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Geometric framework reveals that a moderate protein, high carbohydrate intake is optimal for severe burn injury in mice

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Abstract

Nutritional therapy is a cornerstone of burns management. The optimal macronutrient intake for wound healing after burn injury has not been identified, although high-energy, high-protein diets are favoured. The present study aimed to identify the optimal macronutrient intake for burn wound healing. The geometric framework (GF) was used to analyse wound healing after a 10 % total body surface area contact burn in mice *ad libitum* fed one of the eleven high-energy diets, varying in macronutrient composition with protein (P5–60 %), carbohydrate (C20–75 %) and fat (F20–75 %). In the GF study, the optimal ratio for wound healing was identified as a moderate-protein, high-carbohydrate diet with a protein:carbohydrate:fat (P:C:F) ratio of 1:4:2. High carbohydrate intake was associated with lower mortality, improved body weight and a beneficial pattern of body fat reserves. Protein intake was essential to prevent weight loss and mortality, but a protein intake target of about 7 kJ/d (about 15 % of energy intake) was identified, above which no further benefit was gained. High protein intake was associated with delayed wound healing and increased liver and spleen weight. As the GF study demonstrated that an initial very high protein intake prevented mortality, a very high-protein, moderate-carbohydrate diet (P40:C42:F18) was specifically designed. The dynamic diet study was also designed to combine and validate the benefits of an initial very high protein intake for mortality, and subsequent moderate protein, high carbohydrate intake for optimal wound healing. The dynamic feeding experiment showed switching from an initial very high-protein diet to the optimal moderate-protein, high-carbohydrate diet accelerated wound healing whilst preventing mortality and liver enlargement.

Key words: **Burn injury: Geometric framework: Macronutrients: Mouse models**

Severe burns are the most traumatic and physically debilitating injuries, affecting multiple organ systems and causing significant mortality and morbidity[1,2]. The vast majority of burns occur in low- and middle-income countries, in resource-limited conditions[3]. Nutritional support is a highly accessible, simple and effective intervention, capable of accelerating wound healing, reducing infection, enhancing recovery and attenuating the hypermetabolic response[2,4,5].

The effectiveness of different combinations of macronutrients, energy providing dietary components, that is, protein, carbohydrate and fat, to support recovery after burn injury has been investigated in animal[6–8] and human studies[9–14]. To date, the available evidence is inconclusive in supporting a specific macronutrient regimen, although early, aggressive, high-protein, high-energy diets are favoured[5,15]. Reflecting this uncertainty, the macronutrient composition of enteral diets varies considerably

Abbreviations: BAT, brown adipose tissue; C, carbohydrate; E, extremely; EDL, extensor digitorum longus muscle; F, fat; GF, geometric framework; H, high; iWAT, inguinal white adipose tissue; L, low; M, moderate; P, protein; V, very.

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between burn units across the globe. Consistently, 15–25 % of energy content is provided as protein, but the amount of carbohydrate fluctuates from 40 to 85 % and the amount of fat from 3 to 40 % of total energy[13,16].

Many questions remain to be answered regarding the optimal macronutrient regimen for supporting recovery from severe burns. Should the majority of energy content be provided as carbohydrate or fat? Are there benefits in using low-protein diets? What is the most effective combination of macronutrients? Addressing these questions are important as innovation in nutritional therapy has the capacity to improve multiple aspects of recovery from burn injury[17]. Findings from burns research can also be generalised to complex trauma and critical illness, as burns represent an ideal trauma model[17].

The present study aims to identify the optimal macronutrient intake for wound healing after severe burn injury in a mouse model. It is the first to comprehensively investigate the optimal combination of macronutrients to support recovery from burn injury. Previous investigations have been limited by a one-variable-at-a-time approach to nutrition that fails to recognise the multidimensional nature of nutritional science[18]. Here, this is overcome using the geometric framework (GF), a modelling method which is capable of analysing not only the individual but also the interactive effects of macronutrients[19].

Materials and methods

Geometric framework study experimental model and subject details

Twelve-week-old male BALB/c mice, weighing 25·2 (SEM 1·4) g (Animal Resources Centre; *n* 120; *n* 6 mice/diet, additional mice added to achieve group size of *n* 5–6 if deaths occurred), were housed in the Translation Research Facility, ANZAC Research Institute, a specific pathogen-free facility. The environment was controlled at 24–26°C and 44–46 % humidity under a 12 h light–12 h dark cycle. All protocols were approved by the Sydney Local Health District (SLHD) Animal Welfare Committee (Protocol no. 2013/059) under Australian National Health and Medical Research Council Guidelines for animal experimentation. Each mouse was anaesthetised with 3 % isoflurane, and the dorsal area was shaved. A full thickness contact burn injury (2 × 2 cm, about 10 % total body surface area) was created. On day 2 post-burn, wounds were debrided, which involved excision of necrotic eschar until evidence of bleeding from the wound edge was achieved, with wounds subsequently dressed for 10 d. Day of debridement was defined as day 0. Analgesia (intraperitoneal carprofen 5 mg/kg) was provided daily for 4 d after burn injury.

For the GF study, post-burn injury, animals were randomly allocated and housed individually in standard approved cages and fed *ad libitum* with one of the eleven high-energy diets (Specialty Feeds), varying in macronutrient contribution to net metabolisable energy, but identical in micronutrient and total energy content about 17 kJ/g[20]. The diets varied in protein (5–60 %; casein and methionine), carbohydrate (20–75 %; sucrose, wheat starch and dextrinised maize starch) and fat (20–75 %; soya bean oil) (Table 1). All other ingredients were kept similar. Other ingredients include cellulose, a mineral mix Ca, P, Mg, Na, C, K, S, Fe, Cu, iodine, Mn, Co, Zn, Mo, Se, Cd, Cr, Li, B, Ni and V) and a vitamin mix (vitamins A, D_3, E, K, C, B_1, B_2, niacin, B_6, pantothenic acid, biotin, folic acid, inositol, B_{12} and choline) supplemented to the same levels as AIN-93G. Diets were named according to the generally accepted classifications of macronutrient content and abbreviated as follows: extremely (E), very (V), high (H), moderate (M), low (L), protein (P), carbohydrate (C) and fat (F)[21]. Diets were designed to systematically sample P:C:F diet space with optimal power for fitting surface response models with the GF. A high-protein, high-carbohydrate (HPHC) diet was used as a control as the content was similar to standard chow. From the eleven experimental diets, four were selected for further individual analysis based on wound healing rate, low-protein, high-fat (LPHF) for the poorest, moderate-protein, high-carbohydrate (MPHC) for the best, extremely high-protein, equal carbohydrate and fat (EHPCF) for moderate, and high-protein, high-carbohydrate (HPHC; control).

