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ORIGINAL ARTICLE

TGF-β_1 expression in wound healing is acutely affected by experimental malnutrition and early enteral feeding

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Abstract

Malnutrition is associated with the delay or failure of healing. We assessed the effect of experimental malnutrition and early enteral feeding with standard diet or diet supplemented with arginine and antioxidants on the levels of mRNA encoding growth factors in acute, open wound healing. Standardised cutaneous dorsal wounds and gastrostomies for enteral feeding were created in malnourished (M, $n = 27$) and eutrophic control (E, $n = 30$) Lewis male adult rats. Both M and E rats received isocaloric and isonitrogenous regimens with oral chow and saline (C), standard (S) or supplemented (A) enteral diets. On post-trauma day 7, mRNA levels of growth factor genes were analysed in wound granulation tissue by reverse transcription polymerase chain reaction (RT-PCR). M(C) rats had significantly lower transforming growth factor β(*TGF*-β_1) mRNA levels than E(C) rats (2·58 ± 0·83 versus 3·53 ± 0·57, $P < 0{\cdot}01$) and in comparison with M(S) and M(A) rats (4·66 ± 2·49 and 4·61 ± 2·11, respectively; $P < 0{\cdot}05$). *VEGF* and *KGF-7* mRNA levels were lower in M(A) rats than in E(A) rats (0·74 ± 0·16 versus 1·25 ± 0·66; and 1·07 ± 0·45 versus 1·79 ± 0·89, respectively; $P \leq 0{\cdot}04$), but did not differ from levels in E(C) and M(C) animals. In experimental open acute wound healing, previous malnutrition decreased local mRNA levels of *TGF*-β_1 genes, which was minimised by early enteral feeding with standard or supplemented diets.

Introduction

Growth factors actively participate in the organisation, coordination and mediation of all cellular processes involved in wound healing, such as cellular migration, angiogenesis, matrix synthesis, collagen deposition, formation of granulation and tissue remodelling (1,2).

Transforming growth factor β (TGF-β) has cell-specific effects in this setting by influencing cellular proliferation, differentiation, metabolism and the extracellular matrix (ECM) (3–6). TGF-β_1 is the most abundant isoform in all tissues and cells, and its expression peaks earlier post-wounding than TGF-β_2 or TGF-β_3 expression, suggesting a central role for this isoform in the acute phase of the healing process (7,8).

Key Messages

- previous malnutrition decreased local mRNA levels of *TGF*-β_1 genes
- early enteral feeding with standard or supplemented diets minimised the decrease in wound mRNA levels of *TGF*-β_1 genes in malnourished rats
- supplemented enteral diet was not superior to standard enteral diet in minimising the decrease in wound mRNA levels of *TGF*-β_1 genes in malnourished rats

TGF-β_1 is crucial in wound healing because it influences fibroblast, collagen synthesis and ECM formation, and plays

a critical role in wound strength by promoting the progressive replacement of collagen type III by collagen type I via its ECM remodelling properties (9–11).

Keratinocyte growth factor (KGF) stimulates reepithelialisation and mediates mesenchymal–epithelial interactions to promote epithelial proliferation and migration within the wounded area, which facilitates differentiation of new epidermis (12). Blood platelets secrete platelet-derived growth factor (PDGF) when they adhere to traumatised tissues, which leads to an autocrine and paracrine amplifier effect (12). Angiogenesis in subjacent dermal endothelial cells is induced by vascular endothelial growth factor (VEGF), which is synthesised mainly by keratinocytes around the wound edge (12).

Wound healing is strongly dependent on the availability of energy, protein and micronutrients. Malnutrition is largely associated with a delay or failure of the healing process, but nutritional intervention can mitigate malnutrition and improve wound healing, mainly by increasing collagen deposition after trauma (3).

Specialised diets enriched with arginine and antioxidants have been advocated in this setting and associated with less morbidity and shorter hospital stays (3). Arginine may benefit the healing process by positively affecting microvascular and perfusion changes, protein synthesis, cellular proliferation and signalling via proline and polyamine synthesis, whereas antioxidants can contribute to healing by preventing or attenuating peroxidative damage (3,13–16).

