Protein-Energy Malnutrition Alters Thermoregulatory Homeostasis and the Response to Brain Ischemia

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Abstract: Co-existing protein-energy malnutrition (PEM), characterized by deficits in both protein and energy status, impairs functional outcome following global ischemia and has been associated with increased reactive gliosis. Since temperature is a key determinant of brain damage following an ischemic insult, the objective was to investigate whether alterations in post-ischemic temperature regulation contribute to PEM-induced reactive gliosis following ischemia. Male Sprague-Dawley rats (190-280g) were assigned to either control diet (18% protein) or PEM induced by feeding a low protein diet (2% protein) for 7 days prior to either global ischemia or sham surgery. There was a rapid disruption in thermoregulatory function in rats fed the low protein diet as assessed by continuous recording of core temperature with bio-electrical sensor transmitters. Both daily temperature fluctuation and mean temperature increased within the first 24 hours, and these remained significantly elevated throughout the 7 day pre-ischemic period (p < 0.027). In the immediate post-surgical period, PEM decreased body temperature to a greater extent than that in well-nourished controls (p = 0.003). The increase in daily temperature fluctuation caused by PEM persisted throughout the 7 day post-surgical period (p < 0.001), and this interacted with the effects of global ischemia on days 8 (p = 0.018) and 11 (p = 0.021). The astrocytic and microglial responses induced at 7 days after global ischemia were not influenced by PEM, but this preliminary analysis needs to be confirmed with a more reliable global ischemia model. In conclusion, exposure to a low protein diet rapidly impairs the ability to maintain thermoregulatory homeostasis, and the resultant PEM also diminishes the ability to thermoregulate in response to a challenge. Since temperature regulation is a key determinant of brain injury following ischemia, these findings suggest that the pathophysiology of brain injury could be altered in stroke victims with co-existing PEM.

Keywords: Global brain ischemia, protein-energy malnutrition, rat, reactive gliosis, thermoregulation, two-vessel occlusion.

INTRODUCTION

Despite years of animal research focused on understanding the pathophysiology of ischemic brain injury, there have been challenges in translating much of this work into effective neuroprotective treatments for human stroke victims [1]. Our laboratory has instead focused on investigating the implications of compromised nutritional status as a possible stroke co-morbidity factor. Since a significant proportion of stroke patients have poor nutritional status at the time of insult [2-5], it is crucial to understand what impact this may have on the outcome. Thus, differences in the intracellular events following stroke caused by malnutrition need to be identified as the basis for designing targeted nutritional interventions.

The extent of neuronal damage following an ischemic insult can be strongly influenced by poor nutritional status. Protein-energy malnutrition (PEM), characterized by deficits in both protein and energy status, is present in 12-19% of stroke patients upon admission to the hospital [3-5]. The problem often worsens as a result of post-stroke hospital care to reach prevalence rates as high as 20-35% at one week [4,6], and 35-49% by the time of admission to a rehabilitation unit [7,8]. Clinical evidence, with noted methodological limitations, has demonstrated a correlation between PEM and increased morbidity and mortality following stroke [3-5,9]. A cause-and-effect relationship has been identified with the use of a rodent model of global brain ischemia demonstrating that PEM affects outcome on both a cellular and functional level [10-12].

The extent of neuronal injury following global ischemia is highly temperature-sensitive. Post-ischemic hypothermia can drastically reduce ischemic brain injury [13,14], whereas hyperthermia can aggravate injury [15]. Chronic malnutrition has been reported to impair the ability to maintain thermoregulatory homeostasis [16-18], especially when animals are confronted with thermoregulatory challenges [17]. Therefore, an altered thermoregulatory response induced by PEM could explain some of the changes in the neuronal and glial response following global ischemia previously reported by our laboratory [10,11].

Neuronal damage progresses during the acute period following ischemia by activation of several interrelated secondary mechanisms. Reactive gliosis and inflammation are among two of these processes within a complex cascade of intracellular and extracellular events [19,20]. These two
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processes are also strongly interrelated, since pro-inflammatory mechanisms modulate the activation of glial cells, which in turn produce more inflammatory cytokines [19,20]. Inflammation is an orchestrated response following an ischemic insult that can contribute both to neuronal death and modulation of repair processes [21]. Since the post-ischemic inflammatory response is only partly understood, there has been much debate about its overall effect on outcome. That immunodeficient mice present with less stroke injury [22] suggests that inflammation is more detrimental than beneficial. Temperature regulation can directly alter both the glial and inflammatory responses following global ischemia [20]. One mechanism by which hypothermia attenuates neuronal damage after global ischemia is by reducing microglial activation and nuclear factor κB (NFκB) activation [23]. The mechanisms associated with hyperthermia are less established [15]; however, stroke victims with hyperthermia have increased plasma levels of pro-inflammatory mediators when compared to normothermic patients [24].

