Protein-Energy Malnutrition Causes Deficits in Motor Function in Adult Male Rats

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Abstract

Background: Adult protein-energy malnutrition (PEM) often occurs in combination with neurological disorders affecting hand use and walking ability. The independent effects of PEM on motor function are not well characterized and may be obscured by these comorbidities.

Objective: Our goal was to undertake a comprehensive evaluation of sensorimotor function with the onset and progression of PEM in an adult male rat model.

Methods: In Expt. 1 and Expt. 2, male Sprague-Dawley rats (14–15 wk old) were assigned ad libitum access for 4 wk to normal-protein (NP) or low-protein (LP) diets containing 12.5% and 0.5% protein, respectively. Expt. 1 assessed muscle strength, balance, and skilled walking ability on days 2, 8, and 27 by bar-holding, cylinder, and horizontal ladder walking tasks, respectively. In addition to food intake and body weight, nutritional status was determined on days 3, 9, and 28 by serum acute-phase reactant and corticosterone concentrations and liver lipids. Expt. 2 addressed the effect of an LP diet on hindlimb muscle size.

Results: PEM evolved with time with the LP diet. Total food intake decreased by 24% compared with the NP group. On day 28, body weight and serum albumin decreased by 31% and 26%, respectively; serum α2-macroglobulin increased by 445% (P < 0.05). Forelimb dysfunction (173% increase in adaptive flexed-arm-hang score) developed on day 2 with the LP diet (P < 0.001), whereas abnormal walking (34% decreased incidence of correct hindlimb placement) evolved by day 27 (P < 0.05). The LP diet reduced the cross-sectional area of gastrocnemius medialis (P < 0.05).

Conclusions: PEM in the adult male rat causes a variety of sensorimotor abnormalities that develop at different stages of malnutrition. This model can be used in combination with disease models of sensorimotor deficits to examine the interactions between nutritional status, other treatments, and disease progression.

Keywords: protein-energy malnutrition, performance-based measures, motor function, acute-phase reactants, rat model

Introduction

Adulthood protein-energy malnutrition (PEM) has a negative impact on human well-being. PEM is prevalent in the elderly (1, 2) and often occurs in parallel with neurological disorders affecting hand use and walking ability, such as after stroke or Parkinson disease (3–6). Although adulthood PEM can predict poor motor outcome in hospitalized adults (6, 7), it is not obvious whether it is a direct cause of the functional deficits. Multiple comorbidities can obscure the recognition of the first signs of PEM and its progression, thus confounding the diagnosis of PEM and preventing a timely and appropriate nutritional intervention. Because the clinical manifestations of malnutrition are related to the duration and the degree of nutritional compromise (8), early recognition of malnutrition and knowing the time of onset are critical for defining treatment plans.

Detecting malnutrition at an early stage should allow for the most effective intervention. The Academy of Nutrition and Dietetics and the American Society for Parenteral and Enteral Nutrition recently standardized diagnostic criteria to identify malnutrition in clinical practice. The following set of characteristics was advised: history and clinical diagnosis, physical examination/clinical signs, anthropometric data, laboratory data, food/nutrient intake, and functional assessment (9).
Although it is recommended that multiple measures be used to assess each of the 5 categories, only the hand grip strength test, which efficiently detects severe malnutrition, is routinely used to document a decline in physical function (9). Performance-based measures should not be neglected, given their strong predictive validity for subsequent disability onset (10). Objective and standardized performance-based measures of balance, strength, and walking have been developed for functional evaluation (10, 11) and should be investigated for detecting malnutrition.

No clinical or preclinical study has provided a comprehensive evaluation of sensorimotor abnormalities associated with the onset and progression of adulthood PEM. Thus, grip strength and muscle endurance, balance, and walking ability were assessed in the current study in an adult male rat model of PEM during the progression of malnutrition. PEM was induced by feeding a low-protein (LP) diet. Nutritional status was evaluated by body morphometric measurements, food intake, serum acute-phase reactants, and corticosterone concentrations and the degree of liver steatosis. We hypothesized that a comprehensive functional evaluation and characterization of the time of onset of each of the functional abnormalities would be a powerful approach to recognize the early signs, time of onset, and the degree of severity of PEM.