Table 1. Macronutrient composition of diets*

		Protein		Carbohydrate		
Diet	%P/C/F	Casein	Methionine	Sucrose	Wheat starch	Dextrinised starch
EHPCF	60/20/20	1	0·015	1	0·5	1·5
VHPCF	42/29/29	1	0·015	1	2	1·5
VHPHC	33/47/20	1	0·015	1	5	1·5
VHPHF	33/20/47	1	0·015	1	0·5	1·5
HPHC	26/57/17	1	0·015	1	9·5	1·5
HPCF	23/38/38	1	0·015	1	3·5	1·5
MPHC	14/57/29	1	0·015	1	7	1·5
MPHF	14/29/57	1	0·015	1	2	1·5
LPHC	5/75/20	1	0·015	1	10	1·5
LPCF	5/48/48	1	0·015	1	5	1·5
LPHF	5/20/75	1	0·015	1	0·5	1·5
VHPMC	40/42/18	1	0·009	1	2	1·5

E, extremely; V, very; M, moderate; L, low.
* Experimental high-energy diets (about 17 kJ/g) showing the % total energy of protein (P), carbohydrate (C) and fat (F), and the proportion of protein (casein and methionine) and carbohydrate (sucrose, wheat starch and dextrinised starch) compositions.

Mice were monitored daily for 7 d after burn injury and were culled if they lost >20 % body weight, developed large leg wounds (a leg wound impairing ability to bear weight or which progressed in size over the 7 d without evidence of healing) or scored high on the distress scale. In accordance with SLHD Animal Ethic Committee Guidelines, the definition of 'distress scale' is: 0 = normal; 1 = lack of grooming; 2* = rough coat, nasal/ocular discharge; 3* = very rough coat, abnormal posture, enlarged pupils. Animals were considered to be in distress when they scored either two or three and euthanasia was required. Thereafter, body weight, wound size and food intake were recorded weekly over 35 d. Wound size was measured using the VISITRAK Digital System (Smith & Nephew) and calculated as a % difference compared with the wound size on day of debridement. On day 35 when wounds had completely healed, mice were anaesthetised using a combination of ketamine/xylazine (100 mg/100 mg/kg) and euthanised by cervical dislocation. The extensor digitorum longus muscle (EDL), inguinal white adipose tissue (iWAT), interscapular brown adipose tissue (BAT), liver and spleen were collected and weighed.

Dynamic feeding subjects and methods

The results from the GF study were subsequently used to investigate if outcomes could be further enhanced in a dynamic feeding study. As the GF study showed a mortality benefit with the consumption of a very high-protein diet in the first week after injury, a very high-protein, moderate-carbohydrate (VHPMC; P40:C42:F18) diet was specifically designed. Twelve-week-old male BALB/c mice (*n* 36; *n* 12 mice/diet) were housed in the same conditions and underwent an identical burn injury as per the GF study. After burn injury, mice were randomly allocated to one of the three diets, VHPMC (P40:C42:F18), MPHC (P14:C57:F29) and a dynamic diet. The dynamic diet group was designed to combine and validate the benefits of an initial very high protein intake for mortality and subsequent moderate protein, high carbohydrate intake for optimal wound healing. Mice in the dynamic diet group were fed a VHPMC diet until day 7 and were then fed a MPHC diet. Body composition was assessed using an in-house dual-energy X-ray absorptiometry; wound healing rate and body weight were recorded weekly over 28 d. On day 28, mice were culled and organs (EDL, iWAT, BAT, liver and spleen) were collected and weighed.

Statistical analysis

Data were analysed with the GF, a three-dimensional nutrient space which generates heat maps known as response surfaces[22]. Response surfaces were generated for the variables, wound healed (%), body weight loss (%), EDL, iWAT, BAT, liver and spleen weight (mg/g) using non-linear thin-plate spline procedures in R (version 3.4.3)[23]. The main and interactive effects of protein, fat and carbohydrate intake were tested statistically using General Additive Modelling[23] (online Supplementary Tables S2 and S3). Response surfaces are presented as two-dimensional heat maps, which are slices from the full-fitted three-nutrient surface cut through the median intake for the third nutrient axis[19]. Red areas indicate highest values for the variable which fall as the colour shifts to dark blue. Isolines (black lines) in response surfaces indicate areas of equality for the variable. In some response surfaces, nutritional rails (radials indicating the ratio of macronutrients in the diet) have been superimposed to aid interpretation. When an animal is restricted to a single experimental diet, it can move along this radial by increasing or decreasing food intake but is constrained to consume nutrients in the ratio at which they occur in the diet.

Statistical analysis for body weight loss (%) in surviving and dead mice and organ weights (mg/g) was completed using IBM SPSS V25 with one-way ANOVA and Tukey's *post hoc* test. The differences between select groups and periods for continuous variables such as wound healing (%), body weight loss (%) and food intake (g/week) were analysed using linear regression within the framework of generalised estimating equations. Generalised estimating equation corrects for the bias in the estimates caused by correlation due to having multiple observations from each mouse. Data are presented as mean values with their standard errors.

Justification of sample size: *n* 6/group per time point gave us 98 % power for two-sided α ($P = 0{\cdot}05$) to detect 20 % or greater differences in wound healing rate.

Results

Burn injury wound healing

Response surfaces were generated for wound healed (%) on days 7–28 (Fig. 1(a–d), online Supplementary Table S1). Wound healing was accelerated with increasing carbohydrate intake for days 7, 14 and 21. Mice that consumed carbohydrate:fat in a 2:1 ratio had the fastest wound healing, with about 15 % wound closure on day 7 (inflammatory phase), about 54 % on day 14 (proliferative phase) and about 76 % on day 21 (late proliferative phase). In contrast, the poorest healing occurred when mice consumed the opposite intake with a carbohydrate:fat ratio of 1:2, with wound healing of about 0 % on day 7, about 48 % on day 14 and about 62 % on day 21 (Fig. 1(a–c)). By day 28 (remodelling phase), wound healing was similar in all groups but response surfaces indicated high carbohydrate intake was favourable (Fig. 1(d)). Wound healing was also accelerated by the consumption of protein:carbohydrate in a 1:4 ratio, or protein:fat ratio of 1:2 (Fig. 1(c)). Importantly, response surfaces that showed a high protein intake of >30 kJ/d caused delayed wound healing, with only about 46 % wound closure on day 14 and about 64 % on day 21 (Fig. 1(b) and (c)). Combining this information, an intake consisting of P:C:F in a 1:4:2 ratio was associated with the fastest healing rate (Fig. 1(c)).