We have previously obtained indirect evidence of an altered glial and inflammatory response following global ischemia in protein-energy malnourished rodents [10,11] that appeared to be related to impaired functional outcome as assessed by the open field test [11]. Since this impaired function occurred without an increase in hippocampal CA1 neuronal death, it suggests either that the CA1 neurons remained viable but with aberrant function or that there was injury occurring elsewhere. Given that a short post-ischemic sampling time (10 days) was used for this study and cell death can continue for months [25], it is possible that long-term cell death would have been increased in the malnourished animals due to elevated inflammation. Unusually dramatic reactive gliosis was evident on routine histological staining in one-third of the malnourished gerbils [11]. These animals were also the ones with both the most severe functional impairment (thigmotaxis) and greater than average hippocampal CA1 neuronal death, leading to the hypothesis that PEM enhanced the inflammatory response. A limitation, however, was that the glial cell type was not characterized by immuno histochemical analysis. Another perplexing finding was that the pronounced reactive gliosis, while never apparent after global ischemia in control diet fed gerbils, was evident in only a subset of malnourished animals [11]. While intra-ischemic tympanic temperature was strictly controlled in this study, a possible contributor to variability in reactive gliosis and the extent of ischemic injury is post-ischemic temperature regulation. Since spontaneous variation in post-ischemic temperature occurs in the gerbil bilateral carotid artery model of global ischemia [26], we cannot rule out the occurrence of either hypothermia or hyperthermia.

Other evidence supporting the hypothesis that PEM increases the inflammatory response came from the observation that malnourished gerbils have increased activation of the transcription factor NFκB in the hippocampus [10]. Pro-inflammatory molecules are predominantly regulated at the level of transcription by the critical intracellular mediator, NFκB, which also plays a role in glial cell function [27]. NFκB activation rapidly occurs following ischemic stroke, ultimately escalating inflammation by altering pro-inflammatory gene expression [28,29]. Although there is yet no direct evidence that PEM enhances the expression of NFκB pro-inflammatory target genes following brain ischemia in experimental stroke models or stroke patients, it appears that PEM itself causes a low-grade inflammatory response [30,31].

In the current study, we extend our previous findings to the influence of PEM on global ischemia modeled by two-vessel occlusion (2-VO) in the rat. The first objective was to investigate if the astrocytic (glial fibrillary acidic protein (GFAP)) and microglial (ED-1 and OX-42) responses after global ischemia were altered by PEM. Second, we assessed whether PEM modifies pre- and post-ischemic temperature regulation with the intent of clarifying whether such alterations contribute to PEM-induced reactive gliosis following ischemia.

**MATERIALS AND METHODS**

**Animals**

Forty-three male Sprague-Dawley rats (30-32 day old) (Charles River Canada, QC, Canada) were acclimatized on rat chow for 2 days and then placed on a purified control diet (see below for composition) for 5-6 days. The rats were caged in groups of 2-4 and had food and water available ad libitum. Animals were housed in a temperature and humidity controlled room with a 12-hour light/dark cycle. All animal procedures were in compliance with guidelines of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Committee on Animal Care and Supply.

**Core Temperature Bio-Electrical Sensor Transmitter Implantation**

After acclimation, calibrated bio-electrical sensor transmitters encapsulated in biocompatible silicone (SubCue™ Dataloggers), pre-programmed to record core body temperature every 30 minutes, were implanted aseptically into the abdominal cavity. Rats were anaesthetized with isoflurane (4% induction, 2% maintenance with 1 L/min oxygen) and placed on a heated water blanket. A 2-3 centimeter mid-abdominal incision through the skin and linea-alba was made, allowing insertion of the sterilized, calibrated temperature sensor into the peritoneal cavity. Following suturing of the incision site, a single subcutaneous injection of Ketoprofen (5mg/kg) was given. Temperature sensors remained in situ until the end of the experiment.