Methods

Expt. 1 addressed the influence of PEM on forelimb and hindlimb function in parallel with body weight, food intake, brain size, acute-phase reactants, and serum corticosterone and liver lipid concentrations. The battery of behavioral tests and the repeated-measures design were implemented to reveal both PEM-induced motor abnormalities and possible compensatory adjustments. Expt. 2 assessed the effect of PEM on muscle size. The anatomic terminology used is in agreement with previously published data (12, 13).

Animals

Male Sprague-Dawley rats (11–12 wk old; n = 27) were obtained from Charles River Laboratories. Rats were housed in clear plastic cages (39.5 × 34.6 × 22.7 cm) with absorbent bedding in groups of 2 in a colony room maintained on a 12-h light/dark cycle (0700–1900 h) with controlled temperature and humidity. This work was approved by the University of Saskatchewan’s Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Expt. 1

Experimental design. The design for Expt. 1 is summarized in Supplemental Figure 1. Sixteen male Sprague-Dawley rats, age 12–13 wk, were fed regular rat feed pellets (ProLab RMH 3000 no. 00031495; LabDiet) for 3 d. They were then acclimatized to a modified AIN-93M diet voluntarily reduced food intake, resulting in PEM (15).

Body weight. Body weights were recorded daily for 7 d before and 11 d after diet assignment and twice a week for the remaining experimental period.

Food intake. Food intake was recorded daily on a cage basis (2 rats/cage) throughout the experimental period.

Brain morphometric measurements. The brain was removed from the cranium and weighed before the capture of a digital image of the dorsal view. Brain area (cm²) was measured by using Image J software (NIH).

Biochemical data.

Serial blood sampling. Under ∼0.5% isoflurane anesthesia (1 L O₂/min, ∼1 mL blood was collected between 0900 and 1000 h on days 3 and 9 from the tail vein [BD Insyte; intravenous catheter, 24 gauge × 0.75 inch (0.7 × 19 mm)]. At the end of the Expt., ∼3 mL was collected by cardiac puncture under ∼4% isoflurane anesthesia (1 L O₂/min). Blood was allowed to clot for 30 min at room temperature and then centrifuged at 1300 × g at 4°C for 10 min. Serum was removed and stored at −80°C.

Serum albumin concentration. Serum albumin concentration was determined in triplicate by the bromocresol green method (16). Blank, albumin standard, or serum (15 μL) was added to 3.0 mL bromocresol green reagent (0.15 mmol bromocresol green/L, 0.075 mol succinate buffer/L, 4 g 30% Brij-35/L). After 30 min, absorbance was measured spectrophotometrically at 628 nm (SpectraMax M5). Albumin concentration was determined by fitting the data by linear regression.

Serum corticosterone concentration. Serum corticosterone concentration was analyzed by Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan, by using ImmuChem double antibody corticosterone 121I RIA (MP Biomedicals), which measures total corticosterone. Serum samples (1:5000) were run in triplicate.

Serum α2-macroglobulin concentration. Serum α2-macroglobulin (2AM) concentration was determined by double sandwich ELISA kit (Immunology Consultants Laboratory). Serum samples (1:500) were run in triplicate.

Total liver lipids. Lipid content was determined in duplicate by using the lipid extraction method as described previously (17, 18). Liver lipid weight was expressed as a percentage of liver wet weight.

Functional tests.

