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Barbara Strasser, Gabriele Kohlboeck, Maria Hermanky & Michael Leitzmann

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SHORT COMMUNICATION

Role of dietary protein and exercise on biomarkers of immune activation in older patients during hospitalization

Barbara Strasser[1,3] · Gabriele Kohlboeck[1] · Maria Hermanky[2] · Michael Leitzmann[3]

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Abstract

The aim of this study was to examine the effect of short-term protein supplementation (aiming to consume 1.2 g protein/kg body weight per day) combined with moderate resistance training on 3 days of the week on tryptophan–kynurenine metabolism in 40 older patients with hip fracture. Secondary outcomes for physical recovery were hand-grip strength and chair-rise score. Older patients with hip fracture exhibited higher degrees of immune activation, detected by increased neopterin and kynurenine to tryptophan levels compared with reference values for healthy elderly with no significant differences between those who received the exercise–protein intervention compared to the control. Increasing dietary protein intake during hospitalization did not alleviate the Th1-type immune response in the elderly patient. On the other hand, muscularity per se may affect immune activation responses following injury, as improvements in maximum hand-grip strength with the intervention were related to decreases in neopterin levels.

Keywords Aging · Exercise · Dietary protein · Muscle disuse · Tryptophan–kynurenine metabolism

Introduction

The process of ageing is accompanied by chronic immune activation, and sarcopenia may represent a consequence of a counter-regulatory strategy of the immune system. Thereby, inflammatory cytokines, in particular, Th1-type cytokine interferon-gamma (IFN-γ), induces various biochemical pathways such as tryptophan (Trp) breakdown. Tryptophan deprivation can suppress immune activation processes via restriction of protein biosynthesis and the induction of regulatory T-cells by kynurenine (Kyn) metabolites [1]. The mode of Trp degradation and concentration of Kyn is a function of free Trp concentration, which is in turn influenced by competing amino acids such as leucine, which is a major regulator of muscle protein synthesis [2]. In parallel, IFN-γ also induces the production of neopterin in human monocyte-derived macrophages and dendritic cells, the elevation of which is often linked with conditions of immune activation and inflammation such as frailty [3]. Thus, the activated immune system in older individuals can be detected by increased neopterin and Kyn to Trp levels. Still, little is known about a possible influence of the immunobiochemical pathways on muscle pathophysiology in older patients during muscle disuse and whether higher protein intake and muscle-strengthening exercises may be able to beneficially affect immune activation responses during the early rehabilitation period following injury. Perioperative nutritional interventions may have a positive effect on functional recovery in elderly hip-fracture patients [4] as the majority in this patient group suffer from malnutrition and sarcopenia negatively impacting patients' outcomes [5]. Therefore, the aim of the present randomized controlled trial was to investigate the effect of a combined exercise–protein intervention on immune activation and muscle function parameters in older patients during hip fracture recovery.

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✉ Barbara Strasser
Barbara.Strasser@med.sfu.ac.at

[1] Center for Gastrointestinal Diseases and Metabolism, Medical Faculty, Sigmund Freud Private University, Freudplatz 3, 1020 Vienna, Austria

[2] AUVA Lorenz Boehler Trauma Unit, 1200 Vienna, Austria

[3] Department of Epidemiology and Preventive Medicine, University Regensburg, 93053 Regensburg, Germany