**Diet Assignment**

At 5-7 days following temperature sensor implantation, the rats (190-280g) were randomly assigned to either a protein adequate control diet (CON, 18% protein) or a protein-deficient diet (PEM, 2% protein) (Dyets, Inc., PA, USA) [32] and remained on the respective diet regimen until euthanasia. Rats of this age fed a 2% protein diet voluntarily reduce food intake, resulting in mixed PEM [32]. Diets were modified from the American Institute of Nutrition-93G diet [33] to not contain the antioxidant, tertiary-butyldihydroquinone. Food intake was recorded daily and body weight recorded weekly. The day of assignment to diet was considered experimental day 0, and the experimental timeline is shown in Fig. (1).
Global Forebrain Ischemia

On experimental day 7-8, rats were subjected to either global forebrain ischemia via the 2-VO model of bilateral common carotid artery occlusion combined with hypotension (ISC) or sham surgery (SHAM), as previously modified [34] from Smith et al. [35]. All surgical procedures were performed using aseptic technique. Prior to surgery, animals were fasted (16-20 hours) to achieve blood glucose levels within a consistent range. Animals were anaesthetized with isoflurane (~2% maintenance in 70% N₂O and 30% O₂) and placed on a heated water blanket. During surgery, brain temperature was estimated using a tympanic probe (IT-18 flexible probe; Physitemp Instruments Inc., NJ, USA) and maintained near 37.5°C with an overhead heating lamp outfitted with an infrared bulb (250W). The heating lamp was controlled by an automated feedback temperature controller (CN9500; Omega Engineering Inc., CT, USA) attached to the tympanic probe. Both common carotid arteries were isolated via a 2 centimeter ventral midline incision. The jugular vein was cannulated for blood withdrawal and infusion to induce hypotension during the ischemic period. The tail artery was cannulated for insertion of a blood pressure sensor (PressureMAT PDKTP4-PCS; PendoTech, NJ, USA) for measurement of mean arterial blood pressure (MABP). Arterial blood samples (100 µL) were obtained from the tail artery for measurement of blood gases, hematocrit, and glucose concentration. For induction of ischemia, once blood pressure had reached 35 mmHg, micro-aneurysm clips (S&T Vascular Clamps HD-S; Fine Science Tools, BC, Canada) were applied to both carotid arteries for 10 minutes. During this period, blood was withdrawn into a heparinized syringe warmed by the heating lamp or infused as necessary to sustain MABP at 35-40 mmHg. Clips were removed following the 10 minute occlusion, carotid artery reperfusion was visually verified, and blood was slowly re-infused via the jugular vein. All incisions were sutured, and a bupivacaine dose (2mg/kg) was divided equally among the 3 incision sites and subcutaneously injected. Sham rats were treated identically except that carotid arteries were not occluded and hypotension was not induced. Thus, the four experimental groups generated at this time were: CON-SHAM (n=10), CON-ISC (n=10), PEM-SHAM (n=12), and PEM-ISC (n=11).

Immunohistochemistry

On experimental day 14 (7 days following surgery), rats were anaesthetized under isoflurane and perfused trans-cardially with 0.9% heparinized saline followed by 4% paraformaldehyde. Intact heads were stored at 4°C overnight in paraformaldehyde. Brains were removed and refrigerated for an additional 24 hours in paraformaldehyde. Extracted brains were submerged into a 20% sucrose solution for 3-5 days. Fixed brains were stored at -20°C until sectioning. Coronal sections (14 µm) were taken from the anterior hippocampus and immunolabeled for GFAP, ED-1 and OX-42. To ensure accurate assessment of relative immunohistochemical changes among experimental groups, one section from each of the experimental groups was mounted on the same slide. Thus, each slide contained 4 sections, and all experimental groups were processed under identical conditions.