Of the eleven experimental diets, the diet most closely resembling this 1:4:2 ratio was the moderate-protein, high-carbohydrate diet (MPHC; P14:C57:F29) diet. However, an interesting finding was the observed delay in wound healing in the early inflammatory stage (days 0–3) in MPHC mice, with only about 3 % wound closure, compared with about 20 % in the HPHC control, although this was not statistically significant (Fig. 1(e)). Healing was then found to progress rapidly in MPHC mice with about 75 % of the wound healed by day 14, compared with only about 58 % in LPHF, 65 % in EHPCF. This finding suggests that a high protein intake is essential in the early inflammatory stage of wound healing, but a moderate protein intake is more favourable as wound healing progresses.

Body weight changes

Body weight loss (%) was measured as an indicator of the hypermetabolic response to injury[24]. Protein intake was found to strongly influence body weight loss (%) on all days (Fig. 2(a–d), online Supplementary Table S2). Weight loss was minimised to about 13–14 % for mice with a protein intake over a threshold of about 7 kJ/d (about 15 % total energy intake; red dotted line) up to about 30 kJ/d (Fig. 2(a–d)). This intake was achieved in MPHC, HPHC and EHPCF diets resulting in minimal weight loss in mice fed these diets, when compared with mice on a LPHF diet, that failed to reach the protein threshold, and had rapid, progressive and sustained weight loss of about 30 % over 28 d (Fig. 2(e)).

From day 14, interactive effects between protein, carbohydrate and fat were important (online Supplementary Table S2). On day 14, when wounds were about 50 % healed, the benefit of consuming more carbohydrate than fat was appreciable (Fig. 2(b)). Increasing fat intake from 25 to 50 kJ/d doubled weight loss from about 12 % to about 24 % (Fig. 2(b)). In contrast, increasing

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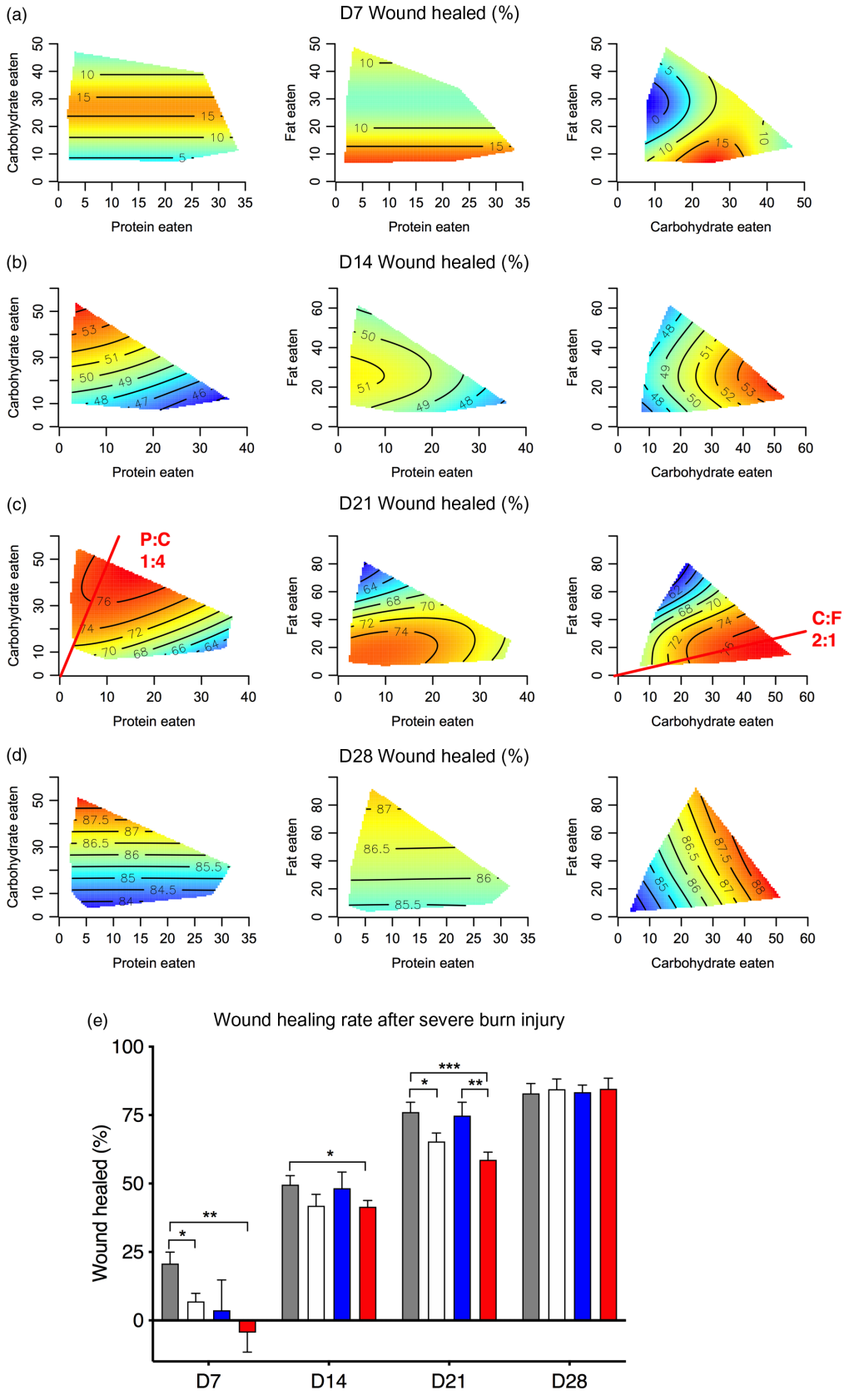

Fig. 1. Wound healed (%) *v.* macronutrient intake: (a–d) response surfaces showing the relationship between wound healed (%) (black numbered lines/isolines) and macronutrient (protein, carbohydrate or fat) intake on *x* and *y* axis (kJ/d) on days 7, 14, 21 and 28 (*n* 66). Solid red lines are nutritional rails with a fixed ratio of macronutrients which maximises wound healing. For each two-dimensional slice, the third macronutrient not included on the *y* and *x* axis is at its median value. (e) Bar graph showing wound healing rate in select diets (*n* 6/group). $^{*}P \leq 0{\cdot}05$, $^{**}P \leq 0{\cdot}005$, $^{***}P \leq 0{\cdot}0005$. Data are mean values with their standard errors (see also online Supplementary Table S1). (e) ▬, HPHC; □, EHPCF; ▬, MPHC; ▬, LPHF. H, high; P, protein; C, carbohydrate; E, extremely; F, fat; M, moderate; L, low.