We reasoned that growth factor synthesis during wound healing could be impaired in poor nutritional status, especially during the acute post-trauma period, and that a specialised diet enriched with arginine and antioxidants would beneficially affect the molecular expression of these healing mediators. Therefore, the objective of this study was to assess the expression of growth factor genes in acute open wound healing under experimental malnutrition and early enteral feeding with standard and specialised (containing arginine, vitamins C and E, selenium and zinc) enteral diets.

Materials and methods

Animals

All animals received appropriate care throughout the experimental procedure, and the animal experimentation protocol was approved by the local ethics committee 'Comissão de Ética para Análise de Projetos de Pesquisa do HCFMUSP'. We used 57 adult male isogenic Lewis rats that weighed 250–350 g. The animals were allowed free access to water and standard rat oral diet (AIN-93M, Rhoster Indústria e Comércio, Brazil) in individual metabolic cages at room temperature for 10 days with regular light cycles before the experimental procedures in order to acclimatise the rats to our laboratory conditions.

Experimental malnutrition

After acclimation, the animals were randomly divided into two groups: eutrophic (E) and previously malnourished (M). The E rats were fed AIN-93M ad libitum; M rats were submitted to dietary restriction, and received 50% of the mean amount of food consumed by E rats. After 14 days of dietary restriction, the M rats had a 12–15% reduction in body weight compared with their initial body weight.

Gastrostomy and cutaneous wounds

All rats were anaesthetised with an intraperitoneal injection of 100 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, São Paulo, Brazil) and 2% xylazine (Rumpum, Bayer, São Paulo, Brazil). After abdominal and dorsal shaving, gastrostomy and four dorsal back full-thickness skin excision wounds (1·3 cm diameter) were made, as described elsewhere (17). The dorsal muscular fascia was exposed (Fig. 1), and the wounds were left uncovered during the post-trauma period.

Post-trauma nutrition

Immediately following surgery, all rats were kept in a fasting state and received 5% dextrose at a rate of 0·3 ml/hour for 12 hours throughout the gastrostomy. After this period, the E and M groups were each randomly distributed into three different subgroups according to the nutritional treatment to be received (Table 1). All animals were fed orally with AIN-93M chow (adjusted for specific total isocaloric and isonitrogenous intakes depending on the group allocation) and saline (0·9%), or two different liquid diets: the standard (Hiper Diet Multifiber, Nutricia, The Netherlands) or a specialised diet (Cubison, Nutricia, The Netherlands). The two enteral diets were isocaloric and isonitrogenous per volume, and had the same amount of dietary fibre, sodium, potassium, chlorine, calcium, magnesium, phosphorus, iron, fluorine, molybdenum, chromium, iodine, carotenoids, thiamine, niacin, pantothenic acid, biotin, choline, and vitamins A, D, K, B16, and B12. The specialised enteral diet had more arginine (0·003 versus 0 g/ml), zinc (0·02 versus 0·012 mg/ml), selenium (0·096 versus 0·057 μg/ml), vitamin E (0·075 versus 0·013 mg/ml) and vitamin C (0·38 versus 0·1 mg/ml) than the standard oral and enteral diets.

Continuous infusion of saline and the enteral diets through the gastrostomy was performed until post-trauma day 7 using

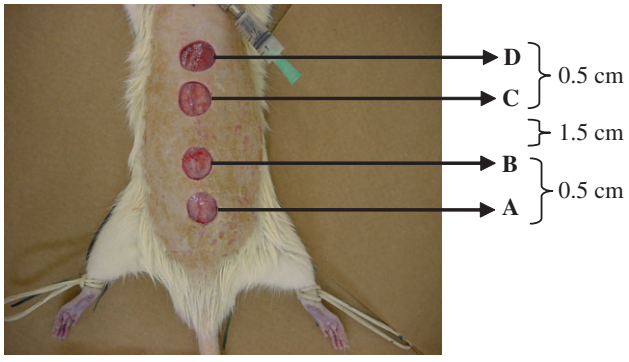

Figure 1 Surgically generated dorsal cutaneous wounds in Lewis rats.