The protocol used for each of the glial markers was identical, except that the sections for OX-42 staining underwent citrate antigen retrieval. Sections were washed in phosphate-buffered saline (PBS) and exposed to 1.0% H₂O₂. Sections were blocked with normal goat serum (5%; Invitrogen, CA, USA), followed by an overnight incubation at 4°C with either polyclonal rabbit anti-GFAP (1:1000, Z0334; DakoCytomation, ON, Canada), monoclonal mouse anti-rat CD68 (ED-1, 1:1000, MCA341R; Serotec, NC, USA), or monoclonal mouse anti-rat CD11b (OX-42, 1:500, MCA275G; Serotec). Slides were washed in PBS and incubated in goat anti-mouse biotinylated secondary antibodies (GFAP, 1:500; ED-1, 1:1000; OX-42, 1:1000; Vector Laboratories, CA, USA). Sections were treated with extravadin (10 µg/ml; Sigma –Aldrich, ON, Canada) and reacted with diaminobenzidine (Vector Laboratories). Quantification was performed by measuring the integrated density value (IDV=sum of pixel values in the region of interest) (AlphaEaseFC Imaging Software, Alpha Innotech) of photographs taken under identical conditions at 400x magnification. The IDV for OX-42 and ED-1 was determined for an isolated region of the hippocampal CA1 pyramidal cell layer (pixel area of 59,985) for both the right and left hemispheres and averaged. The same method was used to obtain the IDV for GFAP, except the stratum oriens and stratum radiatum areas were included with the hippocampal CA1 pyramidal cell layer (pixel area of 367,845). The staining in the CA1 region was normalized against background staining measured as an IDV for the corpus callosum by calculating the ratio, IDV of CA1/IDV of corpus callosum.

Core Temperature Analysis

At the time of euthanasia, the temperature sensors were retrieved and logged temperature data were obtained using
the SubCue™ analyzer software. Alterations in thermoregulatory function resulting from experimental diet and surgical assignment were assessed by examining differences in: a) daily mean temperature, b) daily temperature fluctuation calculated by deducting the lowest core temperature from the highest temperature measured over a 24 hour diurnal cycle, and c) lowest core temperature observed within 8 hours of anaesthetic induction for the surgery.

**Statistical Analysis**

Statistical analyses were conducted using SPSS 17.0 for Windows. All data are presented as mean ± SEM. Pre-surgical temperature data were analyzed using an independent t-test. Two groups were analyzed during the baseline period when all rats were on control diet to demonstrate that there were no initial differences in the groups randomly selected to receive different experimental diets. All post-surgical temperature and immunohistochemical data were analyzed by two-factor ANOVA, and LSD post-hoc tests were performed where indicated. Correlations between hippocampal CA1 injury and the lowest core temperature observed within 8 hours of anaesthetic induction were analyzed by Pearson’s correlation coefficient for data obtained for all rats exposed to ischemia (PEM-ISC + CON-ISC combined) as well as for individual ischemic groups. A probability value of < 0.05 was considered to be statistically significant.

**RESULTS**

Three rats (2 PEM-SHAM, 1 PEM-ISC) were excluded from the study due to surgical complications involving arterial cannulation and maintenance of anaesthesia. Data collected from all experimental rats to assess protein-energy status (food intake, body weight, and serum albumin), intra-ischemic tympanic temperature and blood pressure, the physiological response during surgery (blood gases, blood glucose, and hematocrit), hippocampal CA1 neuron loss, expression of microtubule-associated protein-2, and serum corticosterone concentration are presented in a separate publication [32].

**Thermoregulation**

Fig. (2) shows diurnal rhythm for core temperature as influenced by both PEM and exposure to global ischemia.

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**Fig. (2).** Diurnal rhythm of core temperature as influenced by both PEM and global ischemia. Values are mean core temperature readings taken at 30 minute intervals (n=10). Baseline data were collected for 3 days before assignment to experimental diet (day 0). The pre-surgical fasting occurred on days 6-7, and surgery occurred on day 7.
i) Pre-Surgical Period

There were no significant differences in baseline mean daily core temperature (p=0.063) and daily temperature fluctuation (p=0.366) when all animals were still on the CON diet (Fig. 3A and 3B). After commencement of the diet regimen, mean daily core temperature significantly increased in the group assigned to PEM within the first 24 hours (p=0.006), and remained significant for each of the following days during the pre-surgical period (Fig. 3A, p<0.024). PEM also significantly increased the daily core temperature fluctuation within 24 hours (p=0.027), which remained elevated until surgery day (Fig. 3B, p<0.027). The malnourished rats were warmer than the CON rats during the 12-hour dark period of each day and cooler during the 12-hours of light.

ii) Immediate Pre- and Post-Surgical Periods

The mean core temperatures for PEM and CON animals during 12 hours (8 PM-8 AM) of the 16-20 hour fasting period before surgery are shown in Table 1. The fasted protein-energy malnourished rats were significantly warmer prior to surgery (p= 0.002). Although tympanic temperature was maintained at ~37.5°C throughout the surgical period for all 4 treatment groups, core temperature was altered by dietary treatment in the immediate post-surgical period. Table 1 shows that, irrespective of surgical treatment, PEM independently caused a decrease in the mean lowest core temperature measured within 8 hours following anaesthetic induction (p=0.003). There was no effect of ischemia (p=0.747) nor was there an interaction between diet and ischemia (p=0.241).