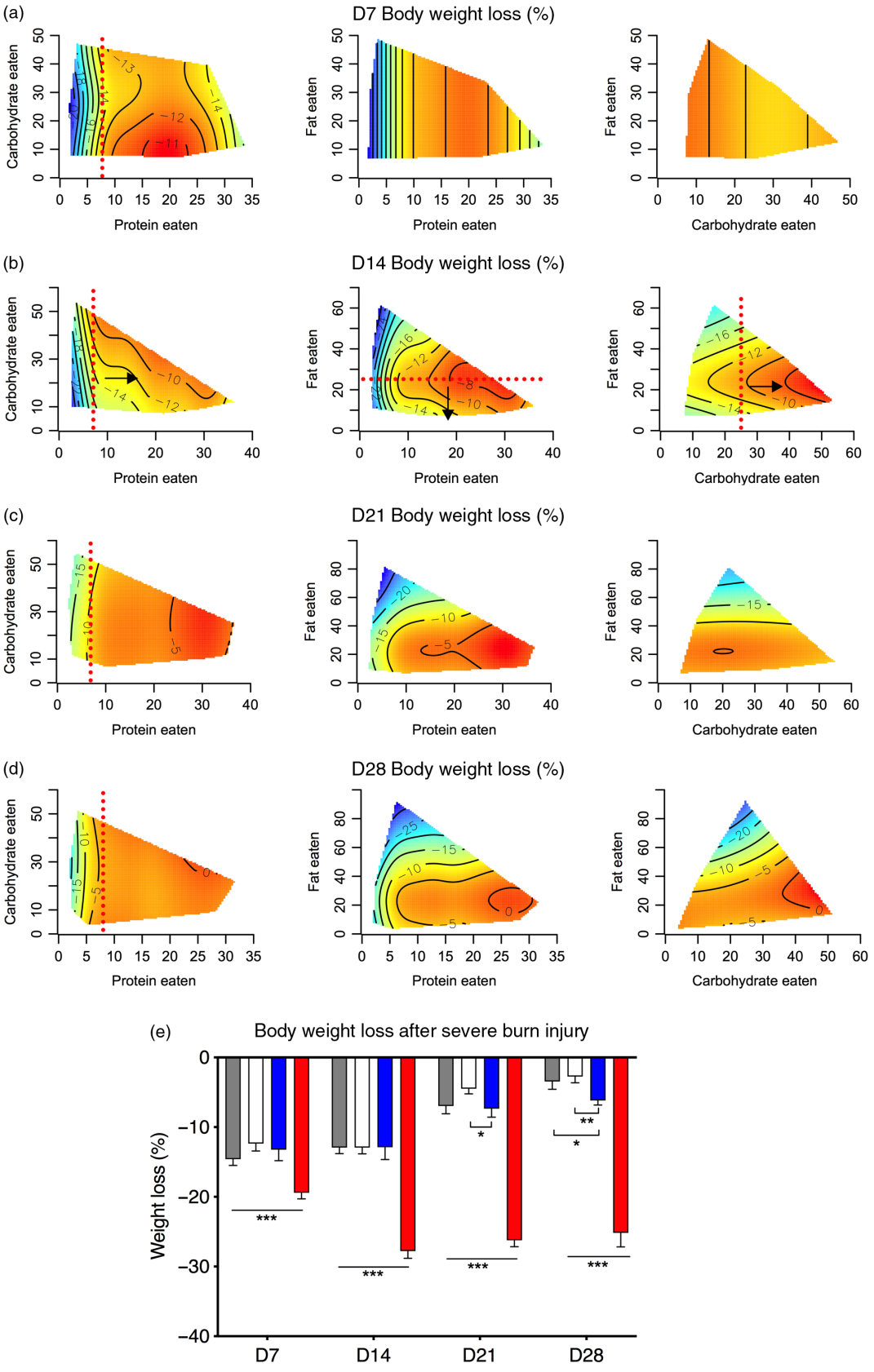

Fig. 2. Body weight loss (%) *v.* macronutrient intake: (a–d) response surfaces showing the relationship between body weight loss (%) and macronutrient intake (kJ/d) on days 7, 14, 21 and 28 (*n* 66). Red lines indicate a nutritional rail with a fixed ratio of macronutrients which maximises the response. Dotted red lines indicate nutritional targets with arrows delineating the desired direction of intake. For each two-dimensional slice, the third macronutrient not included on the *y* and *x* axis is at its median value. (c) Bar graph showing weight loss (%) in select diets (*n* 6/group). $^{*}P \leq 0{\cdot}05$, $^{**}P \leq 0{\cdot}005$. Data are mean values with their standard errors (see also online Supplementary Table S2). ■, HPHC; □, EHPCF; ■, MPHC; ■, LPHF. H, high; P, protein; C, carbohydrate; E, extremely; F, fat; M, moderate; L, low.