Table 1 Experimental groups of isogenic Lewis rats, classified according to nutrition status, oral and/or enteral diet administered after gastrostomy and standardised cutaneous dorsal wound

Groups	Animals (*n*)	Description
N(C)	10	Nourished; gastrostomy with saline + oral diet
N(S)	10	Nourished; gastrostomy with S + oral diet
N(A)	10	Nourished; gastrostomy with A + oral diet
M(C)	09	Malnourished; gastrostomy with saline + oral diet
M(S)	09	Malnourished; gastrostomy with S + oral diet
M(A)	09	Malnourished; gastrostomy with A + oral diet

N, nourished; M, malnourished; C, saline; S, standard enteral diet; A, enteral diet supplemented with arginine and antioxidants.

a micropump infusion (Life Care, Abbott Laboratories, OH) and a graduated burette (Micro Soluset 150/160, Abbott Laboratories, Costa Rica). The infused saline and enteral diets were recorded daily. The oral dietary intake was measured daily by determining the difference between the amount of oral diet added and the residual diet found in the cages after 24 hours. The daily infused and consumed (total intake) energy, nitrogen, arginine, selenium, zinc, vitamin C and vitamin E were calculated.

Percentage body weight variation in the post-trauma period

The variation in body weight during the post-trauma period was calculated by considering the initial (surgery day) and final (post-trauma day 7) body weight. Arithmetic means were calculated for each experimental group.

Granulation tissue sampling

On post-trauma day 7, all rats were anaesthetised with intraperitoneal ketamine hydrochloride and xylazine. Wound tissue containing both epithelium and granulation tissue was collected from the proximal pair of wounds. Samples from granulation tissue collected from each rat were promptly identified, quickly frozen and stored in liquid nitrogen for further analysis of gene expression.

Molecular biology analysis

Total mRNA was extracted using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions and the methods described by Chomezynski and Sacchi (18). The relative quantification of the target genes was based on the endogenous β-*actin* gene, as described by Pfaffl (19). The reactions were performed using Corbett Research Rotor-Gene RG-3000 equipment (Corbett Research, Sydney, Australia) and the SuperScript III Platinum SYBR Green One-Step Quantitect Sybr Green RT-PCR Kit (Invitrogen Life Technologies). Oligonucleotides were designed using the published mRNA sequence (available at www.ncbi.nlm.nih.gov/nucleotide), and primers were designed using the Primer 3 programme.

All primers were synthesised by Invitrogen Life Technologies. The oligonucleotide primers used were as follows: β-*actin* (GenBank accession number C0985) sense primer: 5′-TGT CAC CAA CTG GGA CGA TA-3′, and antisense primer 5′-GGG GTG TTG AAG GTC TCA AA-3′; *TGF*-β_1 (GenBank accession number A3546) sense primer: 5′-ATA CGC CTG AGT GGC TGT CT-3′, and antisense primer 5′-TGG GAC TGA TCC CAT TGA TT-3′; *VEGF* (GenBank accession number D4860) sense primer: 5′-GCC CAT GAA GTG GTG AAG TT-3′, and antisense primer 5′-ACT CCA GGG CTT CAT CAT TG-3′; *PDGF*-α (GenBank accession number S0589) sense primer: 5′-ATG CCT TGG AGA CAA ACC TG-3′, and antisense primer 5′-GTC AAG AAG TTG GCC GAT GT-3′; and *KGF-7* (GenBank accession number J2745) sense primer: 5′-CTG TGG CAG TTG TAA TTG TG-3′, and antisense primer 5′-ACA GGA AGC CCC TTT TGA TT-3′.