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Materials and methods

This study is a secondary analysis from a previously published study which was designed to evaluate the effect of a protein optimized diet on the postoperative course in older patients with hip fracture [6]. In brief, 40 older participants with hip fracture (mean age: 79.6 ± 9.2 years), who were admitted to the Lorenz Boehler Hospital Vienna, were randomly assigned by a computer-generated random number list to an intervention group ($n = 20$) or a control group ($n = 20$). Subjects were excluded if they suffered from chronic renal insufficiency or cognitive impairment. During hospitalization (mean length of stay: 16.4 days), members of the intervention group received a protein-optimized diet with protein-enriched foods (aiming to consume 1.2 g protein/kg body weight per day) plus a protein-enriched drink (providing 300 kcal and 20 g protein per serving, taken once daily as a late meal) and were instructed in moderate resistance training using bodyweight or resistance bands. Muscle-strengthening exercises for the hip, thigh and the upper arm and shoulder muscles were performed in addition to standard therapy on 3 days of the week (30 min per session) under the supervision of a physiotherapist. The control group received standard care with regular non protein-enriched food. Nutritional intake was measured every day during hospitalization using a detailed dietary protocol (OrgaCard Siemantel & Alt GmbH, Germany). The verified food records were entered into the food calculation program Prodi Expert Version 6.3 (Nutri-Science GmbH, Germany) to calculate energy and protein intake according to the German Nutrient Data Base (BLS, Federal Research Centre for Nutrition and Food, Karlsruhe, Germany). In addition, analytical values compiled from food producing firms were used to estimate the protein content of foods. Average energy and protein intake were calculated for each patient during hospitalization. Maximum hand-grip strength was determined using a JAMAR dynamometer (Preston Jackson, USA), and the highest of the three measurements was reported for the dominant hand. Physical functioning was measured with an adapted 30-s chair-rise test with both hands on a reciprocal walker for support. Serum concentrations of free Trp and Kyn were determined by high-performance liquid chromatography (HPLC) and neopterin levels were measured by enzyme-linked immunosorbent assay (BRAHMS Diagnostica GmbH, Germany). The ratio of Kyn/Trp was calculated as an index of Trp breakdown. Laboratory and muscle function parameters were determined at admission, discharge and at 1-month follow-up.

Intention-to-treat analysis was performed using the statistical software package SAS, version 9.4. In dependence of Gaussian distribution, baseline characteristics, biological and functional markers were compared by unpaired Student's t test or Mann–Whitney U-test. Changes in variables between admission, discharge and at 1-month follow-up were analysed by univariate and multivariate repeated measures analysis of variance (ANOVA). A P-value of less than 0.05 (two-tailed) was considered to indicate statistical significance. Data are presented as mean values ± standard deviation (SD).

Results

Baseline characteristics are presented in Table 1. Of the 40 patients enrolled, 35 (12 men, 23 women) had complete data at all time points. Older patients with hip fracture exhibited higher degrees of immune activation, as indicated by biomarker concentrations compared with reference values obtained from a healthy non-frail older population (Table 2, ref. 3). During hospitalization, the intervention group consumed more protein than the control group ($P < 0.01$): 70.5 (SD 13.5) g/day compared with 52.3 (SD 13.9) g/day, which corresponds to a mean protein intake of 1.0 (SD 0.3) g/kg/day vs. 0.8 (SD 0.2) g/kg/day. Furthermore, the intervention group reached a higher energy intake than the control group ($P = 0.016$): 1438 (SD 250) kcal/day compared with 1219 (SD 280) kcal/day. Use of the protein-enriched intervention

Table 1 Baseline characteristics

	Intervention ($n = 20$)	Control ($n = 20$)	P
Age (years)	80.1 (9.3)	79.9 (8.5)	0.986
Sex (female/male)	13/7	13/7	1.000
Height (m)	1.65 (0.07)	1.64 (0.07)	0.861
Body weight (kg)	69.3 (17.3)	67.7 (13.0)	0.741
BMI (kg/m^2)	25.6 (5.9)	24.8 (4.1)	0.625
CRP (mg/dl)	2.6 (1.9)	2.4 (2.3)	0.767
MNA (score)	22.5 (5.9)	23.6 (4.1)	0.508
Mobility aids (pre-injury)	6 (30%)	2 (10%)	0.114
Fracture types (IC/EC)	14/6	11/9	0.882
Length of stay (day)	17.9 (7.7)	15.7 (3.4)	0.261
Medical diagnosis	6 (30%)	7 (35%)	0.343
Cancer	–	3 (15%)	
Congestive heart failure	4 (20%)	3 (15%)	
Chronic kidney disease	1 (5%)	1 (5%)	
Chronic obstructive pulmonary disease	1 (5%)	–	