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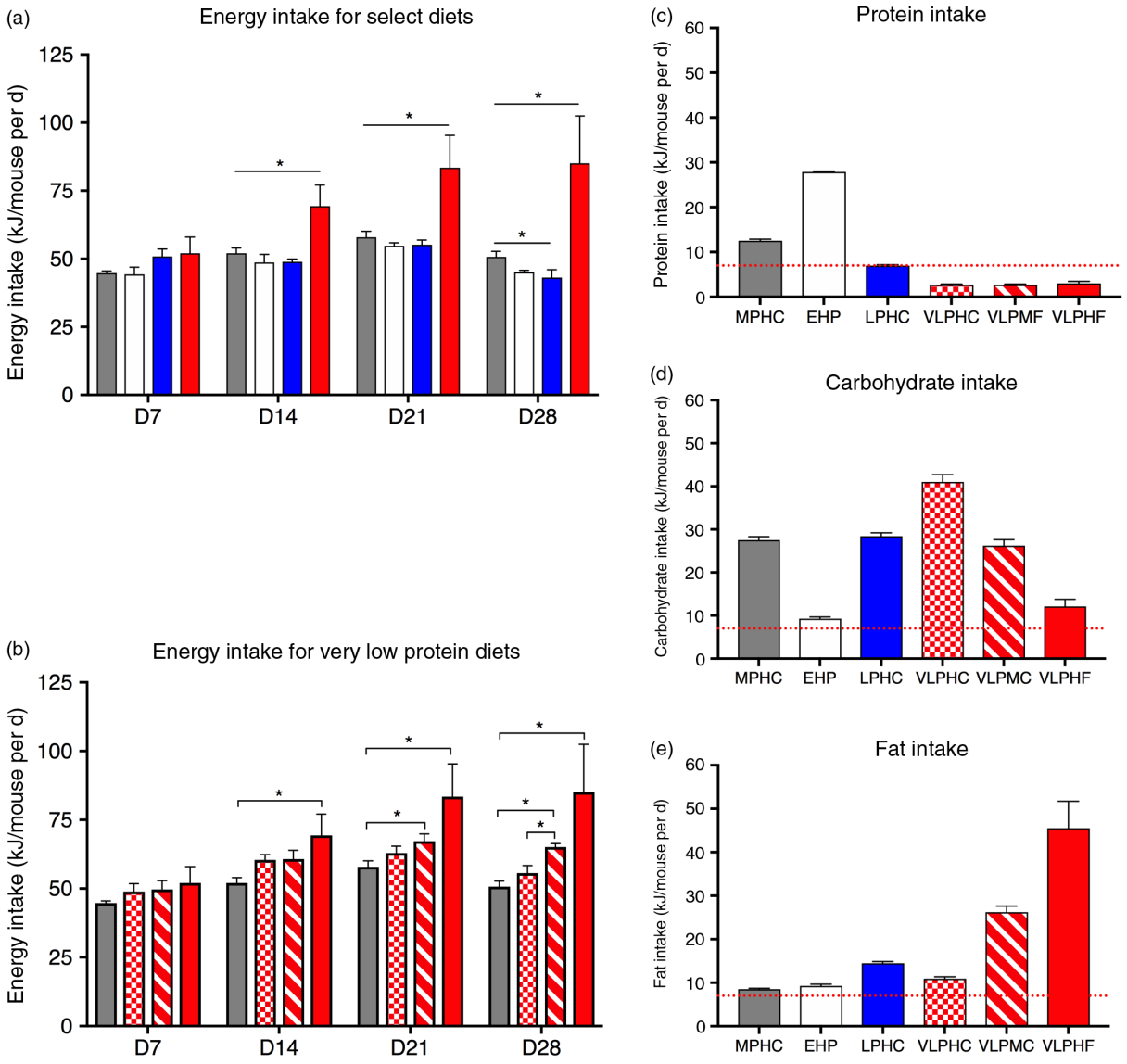

Fig. 3. Macronutrient intake energy, protein, carbohydrate and fat: (a) diets varying in protein intake showing energy intake in the general range of 45–55 kJ/d except in LPHF mice. (b) Energy intake in low-protein diets showing increased intake with higher fat content. (c–e) Intake by individual macronutrients in select and low-protein diets, dotted red line represents intake targets. Optimal intake above red dotted line for (c) and (d) and below red line for (e). $^*P \leq 0{\cdot}05$. Data are mean values with their standard errors n 6/group except LPHF with n 3. (a) ▭, MPHC; ▭, EHP; ▭, LPHC; ▭, VLPHF. (b–e) ▭, MPHC; ▭, VLPHC; ▭, VLPMC; ▭, VLPHF. H, high; P, protein; C, carbohydrate; E, extremely; F, fat; M, moderate; L, low; V, very.

carbohydrate intake from 25 to 50 kJ/d decreased weight loss from about 12 % to 8 % (Fig. 2(b)). There was a strong interaction between protein, carbohydrate and fat, indicating that total energy intake is important, although the benefits from consuming protein >7 kJ/d was still apparent (Fig. 2(c) and (d)).

Food intake analysis

Energy intake was similar in all diets in the range of 45–55 kJ/d per mouse except in the LP diets (5 % P), which were included to show the additional effect of carbohydrate intake in these groups (Fig. 3(a) and (b)). In general, LP mice consumed more energy content in the range of 60–80 kJ/d, with LPHC mice consuming slightly less than LPCF and LPHF mice (Fig. 3(b)). Increasing intake in order to reach protein and carbohydrate targets is a well-observed compensatory phenomenon in mice and many other species including humans[25]. Despite this increase in food intake, LP mice were not able to reach the 7 kJ/d protein threshold (Fig. 3(c)). EHPCF and LPHF diets failed to reach the 25 kJ/d carbohydrate target (Fig. 3(d)), with LPHF additionally consuming over the optimal 25 kJ/d fat intake target (Fig. 3(e)). In LPHF mice, the combination of low protein, low carbohydrate and high fat intake is likely the cause of delayed wound healing, weight loss and high mortality.

Mortality

Nineteen mice died throughout the study, mostly during the first week after burn injury from rapid weight loss and the development of spontaneous leg wounds necessitating culling.

(a) The effect of diet on survival

Diet	P:C:F	Mortality (%)
EHP	60/20/20	0
VHP	42/29/29	0
HPHC	33/48/20	20
HPHF	33/20/48	17
MPHC	26/57/17	33
MPFC	23/38/38	14
LPHC	14/57/29	29
LPHF	14/29/57	43
VLPHC	5/75/20	10
VLPHFC	5/48/48	11
VLPHF	5/20/75	57

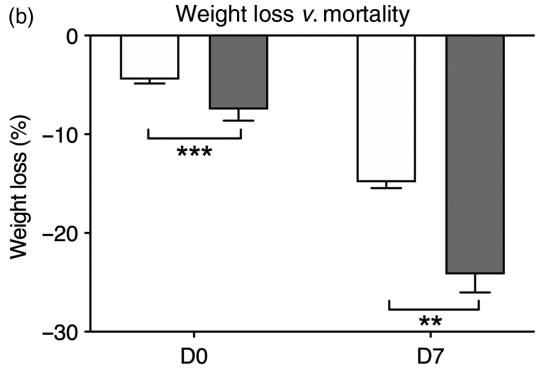

Fig. 4. Mortality in individual experimental diets: (a) no deaths in EHPCF and VHP diets with deaths increasing with lower protein intake. In MP and LP diets % mortality increased with higher fat content. Extremely (E), very (V), high (H), moderate (M), low (L), protein (P), carbohydrate (C) and fat (F). (b) Weight loss in mice surviving *v.* mice that died by day 0 (2 d post burn, day of debridement) and day 7 post-debridement. Total deaths, *n* 19. $^{**}P \leq 0{\cdot}005$, $^{***}P \leq 0{\cdot}0005$. Data are mean values with their standard errors. (b) □, Survived; ■, died.