Bar-holding task. Muscle strength (19) and/or endurance of the forelimbs was quantified with a bar-holding task. A metal bar (3.5 mm thick and 50 cm long) was positioned horizontally 54 cm above a padded surface. The rat was held so that the forelimbs touched the bar, and the hindlimbs were pulled down lightly to stretch out the forelimb and shoulder muscles, enabling the forelimb grasping reflex. Latency was recorded as the length of time the rat could hang on the bar until grip failure or for a preset maximum of 120 s. Strategies used to hang on the bar were also measured as a score reflecting the sum of all complete “pull-ups” with the chin above the bar (score of 1) and partial “pull-ups” with the chin below the bar (score of 0.5). Two days of training (3 trials/d) before the dietary intervention were used to avoid neophobia to the procedure. After dietary assignment, each rat received one 3-trial session on each of the testing days.

Cylinder test. Spontaneous activity and balancing ability were examined in the cylinder test (20). The rat was filmed for 5 min in a transparent clear plastic cylinder of 20 cm diameter and 41 cm height (Supplemental Figure 2).

Spontaneous forelimb activity was quantified as the sum of independent placements for the right and left forelimb and the number of simultaneous forelimb placements made on the inner wall of the cylinder during rearing activity (21). Hindlimb use was quantified as the number
of rearings made with or without supporting the body by leaning with forepaws on the wall. Balancing ability was quantified as the number of rearings without any forelimb support.

**Horizontal ladder walking task.** Skilled and coordinated movements of fore- and hindlimbs were evaluated in the horizontal ladder walking task (22) (Supplemental Figure 3A, B). During the first training phase (days 1–8), the rats were handled and acclimatized to the apparatus; 2) introduced to the food reward (banana-flavored sugar pellets; Bio-Serv no. F05986) in the home cages and in the behavioral apparatus to avoid neophobia and to encourage walking back and forth across the ladder, respectively; 3) food-restricted to motivate them to walk across the ladder, without excessive exploratory activity, in exchange for a sugar pellet reward; and 4) trained in pairs (cage mates; days 1–6) and individually (days 7–8) for 20–40 min. In the second training phase (days 9–14), the diet was provided ad libitum and the rats were trained individually for 10–20 min to promote walking on the ladder without long pauses or hesitations.

On each assessment day, 5 ladder crossings were video-recorded and analyzed. Extra crossings were included if there were multiple stops or hesitations.

**Walking ability.** Skilled limb use was evaluated with a foot-fault scoring system (22) for 1) fore- and hindlimb use (the way the rat placed its foot on the rungs), 2) fore- and hindlimb placement accuracy (number of errors), and 3) forepaw digit use (the way digits were placed on the rung). Forelimb and hindlimb use was rated by using a 7-movement category scale (23), as follows: 1) total miss (0), 2) deep slip (1), 3) slight slip (2), 4) replacement (3), 5) correction (4), 6) partial placement (5), and 7) correct placement (6). Total errors represented any kind of foot slip or total miss defined as a score of 0, 1, or 2. The incidence (%) of each category and total errors was expressed relative to total steps scored. Right and left limb scores were averaged and analyzed separately for fore- and hindlimbs.

**Gait and posture.** To describe possible compensatory body adjustments, lateral views of each rat were captured at a stance phase for one of the forelimbs (24). The estimated position of the hip (h) and the tip of the nose (n) (25) were marked on the acquired images, and the elevation of the hip from the horizontal ladder was measured (Supplemental Figure 3). Body length was measured as the distance between the orthogonal downward projection of the hip (h’) and the tip of the nose. A line drawing connecting n, h, and h’ (Supplemental Figure 3C) was constructed for 5 representative positions for each testing session. The angle between its foot on the rungs, and the cross-sectional area (cm$^2$) was measured from a digital image by using Image J software. Differences in muscle fiber composition (fast- vs. slow-twitching muscles) and functional role (locomotion vs. antigravity) (Supplemental Table 2) were used to interpret the contribution of each muscle to the functional abnormalities identified in the LP group.

