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Clinical Nutrition Experimental

journal homepage: [http://
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Baseline deficiency of the anti-inflammatory eicosapentaenoic acid in cell membranes worsens lean body mass wasting induced by inactivity

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ARTICLE INFO

Article history:

Received 19 January 2017

Accepted 13 April 2017

Available online 21 April 2017

Keywords:

Baseline arachidonic-to-eicosapentaenoic acid ratio

Bed rest

Lean body mass wasting

SUMMARY

Background & aims: Arachidonic (AA) and eicosapentaenoic (EPA) polyunsaturated fatty acids can play respectively a pro- and an anti-inflammatory role. We hypothesized that, at the end of 5-week experimental bed rest, baseline AA/EPA in red blood cells (RBC) membranes, considered the result of dietary fat intake over the previous month, could influence lean body mass wasting in twenty-six healthy volunteers (age: 23.5 ± 0.5 years; body mass index: 22.9 ± 0.5 kg/m²).

Methods: We measured AA and EPA content in RBC membranes at baseline ambulatory conditions and at the end of the study protocol, to verify the PUFA concentrations stability. We assessed changes, between beginning and end of bed, in lean body mass (bioimpedance), insulin resistance (homeostasis model assessment), systemic inflammation (C-reactive protein) and oxidative stress (thiobarbituric acid reactive substances). Volunteers were divided in two groups according to the AA/EPA ratio median value (i.e. AA/EPA = 44): High AA/EPA group (60 ± 3 ; $n = 13$) and Low AA/EPA group (37 ± 1 ; $n = 13$).

Results: At baseline, all analyzed anthropometrical and biochemical indices were similar in the two groups. Bed rest induced a major decrease in lean body mass in High AA/EPA group ($-5.2 \pm 0.5\%$), when compared to Low AA/EPA group ($-3.7 \pm 0.5\%$; $p = 0.03$; ANOVA). Bed rest mediated-changes of insulin resistance, fat mass, systemic inflammation and oxidative stress, failed to

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<http://dx.doi.org/10.1016/j.clnex.2017.04.002>

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show significant interaction with baseline AA/EPA (ANOVA). In pooled data, baseline AA/EPA ratio and percent lean body mass delta changes showed a significant inverse correlation ($n = 26$; $R = -0.50$; $p < 0.01$).

Conclusions: Results suggest that baseline AA/EPA, in RBC membranes, can independently predict lean body mass wasting in immobilized subjects during long term disuse.

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1. Introduction

Sarcopenia affects quality of life and outcome in elderly subjects and in chronically ill patients [1]. Reliable clinical markers able to predict the level of muscle atrophy progression are presently lacking. Physical inactivity and inflammatory response are key factors inducing muscle wasting in different conditions [2]. Inflammation, in particular, is known to trigger muscle wasting in many acute and chronic diseases, as well as during aging and physical inactivity [3,4]. Onset, progression and amplification of inflammatory response rely on metabolites derived either from the proinflammatory n-6 polyunsaturated fatty acid (PUFA) (arachidonic acid, AA), or from the anti-inflammatory n-3 PUFA (docosahexaenoic acid and eicosapentaenoic acid, EPA) [5]. Therefore, the ratio between n-3 PUFA and n-6 PUFA is considered to influence the inflammatory response, in several clinical conditions [6]. An elevated availability of n-6 PUFA in cell membrane has been shown to correlate to inflammatory diseases [6] and to activation of Nuclear Factor-Kappa B, a transcriptional factor controlling protein degradation, through the proteasome-system [7]. On the other side, EPA is known to efficiently inhibit eicosanoids production, thus reducing inflammation and its consequences [8]. PUFA availability and type depends is diet-dependent [9]. Some dietary fats, such as vegetable oils and margarines, contain elevate fractions of n-6 PUFA, while foods such as fatty fish, fish oil and nuts are sources of n-3 PUFA [9]. Recently it has been suggested that n-3 PUFA can improve the protein anabolism in many physiological and pathological conditions [10]. We hypothesized that skeletal muscle catabolism, induced by inactivity or by other conditions associated with sarcopenia, could also be influenced by the AA/EPA ratio. Experimental bed rest is a suitable model to investigate muscle atrophy progression in controlled conditions [11–13]. We measured the body composition changes (bioimpedance analysis) in healthy volunteers before and after 35 days of experimental bed rest and compared them to the AA/EPA ratios, measured on red blood cells (RBC) membranes. Diet composition of the month before the study protocol, assessed in each subject at enrollment, was maintained throughout the study. Insulin resistance (homeostasis model assessment, HOMA), systemic inflammation (high sensitive C-reactive protein, high-CRP) and oxidative stress (thiobarbituric acid reactive substances, TBARS) were also investigated.

