcription using the iScript cDNA synthesis kit (Bio-Rad, Marnes-la-Coquette, France). Quantitative PCR was performed in duplicate for each sample using SYBR Green Supermix (Bio-Rad) for 40 cycles with a two-step program (5 s of denaturation at 96°C and 10 s of annealing at 60°C). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference. The following primers were used: GAPDH-F, 5′-GGCTTCCCGTGT TCC TAC-3′; GAPDH-R, 5′-TGT CAT CAT ACT TGG CAG GTT-3′; IL-1β-F, 5′-AGC AAC GAC AAA ATC CCT GT-3′; IL-1β-R, 5′-GAA GAC AAA CCG CTT TTC CA-3′; TNFα-F, 5′-CCT CCT CTC TGC CAT CAA GA-3′; and TNFα-R, 5′-TGG AAG ACT CCT CCC AGG TA-3′. The expression levels of genes of interest were calculated relative to the expression level of GAPDH. Analyses were performed with BIO-RAD CFX MANAGER 3.0 software (BioRad, Hercules, CA, U.S.A.).

**Statistical analysis**

Data were analyzed using PRISM 5 software (GraphPad, San Diego, CA, U.S.A.). Data were expressed as means ± standard errors of the mean (SEM). Statistical analysis was performed using repeated-measures analysis of variance (ANOVA) and the Mann-Whitney test.

**RESULTS**

**KD pretreatment is associated with attenuation of LPS-induced fever**

Fever induced by LPS appeared 2 h after LPS injection in the control group as described previously. In contrast, body temperature remained below 38.5°C at all time points in the KD group. A significant difference between the body temperature of the two groups was observed starting 2.5 h after LPS injection up to the end of the experiment (repeated measures ANOVA F:15.91; p < 0.0001) (Fig. 1A). The profile of the fever was correlated with plasma IL-1β levels in both diet groups, with a time-dependent increase in IL-1β observed only in controls (1 h after injection 130.8 ± 36.3 pg/ml in control vs. 51.4 ± 9.1 pg/ml in KD [p < 0.05]; 2 h after injection 203.3 ± 65.8 pg/ml in control vs. 171.9 ± 38.8 pg/ml in KD [p < 0.05]; 4 h after LPS injection in Fig. 1C).

**KD pretreatment is associated with a reduction of blood proinflammatory cytokine levels after LPS injection**

Four hours after LPS injection, interleukin 1β (IL-1β) and TNFα plasma levels were significantly lower in the KD group compared to the control group (Fig. 1C, p < 0.01), although no difference was observed before LPS injection. In contrast, 4 h after LPS injection, prostaglandin E2 (PGE2) plasma levels were not different between the two groups.

Four hours after fever induction by LPS, lymphocyte counts in the KD group (Fig. 1B; 2.4 G/L) were lower than in the control group but remained within the normal range (Fig. 1B; 4.4 G/L; p < 0.05), although no difference was observed before LPS injection. Counts of other white and red blood cells did not differ between the two groups either before or after LPS exposure (Fig. 1B).

**KD pretreatment is associated with a reduction of the IL-1β response in the brain after LPS injection**

Four hours after LPS injection, hippocampal mRNA levels for IL-1β were decreased in the KD group compared to the control group (Fig. 1D, p < 0.05). In contrast, mRNA levels for TNFα were not different between the two diet groups.

**KD modifies blood fatty acid profiles**

Due to a major difference in fat intake between the diets (additional material), an expected increase of total fatty acid level was observed after 14 days of KD compared to standard diet (Fig. 2). The levels of the long-chain omega-3 (n-3)
PUFAs EPA (C20:5 n-3) and DHA (C22:6 n-3), which are precursors of some anti-inflammatory agents, were decreased in the KD group (Fig. 2). On the other hand, arachidonic acid (AA; C20:4 n-6), an n-6 PUFAs that is a precursor for the synthesis of some pro-inflammatory eicosanoids, was also reduced by KD.

**Discussion**

Using an LPS-induced fever model, we have revealed the anti-inflammatory properties of KD, as shown by a modulation of the fever response and a decrease in IL-1β levels in the plasma and brain. TNF-α levels in the blood were also lower in the KD group compared to controls.

The role of pro-inflammatory cytokines, in particular IL-1β, has been demonstrated in icotogenesis when central nervous system (CNS) inflammation is present. The implication of peripheral inflammation in seizures and epilepsy has, however, been less well documented. A peripheral increase in IL-1β can elicit blood–brain barrier breakdown, further enabling systemic inflammatory agents to activate inflammation in the brain. Reducing peripheral IL-1β levels delays the onset of status epilepticus in the lithium pilocarpine model. In humans, there are several
conditions where peripheral inflammation contributes to ictogenesis or seizure-induced cell injury. The effect of KD on blood levels of pro-inflammatory cytokines might therefore also be a relevant finding in the epilepsy field. In the immature brain, it appears that inflammation triggers altered seizure susceptibility only when there is a concomitant increase in body temperature. The dual effect of KD on both the increase in body temperature and the increase in IL-1β might thus be of therapeutic interest.

We were able to evaluate the role of KD on inflammation using an LPS-induced fever model. The use of a model of status epilepticus would not have been appropriate because KD exerts anti-ictogenic properties as well as neuroprotective properties. The resulting reduction in the duration of seizure would have been a confounding factor by decreasing the level of inflammation and/or the level of cerebral injury. The decrease in cell injury due to the neuroprotective properties of KD might also modify cerebral inflammation. The absence of a reduction in the counts of white blood cells in the blood (cell count remained in normal ranges) excludes a misinterpretation of cytokine levels. A low level of lymphocytes or of polymorphonuclear cells induced by KD would lead to a lower ability to release/synthesize proinflammatory cytokines.

PUFAs, particularly n-3 PUFAs, have been proposed to be involved in the anti-epileptic actions of KD. The lower levels of EPA and DHA after KD suggest that n-3 PUFA derivatives are not involved in the anti-inflammatory properties of KD. However, we observed a decrease of AA blood levels in KD-fed rats as observed previously observed, suggesting a possible decreased ability to synthesize proinflammatory n-6 derivatives in the KD group.

**CONCLUSION**

KD modulates fever by decreasing peripheral inflammation and brain IL-1β expression. These properties might contribute to the effect of KD in the field of epilepsy, where inflammation contributes to seizure precipitation and/or brain injury. Although we cannot exclude an effect of ketone bodies and/or caloric restriction, the role of a decrease of AA that has been also reported in patients responding to KD, should be explored to better understand the anti-inflammatory properties of KD.

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**DISCLOSURE**

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**REFERENCES**


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

- Table S1. Full description of the diets.
- Figure S1. Mean weights in the two studied groups during the experiment.