**Statistical analysis**

Statistical analysis was performed with the use of SPSS 21. The effect of the LP diet on body weight, food intake, acute-phase reactants, and performance-based measures was investigated by using 2-factorial repeated-measures ANOVA (RANOVA), with time as the within-subjects variable and diet as the between-subjects factor. When the assumption of sphericity was violated, the degrees of freedom were corrected by using the Greenhouse-Geisser estimate of sphericity. Follow-up tests were applied if significant interactions between diet and time occurred. Bonferroni correction was applied to multiple comparisons.

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**Results**

**Expt. 1**

**Body weight.** Body weights in the LP group decreased progressively, whereas those in the NP group increased (P-interaction < 0.001) (Figure 1A). The difference in body weights between groups first reached significance on day 4.

**Food intake.** The LP rats consumed significantly less food than the NP rats (P-interaction < 0.05) (Figure 1B), and this first reached significance on day 8 (P < 0.05).

**Brain morphometric measurements.** There was no difference in brain wet weight between the NP (1.90 ± 0.53 g) and LP (1.90 ± 0.46 g) groups (P = 1.00). The brain area was also comparable between groups (NP: 2.92 ± 0.05 cm$^2$; LP: 2.85 ± 0.03 cm$^2$; P = 0.22).

**Biochemical data.**

**Serum albumin concentration.** The LP diet decreased serum albumin concentrations, and the effect was magnified as malnutrition progressed (P-interaction < 0.001, P-diet < 0.001, P-time = 0.65) (Figure 2A). Serum albumin concentrations in the LP group were 10%, 17%, and 26% lower than those in the NP group on days 3, 9, and 28, respectively (P < 0.05, P < 0.001, and P < 0.05, respectively).

**Serum A2M concentration.** The LP diet initiated a time-dependent elevation in serum A2M concentration (P-interaction < 0.01) (Figure 2B). The A2M concentration in LP rats compared with the NP rats was greater by 194% on day 9 (P < 0.01) and by 445% on day 28 (P < 0.05).

**Serum corticosterone concentration.** Corticosterone concentration is sensitive to any experimental manipulation that generates stress, including handling and blood sampling, and this can yield falsely high serum concentrations and increase group variability (27). Because the response to stress should be most comparable within each rat, the corticosterone concentrations measured on days 9 and 28 were expressed as a percentage...
of the concentrations on day 3, and the relative changes were compared (Figure 2C). There was no significant difference between the NP and LP groups (P-interaction > 0.05). However, a trend for corticosterone concentrations to be greater (40%) in the LP group was evident on day 28 (P = 0.06, 2-tailed t test).

**Total liver lipids.** Liver lipid concentrations were greater in the LP group (8.4% ± 1.4%) than in the NP group (4.0% ± 0.5%) at day 28 (P < 0.05).

**Correlation among indexes of nutritional status.** On day 3, lower body weight was associated only with a lower serum albumin concentration (r = 0.57, P < 0.05). On day 9, body weight continued to be positively correlated with serum albumin (r = 0.78, P < 0.05), and an inverse relation developed with serum A2M concentration (p = −0.60, P < 0.05). Serum albumin and A2M concentrations were inversely correlated (p = −0.65, P < 0.01).

On day 28, body weight continued to be correlated with serum albumin (r = 0.80, P < 0.001) and serum A2M (p = −0.84, P < 0.001) concentrations. Body weight was also negatively correlated with liver lipids (p = −0.62, P < 0.01). There were inverse relations between serum albumin and A2M (p = −0.66, P < 0.01) and serum albumin and liver lipid (p = −0.60, P < 0.05) concentrations. Serum A2M and liver lipid concentrations were positively correlated (p = 0.53, P < 0.05).

Because serum corticosterone, serum A2M, and body weight were also all interrelated on day 28, their correlations are shown in Supplemental Figure 4. Because serum corticosterone is expressed relative to day 3 concentrations, body weight and serum A2M were also calculated as a percentage of day 3 values. The elevations in A2M (p = −0.60, P < 0.05) and corticosterone (p = −0.53, P < 0.05) were significantly related to the decline in body weight (Supplemental Figure 4A). Serum corticosterone and A2M concentrations were positively correlated (p = 0.53, P = 0.05) (Supplemental Figure 4B).