Values are mean ± standard deviation (SD); numbers and percentages

ns non-significant, *CRP* C-reactive protein, *MNA* mini nutritional assessment, *IC* intracapsular, *EC* extracapsular

Table 2 Changes in immune biomarkers and tryptophan over time in 35 older patients with hip fracture compared with 35 healthy non-frail control subjects

	Intervention ($n=18$)			Control ($n=17$)			Reference values[a]
	Admission	Discharge	Follow-up	Admission	Discharge	Follow-up	
Tryptophan (µmol/L)	46.4 (16.3)	47.0 (15.6)	48.4 (18.5)	52.4 (12.1)	50.6 (13.6)	53.3 (9.3)	82.3 (14.9)
Kynurenine (µmol/L)	2.3 (0.8)	2.7 (0.9)	2.7 (1.2)	2.4 (0.8)	2.9 (1.0)*	2.8 (1.2)	2.3 (0.5)
Kyn/Trp (µmol/mmol)	58.6 (33.0)	64.4 (27.5)	60.8 (38.8)	47.8 (19.3)	62.3 (30.6)*	54.0 (26.3)†	37.6 (18.9)
Neopterin (nmol/L)	10.8 (4.7)	16.3 (6.5)*	15.3 (8.7)*	11.5 (8.0)	14.9 (11.7)*	13.4 (8.6)	5.9 (1.8)

Values are mean ± standard deviation (SD)

* Significant time effect ($P<0.05$) compared with admission within a group

† Significant time effect ($P<0.05$) compared between discharge and follow-up within the group

No intervention effect or interaction of intervention and time effects were found ($P>0.05$)

[a] According to Marcos-Pérez et al. [3]

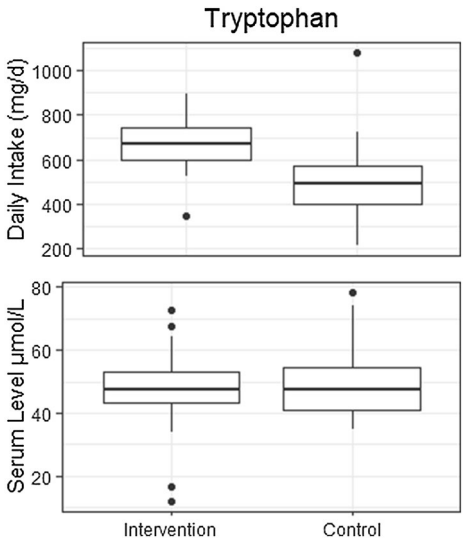

Fig. 1 Dietary intake and serum levels of tryptophan in older patients during hospitalization receiving either protein enrichment ($n=18$) or standard care ($n=17$). Dietary tryptophan levels were calculated according to the German Nutrient Data Base (BLS, Federal Research Centre for Nutrition and Food, Karlsruhe, Germany). The Database contains research results of German Federal Research Centers and universities. In addition, analytical values compiled from food producing firms were used to estimate tryptophan composition of the food

led to an increased daily mean Trp intake in the intervention group compared with the control group ($P<0.01$), while serum Trp levels remained unaltered (Fig. 1 and Table 1). However, the combined short-term exercise–protein intervention did not show an effect on levels of immune biomarkers. No associations were found between dietary Trp intake and serum Trp concentration, immune health parameter (neopterin) or the inflammatory state (C-reactive protein (CRP)). However, a significant association was found between Trp intake and serum Kyn/Trp ratio (reflecting indoleamine 2,3-dioxygenase (IDO) activity) at discharge ($P=0.02$). Hand-grip strength increased with the intervention from 18.6 (SD 6.2) kg to 19.4 (SD 6.2) kg ($P>0.05$) at follow-up but decreased in the control group from 22.5 (SD 8.4) kg to 21.4 (SD 8.1) kg ($P=0.041$), with no significant differences between groups. Chair-rise score improved overall from a mean of 3.4 at admission to 9.4 at follow-up (time effect $P<0.001$, no group effect). In the intervention group, improvements in maximum hand-grip strength from admission to discharge were associated with decreases in neopterin concentrations ($r=0.50$; $P=0.05$). Reductions in neopterin levels explain about 25% of the increase in muscle strength during hospitalization ($R^2=0.25$; $P=0.026$).