2. Methods

Thirty healthy young male volunteers were enrolled to participate to three separated 35-day bed rest studies, each including 10 participants. The experiments were conducted at the Valdoltra Hospital (University of Primorska, Ankarana-Capodistria, Slovenia) in the periods of July–August 2006, 2007 and 2008. The project was approved by the ethical committee of the University of Ljubljana and the experimental protocol is in accordance to the Declaration of Helsinki and its amendments (2002). A written informed consent was obtained by each volunteer upon enrollment. All subjects were physically active before admission to the hospital, none of them was under medication and their body weight and diets had been stable during the previous month.

2.1. Experimental design

The experimental design is fully described elsewhere [14,15]. Briefly, volunteers were admitted at the hospital one week before the bed rest period to allow a dietary and environmental adaptation (ambulatory period). At this time, baseline standard anthropometric measures (body weight and height) and routine medical examinations were performed. Individual dietary habits, relative to the previous month, were assessed through a suitable food frequency questionnaire, while habitual portion sizes were evaluated by a dietitian through household items and a food picture atlas. At the end of the ambulatory period, each subject underwent 35 days of strict bed rest, a model of experimental immobilization during which all daily activities are performed in clinostatic conditions. All volunteers were under frequent periodical medical control and constant nursing assistance. During both ambulatory and bed rest periods, three main meals (breakfast, lunch and dinner) and three snacks were served. To allow the maintenance, during the experimental study, of a diet equivalent to the habitual one, the dietitian prepared specific menus for each subjects, including condiments and fat food categories. Furthermore, the actual food intake at each meal was monitored by the dietitian. Body composition changes (i.e. fat mass, FM and lean body mass, LBM) were assessed by multifrequency bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) before, during, and after the experimental period. A blood sample (5 mL) was collected in the post-absorptive state before and after bed rest (EDTA Tubes, BD Vacutainer, NJ, USA).

2.2. Biochemical analysis

Red blood cells membrane PUFA composition was measured at baseline ambulatory conditions and at the end of the study protocol, to confirm the expected PUFA concentrations stability from the careful dietary control, as previously published [15]. Briefly, fatty acids were extracted from erythrocytes, after cell lysis, by repeated washing with hypotonic solutions and treatment with chloroform-methanol. Methyl-esterification of fatty acids was obtained in a methanol solution containing H₂SO₄. After neutralization, methyl-esters were extracted and resuspended in hexane. Analysis was carried out by gas-chromatography–flame ionization detection (GC-FID; GC 6850 Agilent Technologies, Santa Clara, CA, USA). AA and EPA contents were expressed as percent ratio between area under-the-curve of each selected methyl-ester peak and the sum of all measured methyl-ester peaks. High-CRP was measured with standard methods by a certified external laboratory (Synlab Italia Srl, Italy). TBARS, as index of lipid peroxidation in plasma, was determined using a commercially available kit (Oxitek, ZeptoMetrix Co., Buffalo, NY, USA) with slight modifications, as previously reported [14].

2.3. Data presentation and statistics

Volunteers were stratified according to the AA/EPA ratio assessed at baseline. Subjects with an AA/EPA ratio above the median value were identified as High AA/EPA group, while subjects with lower values were assigned to the Low AA/EPA group. Data are expressed as median values and interquartile range (IQR) reported in parenthesis. Data were log-transformed where appropriate. The differences between the two groups (High-AA/EPA and Low-AA/EPA) were evaluated through the Mann–Whitney non-parametric test for independent samples. The effect of bed rest on analyzed parameters in the two groups, was assessed by repeated measures ANOVA, with activity (baseline and bed rest) as within-subject factor and groups (Low and High AA/EPA groups) as between-subject factor. Statistical significance of correlation analysis was assessed by Spearman's test. $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS software (version 12; SPSS, Inc., Chicago, IL, USA).