**Sensorimotor functional tests.**

**Bar-holding task.**

**Latency.** PEM did not affect latency on day 2 (NP: 20.9 ± 3.5 s; LP: 26.7 ± 3.5 s), day 8 (NP: 15.2 ± 3.3 s; LP: 21.5 ± 1.1 s),
or day 27 (NP: 5.9 ± 2.2 s; LP: 13.7 ± 1.5 s). Although both groups spent comparable time on the bar (P-interaction = 0.36, P-diet = 0.85), latency decreased in successive testing sessions (P-time < 0.001).

**Pull-ups.** The LP rats made significantly more pull-ups to hang on the bar compared with NP rats (P-interaction < 0.05, P-diet < 0.001) (Figure 3). This effect developed by day 2 and was sustained until day 27 (P < 0.05). With successive test sessions, both groups hung more passively on the bar, as shown by the decreasing number of pull-ups with each subsequent test session (P-time < 0.01).

**Cylinder test.**

**Spontaneous activity.** Both groups performed a comparable number of forelimb contacts against the wall (P-interaction = 0.57, P-diet = 0.37), whereas the number of forelimb contacts significantly decreased on day 27 (P-time < 0.001) (day 0: NP, 105 ± 16; LP, 109 ± 10; day 27: NP, 25 ± 4; LP, 40 ± 6). The number of rearings was comparable between the NP and LP groups (day 0: NP, 36 ± 3; LP, 35 ± 2; day 27: NP, 25 ± 2; LP, 30 ± 4) (P-interaction = 0.38, P-diet = 0.51). The number of rearings significantly decreased in both groups by day 27 (P-time < 0.05).

**Balancing ability.** There were no differences in balancing ability between the NP and LP groups (P-interaction = 0.78, P-diet = 0.56). However, the rats from both groups reared without wall support significantly more on day 27 (NP: 12 ± 2; LP: 14 ± 3) compared with baseline rearing activity (NP: 9 ± 2; LP: 9 ± 1) (P-time = 0.05).

**Horizontal ladder walking task.**

**Walking ability.** There were no significant differences between the NP and LP groups on forelimb use, placement accuracy, or digit use (P-interaction > 0.05). However, a statistical trend for the LP rats to have fewer forelimb scores in the “correct placement” category was evident (P = 0.06), and this stemmed from a significant difference between the NP and LP rats on day 8 (P < 0.05) (Figure 4A).

For hindlimb use, although ANOVA did not yield a significant diet x time interaction (P-interaction > 0.05) or time effect (P-time > 0.05), there was a significant diet effect for correct placement (P-diet < 0.001) (Figure 4B) and partial placement (P-diet < 0.05) scores. The LP rats positioned their hindlimbs on the rung either with the wrist or heel instead of using the midportion of the paw. The difference between the NP and LP rats reached significance only by day 27 (P < 0.05). There was neither a diet x time interaction nor a time effect for hindlimb placement accuracy (P-interaction > 0.05, P-time > 0.05). However, the LP group made significantly more errors compared with the NP group (P-diet < 0.001).

**Gait and posture.** The LP rats bent more forward than the NP rats when they walked across the horizontal ladder (Supplemental Figure 3). The LP diet increased the angle quantifying the location of the center of gravity (Figure 4C), and this was dependent on time of exposure to the diet (P-interaction < 0.05, P-time < 0.01). A significant difference between groups was first observed on day 8 (P < 0.05), and this was sustained up to day 27 (P < 0.01).

**Association among indexes of nutritional status and performance-based measures.**

**Bar-holding task.**

**Latency.** A low serum albumin concentration predicted a longer latency on the bar-holding task only on days 2 ($R^2 = 0.36$, $P < 0.02$) and 8 ($R^2 = 0.45$, $P < 0.01$). The A2M concentration did not give a significant regression in relation to latency.