Discussion

Recent findings from the KORA-Age study noted higher concentrations of inflammatory markers in older individuals with lower levels of hand-grip strength, suggesting that inflammation may be involved in the loss of muscle strength or, vice versa, muscularity per se may exert anti-inflammatory effects [7]. The simultaneous presence of inflammation and malnutrition has been shown to reinforce the loss of muscle strength and function during hospitalization [8]. At admission, 10.5% of our patients were malnourished and another 26.3% were at risk of malnutrition. Poor nutritional status is associated with worse outcomes and independently predicts 1 year mortality in elderly hip-fracture patients [9]. Thus, interventions for sarcopenia, including exercise and nutrition, seem to be essential because both have a positive impact on protein anabolism but also influence the inflammatory profile associated with muscle weakness [10]. Beyond that, routine assessment of nutritional status should be included in hip fracture settings with a high prevalence of malnutrition.

In the present study, protein enrichment enabled older patients to increase protein intake to levels that are 80%

of the recommended intake of at least 1.2 g/kg/day [11]; however, this did not affect immune biomarkers and Trp metabolism during the early postoperative period. This may be because geriatric trauma injury is associated with an additional degree of immune stimulation in the elderly, and even the higher protein intake of our intervention group may have been too low for these patients to improve the immune–inflammatory systems. Furthermore, the nature of the additional exercise stimulus using elastic bands may have been too small to overcome immobilization-induced anabolic resistance. Indeed, patients with high levels of inflammation demonstrate lower ability to increase or preserve muscle mass in response to strength training [12].

We found that despite higher dietary Trp intake in the protein supplemented group no effect on serum Trp levels was observed. Mean Trp intake was 668 (SD 127) mg/day and 508 (SD 183.5) mg/day in the intervention and the control group, respectively, which is well above the recommended dietary intake of 250–425 mg/day [13]. It is thus highly unlikely that the observed lower serum Trp values in our patients are caused by insufficient Trp intake. In fact, the results of this study indicate that lower Trp concentrations in hip-fracture patients cannot be attributed to low dietary intake. However, conditions of immune activation and inflammation may play a role, as Trp levels were found to be associated with IDO activity and neopterin concentrations as well as with the serum level of CRP.

This study has several strengths and limitations. A major strength is the randomized controlled study design, the inclusion of old and very old subjects and the use of an objective and standardized test for assessment of muscle strength and function. Limitations of the study are the relatively small sample size and short follow-up time. A major critical drawback of the study is that the intervention group did not reach the minimal amount of protein recommended for this group, i.e. 1.2 g/kg/day, which means that the differences in protein intake between those who received the protein intervention compared to the control were only marginal. Because of practical constraints, it was not possible to blind our participants or the study assessors for the intervention allocation, which could have influenced our results.

Conclusions

In conclusion, short-term muscle disuse resulting from hip fracture results in substantial immune activation in older individuals. Increasing dietary protein intake during hospitalization providing 1.0–1.2 g protein/kg body weight per day does not alleviate the Th1-type immune response in the elderly patient. Muscularity per se may affect immune activation responses following injury, as neopterin levels were found to be associated with muscle strength during the early postoperative period.

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Author contributions BS and MH designed and carried out the study; GK analysed laboratory data and performed statistical analysis; BS wrote the paper; ML revised the article critically; BS and ML had primary responsibility for final content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. This research did not receive any funding from agencies in the public, commercial, or not-for-profit sectors.

Statement of human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at the Lorenz Boehler Hospital Vienna (3/2015) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The purpose, nature and potential risks of the study were explained to the participants before obtaining their written consent.

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