No deaths occurred in the highest protein intake groups (EHPCF and VHPCF) (Fig. 4(a)). The highest mortality of 57 % was observed when a very low protein intake was combined with a high fat and low carbohydrate (VLPHF) intake. Interestingly, morality was only 10 and 11 % in equivalent 5 % protein diet groups (VLPHC and VLPHFC, respectively). This survival difference is likely due to the increased carbohydrate and lower fat content of these diets. Our results also demonstrate that mortality was correlated with weight loss after burn injury (Fig. 4(b)). Mice that died were found to lose significantly greater weight on day 0, with a −4 % weight loss in surviving mice *v.* −7 % in mice that died, and −14 *v.* −24 %, respectively, on day 7.

Muscle, fat, liver and spleen mass

Response surfaces demonstrated a trend to lower EDL mass of about 0·0215 mg/g in mice with a high fat intake of 60 kJ/d, compared with about 0·0230 mg/g with a lower fat intake of 20 kJ/d, although no significant effect was detected with General Additive Modelling analysis (Fig. 5(a), online Supplementary Table S3). A carbohydrate:fat intake of 2:1 or a protein:carbohydrate ratio of 1:4 was associated with decreased iWAT weight at about 7 mg/g (Fig. 5(b)). Interscapular BAT weight was preserved at about 4·2 mg/g in mice also consuming a diet with a carbohydrate:fat ratio of 2:1 or protein:carbohydrate ratio of 1:4 (Fig. 5(c)). Liver weight increased from about 50 to 62 mg/g, and spleen weight increased from about 3·4 to 5·0 mg/g with increasing protein intakes from 5 to 30 kJ/d (Fig. 5(d) and (e)).

Dynamic feeding

There was no difference in wound healing between VHPMC, MPHC and dynamic groups for days 7 and 14 (Fig. 6(a)). Mice in both the dynamic and VHPMC groups were fed the same VHPMC diet (P40:C42:F18) for the first week, with no differences in wound healing rate on day 7. From day 7 however, mice in the dynamic group were subsequently fed a MPHC diet, and by day 21 accelerated wound healing was evident with 77 % wound closure, compared with 70 % in VHPMC and MPHC mice (Fig. 6(a)). This effect continued to day 28 with dynamically fed mice having 93 % wound closure compared with 87 % in VHPMC and 86 % in MPHC (Fig. 6(a)). This accelerated healing rate is photographically represented (Fig. 6(b)) with evidence of faster wound closure on day 21 and 28 in dynamically fed mice. No deaths occurred in the dynamic and VHPMC groups, but 3/12 deaths occurred in the first 2 weeks in the MPHC group.

All groups lost body weight post-burn; however, weight loss was significantly less at 8–10 % and was regained significantly faster in dynamic and VHPMC mice compared with MPHC, who lost about 13 % body weight (Fig. 6(c)). On day 28, VHPMC mice had gained 3 % body weight which was significantly more than dynamic mice who had returned to pre-burn weight at 0 %, and an ongoing 3 % weight loss in MPHC mice (Fig. 6(c)). Analysis of body mass by dual-energy X-ray absorptiometry imaging showed that mice fed the VHPMC diet, although losing a similar amount of 10–14 % lean mass on day 7 as other groups, regained lean mass quickly during the course of healing (Fig. 6(d)). Lean mass improved over the course of wound healing in dynamic and MPHC mice returning close to baseline by day 28 (Fig. 6(d)). Initial fat mass loss was profound in all groups at about 30–40 % (Fig. 6(e)). Dynamic and VHPMC mice recovered fat mass quickly, whilst MPHC mice did not regain fat mass with an ongoing 30 % loss on day 28 (Fig. 6(e)).

Analysis of organ weight showed EDL mass was similar between all groups (Fig. 6(f)). BAT mass was similar between all groups; however, iWAT was significantly increased in dynamically fed mice at 12·5 mg/g compared with 10·3 mg/g in VHPMC mice and 9·1 mg/g in MPHC mice (Fig. 6(g) and (h)). Dynamic fed mice had a significantly lower liver mass at 53 mg/g compared with 62 mg/g in VHPMC mice; however, when compared with liver mass of 57 mg/g recorded in MPHC mice, this was not significantly different (Fig. 6(i)). There was no difference in spleen weight, although this tended to be lighter in dynamic mice at 8·9 mg/g compared with 9·5 and 10·2 mg/g in VHPMC and MPHC mice, respectively (Fig. 6(j)).

Discussion

In the present study, the GF was used to identify the optimal macronutrient intake to support recovery after severe burn injury. The GF study showed firstly that, providing the majority of energy content as carbohydrate rather than fat accelerated wound healing, minimised body weight loss and improved