**Pull-ups.** A low serum albumin concentration also predicted increased pull-up scores on the bar throughout the Expt. (day 2: $R^2 = 0.27$, $P = 0.05$; day 8: $R^2 = 0.29$, $P < 0.05$; day 27: $R^2 = 0.35$, $P < 0.02$).

**Horizontal ladder walking task.**

**Walking ability.** A high serum A2M concentration predicted a lower occurrence of correct placement of forelimbs on day 8 ($R^2 = 0.64$, $P < 0.001$) and of hindlimbs on day 27 ($R^2 = 0.46$, $P < 0.01$). No relation was observed between A2M concentration and the incidence of hindlimb partial placement.

**Gait and posture.** The postural change (forward shift in the center of gravity) was predicted by a low serum albumin concentration on day 8 ($R^2 = 0.34$, $P < 0.05$) and day 27 ($R^2 = 0.39$, $P < 0.05$). The A2M concentration was also a good predictor of this abnormal gait on day 8 ($R^2 = 0.41$, $P < 0.05$). High liver lipid content was also prognostic on day 27 ($R^2 = 0.26$, $P < 0.05$).

**Expt. 2**

**Muscle size.** The gastrocnemius muscle cross-sectional area was significantly smaller in the LP rats compared with the NP rats (P < 0.01) (Figure 5) due to a decrease in gastrocnemius medialis (P < 0.05). No difference was found for soleus or plantaris muscles.

**Discussion**

Using well-established behavioral tasks (28), we explored motor abnormalities that developed on initiation and progression of PEM in the adult male rat. Bar-hanging, cylinder, and horizontal ladder walking tasks were used to evaluate handgrip strength (19, 29), balancing (21), and walking ability (22, 30) in parallel with brain and body morphometric measures and acute-phase reactants. Specific sensorimotor abnormalities developed at different stages of PEM, indicating their utility for detecting the onset and progression of PEM.

The evolving deterioration in nutritional status with the LP diet was assessed by the temporal profile of food intake, body weight, 2 serum acute-phase proteins (albumin and A2M), and liver lipid content. In addition, the relative serum corticosterone concentration was measured because it can be increased as part of the hormonally mediated adaptive response to malnutrition (27, 31). Protein deficiency developed within a few days, as shown by weight loss and a decline in serum albumin. As the malnutrition advanced in severity, serum A2M increased in...
parallel with further decreases in body weight and serum albumin. The liver steatosis further supports the presence of protein deficiency (32). The latter transformed to PEM as the rats voluntarily reduced food intake. Although serum corticosterone was not significantly elevated by the LP diet, concentrations were correlated with body weight and A2M. The model is clinically relevant for future studies aimed at elucidating the mechanistic effects of PEM on recovery after stroke, because the pattern of body weight, food intake, and serum albumin changes reflects that of such patients (33).

Serum acute-phase proteins such as albumin are being abandoned as clinical diagnostic tools for PEM because they have poor specificity and are altered by disease-induced inflammation (34). Nonetheless, proteins such as albumin reflect the combined effects of nutrition and inflammation (35, 36). This negative acute-phase protein is also influenced by dietary protein supply (37). The rapid decline in serum albumin supports its value for detecting acute malnutrition onset in rat models, whereas the small additional decrease with advancing PEM limits its sensitivity for monitoring malnutrition severity. This may be related to a reduced fractional catabolic rate that defends the albumin pool (38). At the advanced stage of PEM, A2M increased strongly and correlated with body weight, and thus it is a more sensitive indicator of progressing malnutrition than albumin.

The combination of decreased serum albumin and increased A2M provides evidence that PEM independently initiates an acute-phase response in the adult rat, as previously reported in younger rats (18). Given species-specific differences in the extent to which synthesis of selected proteins change in response to an acute-phase stimulus, A2M and albumin were chosen as sensitive indexes for the rat (39–41). Such alterations in these proteins (37, 39) may reflect a low-grade inflammation that is metabolically triggered by PEM (42, 43). Although cytokines were not measured, elevated serum IL-6 concentration was previously reported in PEM, and this could drive excessive A2M production (43, 44).