iii) Post-Surgical Period

The only significant difference in mean daily temperature in the post-surgical period was an independent decrease on day 7 (p=0.004) and an increase on day 9 caused by ischemia (p=0.02, Fig. 4A). There were no effects of PEM nor an interaction on any experimental day (p>0.05). The daily temperature fluctuation remained significantly increased by PEM throughout the 7 day post-surgical period (Fig. 4B). This was an independent effect of PEM (p<0.001), with the exception of an interaction on days 8 (p=0.018) and 11 (p=0.021). On day 8, the PEM-SHAM animals had a significantly greater temperature fluctuation compared to the other three experimental groups (p<0.001). On day 11, ischemia increased the temperature fluctuation only when the rats were malnourished (p<0.015). There was also an independent decrease caused by ischemia on day 9 (p=0.002).

Table 1. Core Temperature Measurements in the Immediate Pre- and Post-Surgical Periods

<table>
<thead>
<tr>
<th>Pre-Surgery</th>
<th>CON</th>
<th>PEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-hour Fasting Mean Temp (°C)</td>
<td>37.4 ± 0.1</td>
<td>37.7 ± 0.1*</td>
</tr>
<tr>
<td>Post-Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest Core Temp within 8h A°I (°C)*</td>
<td>35.9 ± 0.1</td>
<td>36.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>CON-SHAM</td>
<td>CON-ISC</td>
</tr>
<tr>
<td></td>
<td>35.6 ± 0.2</td>
<td>35.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>PEM-SHAM</td>
<td>PEM-ISC</td>
</tr>
</tbody>
</table>

Data are mean (±SEM). n=20 for pre-surgical data, and n=10 for post-surgical groups. *Indicates a significant difference from CON group by independent t-test (p<0.002). *Indicates a significant independent effect of diet (p=0.003) by 2-factor ANOVA.
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Fig. (4). Post-surgical mean (± SEM) core temperature (A) and daily core temperature fluctuation (B) for CON-SHAM, CON-ISC, PEM-SHAM, and PEM-ISC (n=10). Experimental day 7 is 0-24 hours after surgery. For mean daily core temperature, an independent effect of ischemia occurred on days 7 and 9 (§). For daily temperature fluctuation, there was an independent effect of PEM (*), with the exception of an interaction on days 8 and 11 (£) and an independent effect of ischemia on day 9 (§).

Fig. (5) shows representative data to illustrate the influence of the extent of hippocampal injury on the diurnal rhythm of core temperature during the post-ischemic period in well-nourished rats. In general, CON-ISC rats presenting with unilateral hippocampal damage tended to have diurnal cycles similar to those of CON-SHAM rats (Fig. 5A). With extensive bilateral hippocampal CA1 injury, there was a disruption in the diurnal cycle of CON-ISC rats (Fig. 5B). The disruption lasted for 1-2 days, at which time the cycle returned to a normal pattern. This pattern of disruption in relation to extent of injury was observed in some of the PEM-ISC group, but the observation was not as consistent.

Immunohistochemistry

A separate report describes hippocampal CA1 cell counts from the animals in this study [32]. Extensive bilateral hippocampal CA1 injury after 2-VO surgery was evident in 7 CON-ISC animals, with 3 rats demonstrating unilateral

Fig. (5). Post-ischemic diurnal pattern for core temperature as influenced by extent of hippocampal injury in rats exposed to control diet and global ischemia. Representative patterns are shown for a CON-ISC rat with unilateral hippocampal damage (A) and a CON-ISC rat with extensive bilateral damage (B).
Surprisingly, only 5 rats in the PEM-ISC group had bilateral hippocampal CA1 injury, while 1 rat demonstrated unilateral CA1 damage and 4 rats sustained no CA1 injury [32].

There were no differences in background staining in the corpus callosum caused by surgical treatment (p>0.05) or diet assignment (p>0.05) for any of the three glial markers (GFAP, ED-1, OX-42).