Statistical analysis

The distributions of all data were tested for normality. Statistical analysis was then performed using analysis of variance (ANOVA) with the experimental factors of nutritional status (E versus M) and the two different enteral solutions delivered through the gastrostomy (S versus A). ANOVA was preceded by logarithm transformation of the mRNA levels, which was required for adequate mRNA level matching to normal profile data. When a significant interaction was found, post hoc analysis (Newman–Keuls test) was used to compare differences among independent groups. An alpha-level of 0·05 was used to determine overall significance. Values are reported as mean ± SE.

Results

Total energy and nitrogen intake

For eutrophic animals, there was no difference in the total caloric intake among the experimental groups, but the A group ingested higher amounts of nitrogen than the C and S groups. For malnourished animals, there was a higher intake of calories in the C group than in the S and A groups, and a tendency towards lower nitrogen intake in the S group than in the C and A groups ($P \geq 0{\cdot}051$). The data describing total calories and nitrogen intake among the experimental groups are described in Table 2.

Arginine and antioxidant intake

For both studied nutritional status, animals from A group had a total intake of arginine, selenium, zinc, vitamins C and E that was higher than those from the C and S groups. However, there was no difference in the intake of these nutrients between

Table 2 Total of calories (kcal/day) and nitrogen intake (g/day) and percentage of body weight variation in post-trauma period*

Groups		Calories	Nitrogen	Body weight
E	C	53·31 ± 7·66	0·30 ± 0·04	0·40 ± 9·56
	S	52·08 ± 5·27	0·30 ± 0·01	−1·19 ± 3·17
	A	55·19 ± 6·43	0·42 ± 0·04†	1·52 ± 4·68
M	C	67·32 ± 12·23‡	0·38 ± 0·07	9·32 ± 7·98‡
	S	54·35 ± 10·30	0·31 ± 0·06	1·15 ± 9·04
	A	48·29 ± 5·01	0·37 ± 0·04	2·03 ± 3·34

E, eutrophic; M, malnourished; C, oral diet + saline; S, oral diet + standard enteral diet; A, oral diet + specialised enteral diet.
*Data presented as mean ± standard deviation.
†$A > C$ and S ($P \leq 0{\cdot}000$).
‡$C > S$ and A ($P \leq 0{\cdot}036$ for calories; $P = 0{\cdot}048$ for body weight).

the malnourished and eutrophic rats from A group. For both studied nutritional status, animals from the C and S groups were similar in terms of zinc intake, but the C group had a higher intake of arginine and vitamin E and a lower intake of selenium and vitamin C than the S group. The data describing total arginine and antioxidant intake among the experimental groups are shown in Table 3.

Percentage of body weight variation in the post-trauma period

On post-trauma day 7, most of the animals had gained weight compared with the first post-trauma day. There were no significant differences in the percentage of body weight gain among the eutrophic animals. For malnourished animals, the C group had a higher weight gain compared with the A and S groups. The data regarding the percentage body weight variation among the experimental groups are described in Table 2.

Gene expression analysis

When we compared malnourished with eutrophic rats from control groups, malnutrition was associated with lower mRNA expression levels of *TGF*-β_1. For the same nutritional status, malnourished rats from the control group also had lower expression levels of *TGF*-$\beta 1$ than malnourished animals treated with both studied enteral diets. For animals from A group, those malnourished had lower mRNA expression levels of *VEGF* and *KGF-7* than those eutrophic. The data describing growth factors mRNA expression are listed in Table 4.

Discussion

Malnutrition is associated with delay or failure of healing, but little is known about the molecular mechanisms underlying this effect (6). This study is part of a large study that aimed to assess the effect of malnutrition and specialised enteral diet on wound healing. Partial data concerning tissue, cell and collagen alterations in wound healing were previously reported and the present data comprises the mechanistic approach of these alterations (17). In this study, we show that malnutrition was accompanied by decreased wound mRNA levels of *TGF*-β_1, which was minimised by post-trauma short-term feeding with standard or supplemented enteral diets in experimental acute open wound healing.