The onset of malnutrition was accompanied by specific motor deficits that evolved with time of exposure to the LP diet. The almost immediate response was forelimb muscle dysfunction detected by the bar-holding task. Forepaw grip strength (19, 29) was measured because diminished handgrip strength is a validated predictor of suboptimal nutritional status in clinical populations (45, 46). During bar hanging, the NP rats more often used a grip around the bar between digits and palm to initiate a “straight-arm hang.” This movement uses multiple sets of muscles, including flexor digitorum superficialis and flexor digitorum profundus in the paw (47, 48), wrist flexors, brachioradialis and extensor carpi radialis muscles in the forearms, and deltoid (49) and spino- and clavo-trapezius (50) muscles in the shoulders. Forelimb muscle dysfunction in LP rats was identified by increased use of an adaptive bar-hanging strategy in which the rats used more “flex-arm hangs” (pull-ups) intermittently with “straight-arm hangs.” Because paw-grip duration was unaffected by an LP diet, irrespective of strategy used to hang on the bar, we propose that the paw or wrist flexors were less affected (48). Muscle weakness may stem from the high

**FIGURE 4** A temporal characterization of skilled forelimb (A) and hindlimb (B) use and body posture (C) in the horizontal ladder walking task in adult male rats fed an LP or an NP diet for 28 d. Values are means ± SEMs, n = 8 for each group. **** Difference between NP and LP groups: *P < 0.05, ***P < 0.001. B, baseline; LP, low-protein; NP, normal-protein.

**FIGURE 5** Cross-sectional area of hindlimb muscles in adult male rats fed an LP or an NP diet for 26 d. Values are means ± SEMs, n = 4 (NP) and n = 7 (LP). **** Difference between NP and LP groups: *P < 0.05, ***P < 0.01. GC, gastrocnemius; GCl, gastrocnemius lateralis; GCm, gastrocnemius medialis; LP, low-protein; NP, normal-protein; Pl, plantaris; Sol, soleus.
fatigability of the forearm muscles, brachioradialis, and extensor carpi radialis, with their higher energy demand (51). To circumvent this problem of muscle fatigue, the LP rats may compensate by relying more on a “flex-arm hang” that engages the deltoid and spinop- and clavo-trapezius muscles in the shoulders and other flexors in the forearms, which have a lower energy demand (51, 52). Compensation is a well-described phenomenon of postural adjustments made in response to sensorimotor deficits, such as after stroke, in an attempt to restore performance (53, 54).

Selective atrophy of fast-twitching muscle fibers may be responsible for forelimb weakness. The forearm extensor muscles are composed of a greater number of fast-twitching muscles, whereas slow-twitching muscles contribute more to the deltoid and spinotrapezius muscles of the shoulders (51). Dietary protein deficiency has been reported to cause larger reductions in the diameter of fast-twitching fibers (55) and ultrastructural myofilbril damage associated with glutathione depletion (56). Although forearm muscle size was not investigated in the rats tested in the bar-holding task, the effect of the LP diet on hindlimb muscles was examined in Expt. 2. The LP diet reduced the cross-sectional area of gastrocnemius medialis, which has a higher number of fast-twitch fibers, whereas that of the plantaris and soleus muscles was unaltered. Although these rats had been exposed to surgical manipulation 28 d earlier, we propose that these muscle changes primarily reflect the effects of the dietary intervention. The catabolic effects associated with surgery (57) appear to have been limited, because the key features of PEM caused by the LP diet (decreases in body weight, food intake, and serum albumin concentration of 23%, 21%, and 21%, respectively (X Li, M Alaverdashvili, PG Paterson, unpublished data, 2015)) were comparable to those observed in Expt. 1 (30%, 24%, and 26%, respectively).