Due to the unexpected variability in the CA1 neuronal damage, only rats with bilateral hippocampal damage were included in analysis to provide a preliminary assessment of the effect of PEM. Fig. (6) shows images of the CA1 region immunostained for GFAP, ED-1 and OX-42 on experimental day 14 for all four experimental groups. The images for the CON-ISC and PEM-ISC groups are representative of sections from rats sustaining bilateral CA1 injury in which CA1 cell counts are similar. Semi-quantification analyses revealed that global ischemia significantly increased GFAP (p<0.001; n=10 CON-SHAM, n=7 CON-ISC, n=10 PEM-SHAM, n=5 PEM-ISC), ED-1 (p<0.001; n=10 CON-SHAM, n=7 CON-ISC, n=10 PEM-SHAM, n=5 PEM-ISC) and OX-42 (p<0.001; n=7 CON-SHAM, n=5 CON-ISC, n=7 PEM-SHAM, n=3 PEM-ISC) staining, but were unaffected by dietary treatment (GFAP p=0.607; ED-1 p=0.195; OX-42 p=0.171), nor was there an interaction (GFAP p=0.056; ED-1 p=0.152; OX-42 p=0.132).

Correlations

Correlation analysis was performed to determine if the inconsistency in CA1 hippocampal injury was related to a post-ischemic drop in core temperature. The lowest core temperature within 8 hours of anaesthetic induction was correlated with total CA1 cell counts reported in [32] for: 1) CON-ISC and PEM-ISC groups combined, and 2) CON-ISC and PEM-ISC groups separately as shown in Table 2. Results from correlation analysis detected a significant negative correlation between hippocampal injury and the drop in temperature when only CON-ISC animals were included in the analysis (r = -0.897, p<0.001). Thus, the decrease in neuronal damage was limited to the CON-ISC animals that experienced a drop in core temperature.

Table 2. Correlation of Post-Ischemic Hippocampal CA1 Total Cell Counts with Post-Surgical Decline in Core Temperature*

<table>
<thead>
<tr>
<th></th>
<th>CON-ISC + PEM-ISC (n=20)</th>
<th>CON-ISC (n=10)</th>
<th>PEM-ISC (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core Temperature (°C)</td>
<td>-0.352</td>
<td>0.127</td>
<td>-0.897</td>
</tr>
<tr>
<td></td>
<td>p=0.010</td>
<td>p=0.978</td>
<td>p&lt;0.001</td>
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*Detected by Pearson’s Correlation Coefficient. *Lowest core temperature observed within 8 hours following surgical anaesthetic induction.
DISCUSSION

The major finding from this study was the rapid effect of a change in experimental diet on thermoregulation. Both daily temperature fluctuation and daily mean temperature were increased consistently within the first 24 hours after assignment to the low protein diet. As malnutrition developed, both parameters remained significantly higher than those of control diet fed rats throughout the pre-ischemic period. During this period, the greatest differences between the two experimental groups were 0.90°C for temperature fluctuation on experimental day 3 and 0.35°C for mean temperature on day 1.

This disruption in thermoregulatory response is in agreement with previous reports. However, the extent of the response and the trend in temperature patterns vary, with both warming [17,18] and cooling [36-38] responses reported. These discrepancies are likely due to differences in species, method used to measure temperature, age, severity of malnutrition, extent to which protein and/or energy were limiting, and stage of adaptation to the nutrient deficiency. Most studies in malnourished rodents have been well-controlled, although findings cannot be specifically attributed to protein-energy status in those studies in which the control and deficiency diets are not matched for all other nutrients [36]. The technique by which temperature data are obtained is a key factor, since most studies rely on single periodic measurements that fail to highlight changes in diurnal cycles. Furthermore, when conclusions have been drawn from acute rectal monitoring in rodents, this type of measurement can yield artificial temperature data by triggering stress-induced hyperthermia [39]. An advantage of implantable temperature transmitters, as used by us and others [18,36], is that accurate chronic readings highlighting diurnal cycles can be obtained in unanaesthetized animals without inducing a stress response.

Our core temperature results are comparable to the findings from Castañón-Cervantes et al. [18], who observed chronic occipital-cortex temperature regulation in rats exposed to protein malnutrition during brain development and into adulthood. Analogous to our core temperature findings, malnourished Sprague-Dawley rats presented with an altered circadian rhythm characterized by higher-amplitude daily cortical temperature rhythms. Mean daily cortical temperatures were also higher in young malnourished rats. However, this altered thermoregulatory response was not significant in malnourished old rats, demonstrating that age is another variable that may explain discrepancies among studies.