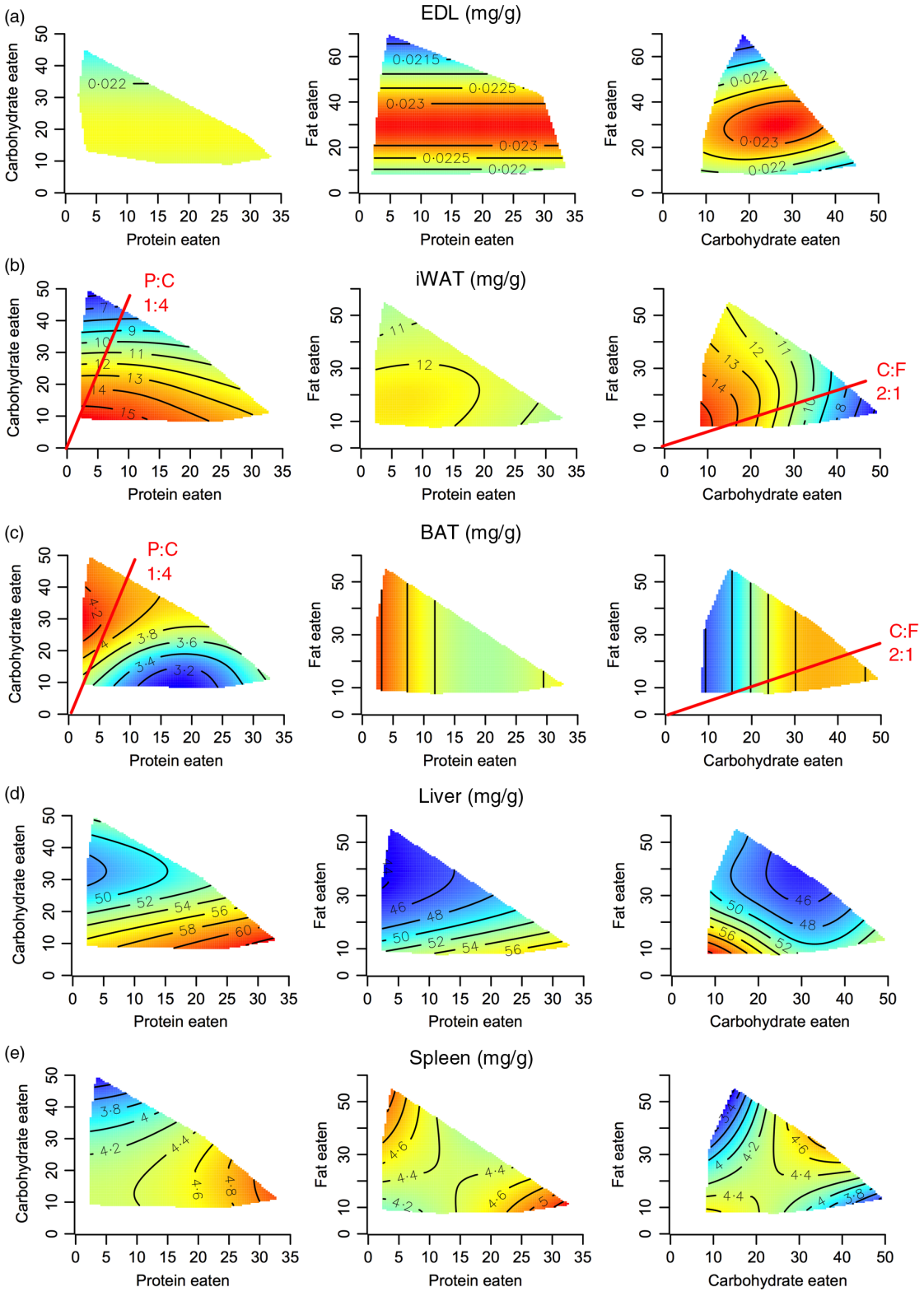

Fig. 5. Analysis of organ weight: (a–e) response surfaces showing the relationship between macronutrient intake and extensor digitorum longus muscles (EDL, mg/g), inguinal white adipose tissue (iWAT, mg/g), brown adipose tissue (BAT, mg/g), liver (mg/g) and spleen (mg/g) on day 35 after burn (*n* 66). Solid red lines indicate a nutritional rail with a fixed ratio of macronutrients which maximises the response. For each two-dimensional slice, the third macronutrient not included on the *y* and *x* axis is at its median value (see also online Supplementary Table S3). M, moderate; P, protein; H, high; C, carbohydrate; V, very; F, fat.

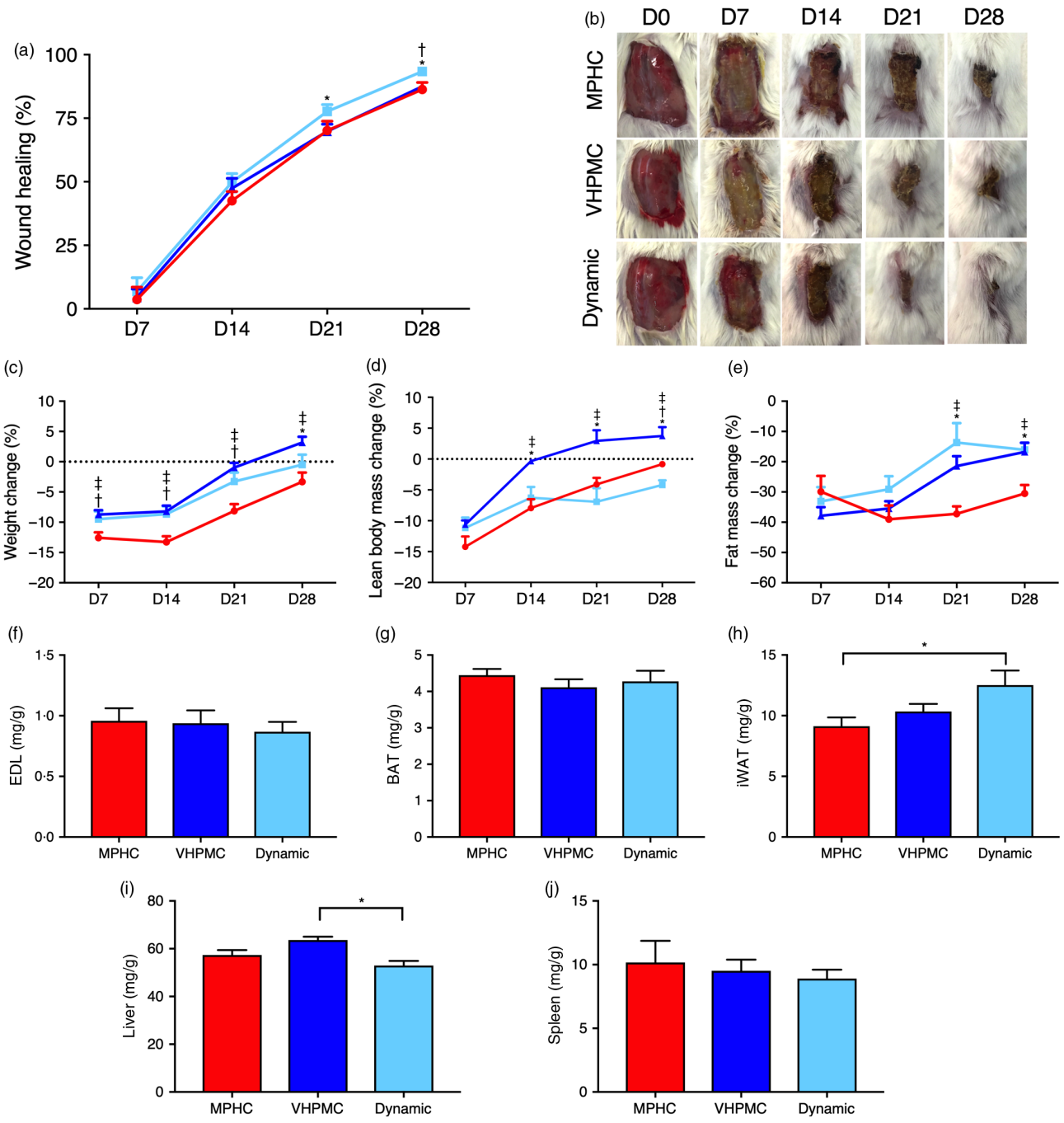

Fig. 6. Dynamic feeding experiment: (a) dynamic feeding involving changing the diet from a VHPMC to a MPHC diet on day 7 significantly accelerated wound healing on days 21 and 28, pictorially represented in (b). Changes in (c) body weight, (d) lean and (e) fat mass over the course of wound healing. Organ weight analysis in mg/g for (f) extensor digitorum longus muscles (EDL), (g) brown adipose tissue (BAT), (h) inguinal white adipose tissue (iWAT), (i) liver and (j) spleen. $P < 0{\cdot}05$ * dynamic *v.* VHPMC, † dynamic *v.* MPHC, ‡ VHPMC *v.* MPHC (*n* 12 mice/diet). Data presented as mean values with their standard errors. (a, c–e) —●—, MPHC; —▲—, VHPMC; —■—, dynamic. M, moderate; P, protein; H, high; C, carbohydrate; V, very.