Although the abnormal bar-hanging pattern provides evidence for forelimb muscle weakness (29), we detected no change in the time that the grip could be sustained. This was unexpected, because a bar-holding task or measurement of grip force with a transducer (such as is measured with a dynamometer in patients) can equally reveal abnormalities in rodent paw grip strength (29). Although measurement protocols are inadequately standardized for human (45) or rodent (19) studies, grip strength is influenced by posture and joint position (46). The recommended position for patient measurements generates minimum grip strength (46). Our measurements, with the rat shoulders flexed at 180° with elbows fully extended, would yield maximum grip strength (58), which may have caused a ceiling effect. Also, measurements made in clinical settings are for a crushed grip produced by the proximal phalanges flexed by the action of digitorum superficialis, whereas the bar-holding task measures a support grip produced both by the distal phalanges flexed by digitorum profundus and proximal phalanges flexed by digitorum superficialis (48, 51, 59). Because there is better leverage for the latter, deeper muscle, the stronger support grip may have reduced the sensitivity of latency for detecting an effect of the LP diet.

During skilled locomotion, the LP diet caused a deficit in hindlimb function and postural changes, whereas forelimb function was less affected. LP rats appeared to bear more body weight on their forelimbs instead of supporting the load approximately equally on fore- and hindlimbs, as reported for well-nourished rats (24). This resulted in a forward shift of the center of gravity and abnormal posture to compensate for the affected hindlimb functions. A similar shift was reported with rat hindlimb malfunction after spinal cord injury (24). Kinematic measurements would be needed to determine if changes in ground reaction forces are responsible for this abnormal posture. The postural changes induced by the LP diet were not accompanied by a deficit in balancing ability in the cylinder task or reduced size of the soleus (antigravity) muscle. However, our measurements do not rule out effects on balance, because we assessed only voluntary (comfortable) rearing. Whether PEM affects the ability to balance in tasks requiring involuntary (forced) standing, such as a one-legged stance task in humans (60), needs further investigation.

Whereas forelimb muscle dysfunction on bar hanging developed acutely in rats confronted with an LP diet, the walking and postural abnormalities developed at later stages. The smaller muscle mass of the forelimb, with its lower grip strength relative to that of the hindlimb (19), may account for the earlier effects on bar hanging. Alternatively, the higher physical load and metabolic activity required for forced hanging may have contributed to the different temporal profile. These findings highlight the importance of selecting the most sensitive functional tests for detecting deficits at different stages of PEM. Whether comprehensive functional evaluation with the use of a battery of tests can differentiate PEM-induced motor deficits from those caused by common comorbidities, such as stroke (3), needs further investigation.

Sensorimotor abnormalities can be generated by alterations in brain and/or muscle. Whereas the contribution of PEM-induced muscle wasting to reduced muscle strength is widely documented (45), it is not clear if adulthood PEM initiates brain changes that induce and/or influence the sensorimotor deficits associated with muscle changes. Region-specific structural changes in neurons in the brain have been reported with adulthood PEM or protein deficiency (61, 62). Although the LP diet changed neither brain weight nor size in this adult model of PEM, a comparable dietary protocol augmented stroke-induced abnormalities in forelimb function in a skilled locomotion task (M Alaverdashvili, X Li, PG Paterson, unpublished data, 2014). Thus, altered neuronal and glial function may also contribute to the sensorimotor abnormalities. The contribution of brain mechanisms, such as plasticity and low-grade inflammation, to PEM-induced functional abnormalities will be investigated in future studies.

In summary, adulthood PEM caused by feeding an LP diet induces abnormalities in forelimb and hindlimb functions. The various sensorimotor abnormalities develop at different stages of the malnutrition. This adulthood model of PEM should be useful in combination with disease models of sensorimotor deficits for examining the interactions between nutritional status and other rehabilitation or pharmaceutical treatments in such disorders. Our findings further suggest that increased use of performance-based measures could enhance the diagnosis of onset and severity of PEM.

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