Table 3 Total arginine and antioxidant daily intake in post-trauma period*

Groups		Arginine (g)	Selenium (μg)	Zinc (mg)	Vitamin C (mg)	Vitamin E (mg)
N	C	0·072 ± 0·008	0·003 ± 0·0003	0·56 ± 0·06	0·0 ± 0·0	1·20 ± 0·14
	S	0·023 ± 0·003	2·345 ± 0·2169	0·67 ± 0·04	4·1 ± 0·4	0·92 ± 0·04
	A	0·412 ± 0·025	4·131 ± 0·2690	1·07 ± 0·06	16·6 ± 0·8	3·64 ± 0·21
M	C	0·075 ± 0·010	0·003 ± 0·0004	0·58 ± 0·08	0·0 ± 0·0	1·25 ± 0·17
	S	0·030 ± 0·004	2·115 ± 0·2261	0·68 ± 0·05	3·7 ± 0·4	0·98 ± 0·08
	A	0·403 ± 0·020	4·089 ± 0·2040	1·02 ± 0·04	16·1 ± 0·8	3·53 ± 0·18

N, nourished; M, malnourished; C, oral diet + saline; S, oral diet + standard enteral diet; A, oral diet + specialised enteral diet.
*Data presented as mean ± standard deviation. Arginine and all antioxidant for N and M groups: A > C; A > S ($P < 0{\cdot}001$). Arginine and vitamin E: E(C) > E(S); M(C) > M(S) ($p < 0.001$). Selenium and vitamin C: E(C) < E (S); M(C) < M(S) ($p < 0.001$).

Table 4 Growth factor mRNA expression measurements in granulation tissue on post-trauma day 7†

Groups		TGF-β_1	VEGF	PDGF-α	KGF-7
Nourished	C	3·53 ± 0·57	1·27 ± 0·97	1·45 ± 1·15	1·50 ± 0·72
	S	4·06 ± 1·58	0·95 ± 0·46	1·46 ± 1·12	1·71 ± 1·16
	A	3·78 ± 1·85	1·25 ± 0·66	1·02 ± 0·45	1·79 ± 0·89
Malnourished	C	2·58 ± 0·83*	1·21 ± 0·70	1·24 ± 0·51	1·27 ± 0·78
	S	4·66 ± 2·49	0·97 ± 0·37	0·97 ± 0·60	1·24 ± 0·50
	A	4·61 ± 2·11	0·74 ± 0·16*	1·08 ± 0·62	1·07 ± 0·45*
ANOVA		M(C) < N(C)* M(C) < M(S)** M(C) < M(A)**	M(A) < N(A)***	No difference	M(A) < N(A)***

*$P < 0{\cdot}01$; **$P = 0{\cdot}05$; ***$P < 0{\cdot}05$.
C, oral diet + saline; S, oral diet + standard enteral diet; A, oral diet + specialised enteral diet.
†Data expressed in arbitrary units (AU).

We choose to assess growth factor gene expression in wounds under malnutrition by using experimental cutaneous open wounds in isogenic rats because this model enables us to verify all the phases of healing (reepithelialisation, formation of granulation tissue and wound contraction) that can be modulated by growth factors and minimises possible genetic variations between the animals that could influence gene expression (20,21). We performed four cutaneous wounds, but only analysed the proximal pairs. The distal pair wounds were evaluated for cell and tissue markers that can be better observed after longer periods than 14 days, and which data were previous reported (17). In addition, the experimental malnutrition model, with an average weight loss of 15%, causes enough protein depletion to prioritise the use of supplied nutrients to wound healing, while avoiding a severe malnutrition status (>20% weight loss), in which organic maintenance can take precedence (22).

Malnutrition was associated with decreased *TGF*-β_1 mRNA expression in wounds on post-trauma day 7. *TGF*-β_1 expression during wound healing was scrutinised previously in several reports. It is known that skin injury promotes an imbalance of *TGF*-β*1*, *2* and *3* expression 24 hours post-trauma, and also on the fifth to seventh day after trauma (9). However, to the best of our knowledge, this issue was not assessed under malnutrition status (23).