We found a prominent difference between the effects of the low protein diet on the daily temperature fluctuation and mean temperature during the pre-ischemic period. During the first three days after exposure to a low protein diet, rats increased both their daily temperature fluctuation and mean temperature. Following this initial period, the increase in temperature fluctuation remained constant and appeared to be permanent, whereas the increase in mean temperature began to decline after the second day. During the initial three day period, malnourished animals were warmer than control animals while awake, but had similar core temperatures while asleep. As time advanced, malnourished rats became cooler during the sleep period, resulting in more normalized mean core temperatures. This suggests that malnourished rats were able to adapt to the initial warming period by adjusting their temperature fluctuation. The increase in amplitude of daily temperature fluctuation was sustained throughout the post-surgical period. In contrast, the malnutrition-induced increase in mean daily temperature did not re-establish after surgery, suggesting that it was an acute response. Due to the short post-surgical period, we cannot rule out the possibility that a change in mean core temperature would re-establish with chronic PEM. Although the long-lasting effects of PEM on thermoregulation are rarely studied, one previous study revealed a persistent effect of chronic protein malnutrition on circadian rhythm for 28 days [18].

Our study did not address the mechanisms responsible for the temperature changes observed in the protein-energy malnourished rat. Body temperature is tightly regulated, with afferent thermal sensing receptor input sent from the skin surface, deep abdominal and thoracic tissues, spinal cord, and brain to the major thermoregulatory regulator, the hypothalamus [40]. Temperatures exceeding the warm- or cold-response thresholds activate the thermoregulatory defense (efficient) responses of sweating, vasodilation or vasoconstriction, non-shivering thermogenesis, and shivering. Behavioural responses, such as altering locomotion, are another form of defense. We propose that the effects of PEM on thermoregulatory defense are complex, involving heat production and loss, and are dependent upon the characteristics of the deficiency as discussed above.

The immediate temperature effects of the low protein diet may reflect a behavioural stress response similar to what has been described with rat handling [41]. We hypothesize that the immediate increase in mean core temperature is contributed to by an acute stress-induced rise in locomotor activity reflecting increased foraging caused by exposure to suboptimal diet. The data indirectly support this, since during the initial period of exposure to low protein diet, the largest temperature change in malnourished rats was during the awake cycle. Simultaneous measurements of the activity and temperature circadian rhythms would address this theory. The sustained increase in amplitude of the diurnal temperature cycle, however, suggests that alterations in thermoregulatory defense are also related to the adaptive metabolic and behavioural adjustments that occur as malnutrition develops [42].

PEM may exert direct effects on central regulation of temperature by altering specific hypothalamic nuclei that sense nutrient level and alter feeding and metabolic rate (reviewed in [43]). In addition, the altered thermoregulatory amplitude suggests that the low protein diet could be interacting with the hypothalamic suprachiasmatic nucleus (SCN). The latter regulates the circadian rhythms for many processes including sleep-wake cycles, feeding, body temperature, and locomotor activity; an alteration in one cycle can influence that of another [44]. Increases in rapid eye movement (REM) sleep, which attenuate thermoregulatory responsiveness (reviewed in [45]), have been reported with chronic malnutrition [46]. The latter can also modify the phase relation of core temperature to the motor activity oscillation [18]. It has even been suggested that a low protein diet can modify the cellular composition of the SCN,
resulting in a weaker coupling force among oscillators [47]. However, unlike our investigation, all of these studies initiated protein deficiency during brain development. Nevertheless, influences could also be indirectly exerted through modifications in neuroactive peptides and hormones. PEM induces a host of endocrine changes that regulate heat production and basal metabolic rate, including increased glucocorticoids [42]. Although we did not detect elevated corticosterone levels in these malnourished rats [32], the unphysiologically high values measured are likely indicative of sampling stress, which can mask an increase caused by malnutrition [48].

Protein-energy malnourished rats also showed a greater decrease in core temperature immediately following anaesthesia, and this was independent of the type of surgery. These data demonstrate that extra precautions need to be taken when studying PEM coupled with any surgical model. Rodents are highly susceptible to hypothermia induced by anaesthesia [49]. Despite the use of a circulating water blanket and heat lamp in the present study, all four experimental groups experienced a decrease in core temperature either during or immediately after anaesthesia. The greater tendency in the malnourished group may have been related to decreased heat conservation resulting from reduced thermal insulation from adipose tissue. None of the rats were able to seek a warmer environment (that is, huddle with cage-mates) due to single-housing during the recovery period. This finding is in agreement with others’ reports on the inability of malnourished rats to cope with environmental temperature stressors. Sprague-Dawley rats fed a protein-restricted diet for 6 weeks were not able to respond to the same level as control animals when subjected to a mild cold challenge. Following a 90-minute exposure period to an 18-19ºC environment, the malnourished animals were on average 2.5ºC cooler and took approximately twice the length of time to recover from the stressor [17]. A similar trend has been reported in malnourished elderly patients [38].