mortality after severe burn injury. Secondly, a protein intake target was identified, above which, body weight loss did not improve, wound healing was delayed and liver and spleen weight increased. Thirdly, there was evidence that the optimal nutritional regimen for supporting wound healing in a mouse model of severe burn injury is to initially consume a very high-protein diet (VHPMC) followed by a moderate protein, high carbohydrate intake, with a P:C:F ratio of 1:4:2 (MPHC). This hypothesis was confirmed in the dynamic study which showed switching from a very high-protein diet to the optimal MPHC diet was ideal, with no deaths or enlargement of the liver and accelerated wound healing.

Carbohydrate intake in the present study was the key macronutrient for regulating burn injury outcomes. Wound healing was accelerated by a high carbohydrate intake and was maximal with a 2:1 ratio of carbohydrate:fat. Body weight loss was minimised

in mice also fed with a high carbohydrate intake in a 2:1 ratio with fat. Although protein intake was identified as being primarily responsible for improving survival, a high carbohydrate intake improved survival within groups where the protein intake was moderate (15 %) or low (5 %), suggesting a survival advantage with high carbohydrate intakes when protein is scarce. Higher carbohydrate intakes were associated with greater reserves of BAT but decreased iWAT, indicating a reduced thermogenic demand and increased metabolism of WAT[2,26,27].

There are a number of reasons by which a high carbohydrate intake may exert benefit. At the local wound level, epidermal migration and fibroblast proliferation are sensitive to glucose[28]. Immune cells also obligate glucose consumers and have a fundamental role in wound healing processes[29–31]. Studies of burn patients have shown that high-carbohydrate diets preserve lean body mass by sparing protein consumption in gluconeogenic pathways and increase insulin levels, encouraging anabolic processes[9,32]. Although the field is lacking in large well-designed randomised clinical trials, a Cochrane review found high-carbohydrate diets decreased the risk of pneumonia in burn patients, with a trend to decrease mortality[12].

Providing energy content primarily from fat was found to be less beneficial. High fat intake was associated with delayed wound healing, increased body weight loss, EDL atrophy and increased mortality. Severe endocrine abnormalities associated with the hypermetabolic response considerably reduce the ability of burn patients to use fat as an energy source[1]. For this reason, diets low in fat but containing the essential and *n*-3 fatty acids are believed to be beneficial for burn patients[32]. Furthermore, high-fat diets are associated with hyperlipidaemia, hypoxaemia, higher infection rates and post-operative mortality[9,14].

High protein intake was associated with minimal weight loss and reduced mortality, especially during the first week after burn injury. Response surfaces identified a protein target above which, limited further improvement in body weight loss was gained. The balanced intake of protein and carbohydrate in a 1:4 ratio maximised wound healing on days 14 and 21. Importantly, providing further protein without increases in carbohydrate was associated with reduced wound healing. This was most obvious on day 14 and 21, stressing the importance of maintaining a high carbohydrate intake with a high protein intake.

Current clinical nutritional regimens for burn patients focus on providing long-term, high-protein diets[12,15]. Severe burn patients have a significant protein loss of up to 150–250 g/d as lean body mass is the major energy source in a hypermetabolic state[33–35]. The development of protein deficiency has various serious clinical implications including, immunodepression, prolonged mechanical ventilation and delays in rehabilitation[36]. Wound healing is also compromised due to deficits in a variety of wound healing mechanisms which are protein-dependent[37]. Meeting protein intake targets of about 1·5 g/kg per d is essential for improving outcomes for burn and critical care patients; however, there is evidence that whilst meeting protein targets is important, over supply of protein may be ineffective and can have negative side effects[15,35,38]. Protein when overabundant can induce acute renal failure, uraemia and metabolic acidosis, requiring renal replacement therapy eventually leading to increased mortality[1]. Recent evidence in critical care research has also indicated that better outcomes may be achieved in patients who receive less protein[39].

In the present animal experiment, high protein intake was associated with increased liver and spleen weight. Previous research investigating the effect of long-term macronutrient intake on splanchnic and hepatic lymphocytes has shown that immune function can be altered by macronutrient intake[40]. In burn patients, hypermetabolism is associated with an increase in liver, spleen and kidney size[14]. Increased liver size, particularly from fatty infiltration, is associated with immune dysregulation, sepsis and higher morality[4,41]. High protein intakes causing enlargement of the liver and spleen in the present study may indicate greater immune dysfunction in burn trauma and is of significant consideration.

Dynamic feeding represents a novel nutritional regimen to further improve outcomes in burn patients. In this mouse experiment, combining the mortality benefit of an early VHPMC and the later wound healing benefits of the optimal MPHC diet on day 7, resulted in no deaths, accelerated wound healing, reduce liver and spleen size and preserved iWAT in dynamically fed mice. However, the slight reduction in LBM recovery observed in mice on this regimen must also be considered. Determining the local mechanisms by which dynamic feeding improves wound healing, such as increasing epidermal migration, cell proliferation, collagen deposition and vascularity will be a priority for future research.

Data presented here are derived from a mouse model, and some differences regarding wound healing processes and metabolic profiles exist between humans and mice[42]. These results must therefore be interpreted carefully; however, mouse models are key to developing an initial understanding of physiological processes and possible novel interventions, as unavoidable confounding variations prevalent in clinical research can be overcome. Translational human studies exploring the benefits of dynamic feeding *v.* high-protein, high-energy diets in current clinical use are possible after further mechanistic studies are completed and may provide new avenues of nutritional management for burn patients. Advances in nutrition and burn care may also potentially be applied to other conditions where hypermetabolism is present, such as critical illness, polytrauma and sepsis, as severe burns are a good example of a universal trauma model[17].

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conducted the research; J. J. H., R. J. P., M. D'S. and Y. W. analysed the data; J. J. H., R. J. P. and Y. W. wrote the paper; D. G. L. C., Z. L., P. K. M., C. N., S. M. S.-B., M. G. J. and S. J. S. edited the paper; Y. W, P. K. M. and S. M. S. B. supervised the project.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000276

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