Nutritional deficiencies impact wound healing by impeding fibroblast proliferation, collagen synthesis and epithelialisation (24,25). Animals with acute protein-calorie malnutrition had reduced sponge hydroxyproline contents, indicating diminished wound collagen accumulation and decreased wound gene expression of type III, but not type I, collagen (26). We previously reported decreased wound gene expression of type III but not type I collagen, and an inhibition of the increase in the fibroblast cell contingent between post-trauma days 7 and 14 in experimental acute open wound healing (17).

TGF-β_1 is involved in ECM formation and subcutaneous administration of this growth factor in rats promotes reepithelialisation and proliferation of fibroblasts and keratinocytes, and increases deposition of collagen (9,27). Increased mRNAs for collagen types I and III were observed in microdissected airways 1 week after intratracheal instillation of TGF-β_1 in BALB/C mice (28). In a full-thickness skin model, Yavuz *et al*. (29) demonstrated that the intact skin of diabetic rats had sparsely distributed regular collagen fibres in the granulation zone, and loss of the regular collagen fibre pattern associated with weak *TGF*-β_1 expression when compared with healthy controls. The restoration of growth factor *TGF*-β_1 expression by treating these animals with aminoguanidine improved wound healing and preserved collagen ultrastructure (29). Therefore, the reduction of wound *TGF*-β_1 gene expression in malnourished rats that we observed may contribute to a better understanding of the molecular mechanisms involved in decreased wound collagen production associated with malnutrition during healing (17,26).

In addition, the lack of consistent *TGF*-β_1 mRNA levels under malnutrition may also be associated with the prolonged inflammatory response and impaired neovascularisation in wounds of nutritionally depleted animals previously reported by us and others (17,30). Active TGF-β_1 elicits the rapid chemotaxis of monocytes to the wound site (31). Besides to decreases in collagens III and I deposition, the exogenous addition of neutralising TGF-β_1 antibodies to cutaneous wounds reduced the monocyte and macrophage inflammatory profile, as well as neovascularisation and fibronectin (10). These findings could traduce a delay of monocyte and macrophage recruitment to wound in the presence of reduced levels of TGF-β1. Interestingly, TGF-β knockout animals develop a severe wasting syndrome with an intense and prolonged inflammatory response in the granulation tissue (32). In 10-day-old TGF-β knockout mouse models, wounds have characteristic inflammation, along with a slight decrease in granulation tissue (33).

It is worth noting that, although the total intake offered for all the animals was homogeneously isocaloric and isonitrogenous, there were higher ingestion of calories and nitrogen in M(C) and E(A), respectively, in comparison to the other experimental groups. However, these differences might not be significant enough to benefit the expression of *TGF*-β_1 mRNA because M(C) had decreased *TGF*-β_1 mRNA in relation to those malnourished groups fed with enteral diets; and E(A) did not change *TGF*-β_1 mRNA in comparison to the other eutrophic groups.

In this study, nutritional therapy with commercially available enteral diets prevented the *TGF*-β_1 mRNA depletion found in malnutrition states. These results confirm the importance of early nutritional therapy in the malnutrition states that occur after surgery or trauma (6).

The main difference between the two enteral diets used in the present study was supplementation with arginine and micronutrients that are thought to assist with the healing process. However, the introduction of these elements in the enteral formula failed to increase growth factor expression; importantly, it decreased *VEGF* and *KGF* expression in the malnourished animals.

The decreased mRNA levels of *VEGF* and *KGF* in malnourished animals fed with specialised enteral diet did not occur in the corresponding malnourished animals in the normal diet control group. Although supplementation was associated with diminished levels of *VEGF* and *KGF* mRNA in malnourished animals, this did not appear to jeopardise wound healing, because the previously reported data showed that on post-trauma day 14 wound closure was not impaired in malnourished animals fed with specialised enteral diets in relation to those fed with oral control and standard enteral diets (17).