Unlike a previous report [50], exposure to global ischemia induced by 2-VO also affected temperature regulation during the post-surgical period. Following the immediate cooling period, there was a distinct recovery phase, which differed depending on experimental group and the extent of neuronal damage. Independent of diet, SHAM animals returned to regular diurnal oscillations immediately following the anaesthetic induced cooling period. CON-ISC animals with extensive hippocampal damage tended to present with distorted diurnal rhythms that lasted approximately 48 hours whereas CON-ISC rats with unilateral hippocampal damage regulated temperature similarly to SHAM animals during the recovery phase. This relationship between disrupted diurnal cycle and extent of hippocampal injury was more ambiguous in the PEM-ISC rats, presumably because of the independent effects of PEM on thermoregulation.

An unfortunate and unexpected finding of the current study reported in detail elsewhere [32] is that co-existing PEM increased the inconsistency of the 2-VO model of global ischemia. This may be a consequence of PEM altering multiple physiological variables, since there was no one clear contributor [32]. The decline in core temperature in the immediate post-anaesthetic period presented here also does not offer an explanation. The mean lowest core temperature in PEM-ISC rats during the 8 hours following anaesthetic induction was 0.8°C lower than the corresponding mean of CON-ISC animals. However, a negative correlation between temperature and the number of surviving CA1 neurons, while detected for the CON-ISC animals, was not evident in the malnourished rats exposed to ischemia. Thus, the decrease in CA1 neuronal damage was not explained by a drop in core temperature. Multiple variables can affect the efficacy of post-ischemic hypothermia for neuroprotection, including the duration, rate and severity of temperature drop, as well as the type of stroke [13]. The majority of protein-energy malnourished rats experienced a brief post-ischemic drop in temperature, and it is unlikely that the temperatures were low enough to provide significant neuroprotection. Greater and more prolonged drops in temperature are needed to significantly mitigate injury [13].

This failure to achieve a consistent model of global ischemia [32] has confounded the assessment of glial response and prevents us from drawing conclusions on whether PEM exacerbates reactive gliosis after global ischemia through alterations in temperature regulation. A preliminary assessment was conducted exclusively on rats presenting with extensive bilateral hippocampal damage. The extent of gliosis appeared to mirror the degree of neuronal injury, but the striking increase in reactive gliosis previously observed in protein-energy malnourished gerbils after global ischemia [11] was not observed. While the glial response significantly differed between SHAM and ISC groups, PEM had no effect on GFAP, ED-1 and OX-42 expression. Since the model of brain ischemia was confounded and evaluating only those rats with bilateral hippocampal injury can introduce bias, our preliminary analysis of the effects of malnutrition on the glial response is inconclusive and should be re-addressed in future with a reliable ischemia model.

CONCLUSIONS

Exposure to a low protein diet rapidly impairs the ability to maintain thermoregulatory homeostasis. Furthermore, when confronted with the thermoregulatory challenge associated with anaesthesia, protein-energy malnourished rats were less able to adapt than well-nourished animals and thus presented with a greater transient drop in core temperature. Since temperature regulation is a key determinant of brain injury following ischemia, these findings suggest that the pathophysiology of brain injury in malnourished stroke victims could be altered and that these patients might require distinct treatment. Although our preliminary analysis of the influence of PEM on the glial response to global ischemia is inconclusive, this question should be re-addressed in a different model of brain ischemia since other evidence suggests that PEM lowers the setpoint for inflammation.

ABBREVIATIONS

2-VO = Two-Vessel Occlusion
AIN-93G = American Institute of Nutrition 93 Growth Diet
ANOVA = Analysis of Variance
CA1 = Cornu Ammonis 1

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CON = Control Diet
GFAP = Glial Fibrillary Acidic Protein
ISC = Ischemia
LSD = Least Significant Difference
MABP = Mean Arterial Blood Pressure
NFkB = Nuclear Factor Kappa B
PEM = Protein-Energy Malnutrition
REM = Rapid Eye Movement
SCN = Suprachiasmatic Nucleus
SEM = Standard Error of the Mean
SHAM = Sham Surgery

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