The current results do not support the hypothesis that arginine may have beneficial effects on the healing process by affecting microvascular and perfusion changes (34). Although dietary arginine supplementation was associated with increased protein synthesis, cellular proliferation, and signalling via proline and polyamine synthesis in other reports, environmental and dietary variables were not controlled in these reports, as they were in this experimental study (35,36). The pool concentration of arginine is important in wound healing, particularly because it is a precursor of nitric oxide (NO). However, arginine levels did not appear to be affected by supplementation or previous nutritional status (37,38). One possible explanation is that healing becomes the highest priority

in injured animals, and therefore metabolic pools of amino acids are utilised by this process over other functions such as control of the inflammatory process.

In clinical practice, the use of enteral supplements/formulas containing arginine with other pharmaconutrients (n-3 fatty acids and RNA) is currently recommended to patients undergoing elective major surgery to decrease infections, hospital stay and also to promote wound healing potentially, with a significant reduction in suture dehiscence, but without overall effect on mortality compared with standard care (39–41). In addition, a recent meta-analysis concluded that this arginine-supplemented enteral diet for patients undergoing elective surgery for gastrointestinal cancer is an effective and cost-saving intervention (42). A more detailed evaluation of arginine supplementation in wound healing would be of interest in subsequent studies.

Vitamins and minerals can potentially enhance wound healing. Vitamins A, C and E; zinc; and selenium may be associated with the prevention or attenuation of peroxidative damage (3). Vitamin and mineral deficiencies are known to impair the normal wound healing process, and these deficiencies can be corrected with supplementation (3). Thus, new specialised diets enriched with arginine and antioxidants (vitamin C, E, selenium and zinc) have a potential role in improving healing in chronic cutaneous wounds and have been associated previously with lower morbidity and shorter lengths of hospital stay (40,43–45). Supplementation with micronutrient antioxidants is currently advocated in some surgical patients. For example, post-bariatric surgery patients with micronutrient levels below 50% of the recommended daily allowances may benefit from vitamin and oligoelement supplementation prior to body contouring surgery (46).

We did not observe any differences among nutritional status or post-trauma treatment with regard to *PDGF* expression. This result may be associated with specific characteristics of *PDGF*, such as site of production and peak levels of expression. *PDGF* is mainly found in the epidermis, is produced early after trauma (around post-trauma day 3, during the inflammatory phase), and stimulates chemotactic agents for neutrophils and the proliferation of fibroblasts (47). Despite the early peak of *PDGF* expression, we studied this growth factor because malnutrition could alter its kinetics.

This study has limitations that deserve to be discussed. The skin repair process was evaluated in rodents and similar results may therefore not be achieved in humans. In addition, the two different enteral diets evaluated are available for human treatment and were not designed to attend the specific nutritional needs of rodents to improve wound healing. Our skin wound model was acute and excisional, and similar results may not occur in other chronic and/or incisional models that may involve different mechanisms of wound repair. Growth factors are continuously released during wound healing, but we only studied molecular alterations on post-trauma day 7 because this study was designed to evaluate molecular mechanisms that could be enrolled in tissues, cells and protein changes previously observed after 14 days of wound healing (17).

Taken together, the data of this study suggest that wound healing impairment in malnourished animals may be due in part to a local decrease in *TGB*-β_1 expression that can be restored by early enteral nutrition. However, we failed to find any potential benefits to a specialised enteral diet that was enriched with arginine and antioxidants to increase wound growth factor expression. Previous studies reported that supplemented nutritional support improves cutaneous open wound healing; however, in light of our results, this may have been due to metabolic pathways that do not depend on the relative concentrations of the growth factors that we investigated (48). The strict conditions under which supplementation is beneficial should be explored in future studies.

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CCA participated in the study design and conception, carried out all experimental assays, interpreted data and wrote the manuscript. RST performed data analysis and interpretation, and wrote the manuscript. RG participated in the acquisition and analysis of data, revised the manuscript critically and gave final approval to the version to be published. MMB participated in the study design and conception, oriented the acquisition and analysis of data, revised the manuscript critically and gave final approval to the version to be published. AFL participated in the study design, revised the manuscript critically and gave final approval to the version to be published. DNL participated in the design and conception of the study, oriented the experimental assays regarding surgical procedures, performed analysis and interpretation of the data, and revised and approved the manuscript.

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