3. Results

All participants concluded the bed rest period, although four were excluded from analysis: Two subjects were excluded for technical problems in the assessment of baseline erythrocyte membrane composition and two because defined as outliers considering their fat mass gain during bed rest (>2 kg). The remaining volunteers (age: 24.0 yrs IQR 23–25; body mass index, BMI: 23.2 kg/m² IQR

21.3–25.1) were stratified according to the AA/EPA ratio assessed at baseline. Subjects with an AA/EPA ratio above the median value (i.e. AA/EPA = 44) were identified as High-AA/EPA group (57, IQR 51–68), while subjects with lower values were assigned to the Low-AA/EPA group (38, IQR 33–41). No significant difference was found in AA/EPA ratio, assessed before and after 35 days of bed rest, within and between subjects ($p > 0.05$). Baseline AA content in the High (18%, IQR 16–18) and Low (17%, IQR 15–18) AA/EPA groups was not significantly different ($p = 0.43$), while baseline EPA content in the High-AA/EPA group (0.32, IQR 0.27–0.36) was significantly lower than in the Low-AA/EPA group (0.47, IQR 0.36–0.52) ($p < 0.015$). Body weight, height and composition (FM and FFM) were not different, at baseline, between High and Low AA/EPA groups (Table 1). A significant bed rest effect in decreasing lean body mass was shown (Table 2). As evidenced by the significant group \times bed rest interaction, bed rest induced a major decrease of lean body mass in the High AA/EPA group when compared to Low AA/EPA group [–5.2% (–6.9 to –4) and –3.9% (–5.3 to –2.8) respectively, $p = 0.03$, non-parametric Mann–Whitney test] (Table 2). Bed rest significantly increased fat mass and HOMA index, however no group \times bed rest interaction was demonstrated. Neither bed rest effect nor group \times bed rest interaction is demonstrated for TBARS or high-CRP plasma concentrations (Table 2). Baseline AA/EPA ratio and percent lean body mass changes, mediated by bed rest, in pooled subjects showed a significant inverse correlation (Fig. 1).

4. Discussion

Markers able to predict muscle atrophy following long term exposure to stressors such as physical inactivity, are lacking. We evaluated 26 volunteers enrolled in three bed rest studies (Valdoltra bed rest 2006, 2007 and 2008) [14–16]. The present work was aimed to investigate in healthy volunteers, the associations between change in lean body mass after 5 weeks of inactivity and the AA/EPA ratio in erythrocyte membranes at baseline. This allowed us to identify a group of subjects with an AA/EPA ratio higher (High AA/EPA) or lower than the median value (Low AA/EPA) than the median value (i.e. AA/EPA = 44). The significant difference of mean AA/EPA values between the two groups reflects variability in previous dietary fat intake. Lean body mass wasting in the High AA/EPA group was markedly and significantly greater when compared to the Low AA/EPA group (Table 2), suggesting that dietary fat intake with a lower n-6 AA/n-3 EPA ratio, before a period of inactivity can worsen atrophy progression in the unloaded muscles. These results are confirmed by the significant linear correlation between baseline AA/EPA ratio, in RBC membranes, and lean body mass wasting, in pooled subject data (Fig. 1). This correlation does not seem to be influenced by other factors potentially related to lean body mass wasting, such as changes in energy balance, insulin resistance [16] and whole body stress response [14]. Our results are in perfect agreement with a previous publication which showed that cancer patients with a lower baseline EPA availability had a greater muscle mass wasting, following chemotherapy treatment [17]. Even though additional studies are needed, our results, and previously published evidences [17], suggest that baseline AA/EPA in RBC membranes can be considered a potential predictive marker of muscle atrophy after a catabolic period. In the present work we can hypothesize that baseline fatty acid membrane composition could affect lean body mass wasting, during inactivity, by a modulation of the inflammatory response, mediated by PUFA families [18]. Inflammation is known to contribute to muscle atrophy [18]. Studies performed in animal models showed

Table 1
Volunteers characteristics.

	Low AA/EPA group	High AA/EPA group	<i>p</i>
Body weight (kg)	73 (70–77)	77 (73–85)	0.17
Height (cm)	183 (175–185)	181 (172–182)	0.20
LMB (kg)	61 (59–64)	64 (61–67)	0.69
FM (kg)	10 (9–12)	12 (11–14)	0.22

LMB, lean body mass; FM, fat mass. Data are expressed as median values and in parenthesis interquartile range. Data were log-transformed where appropriate. Volunteers were stratified accordingly to AA/EPA ratio assessed at baseline. Subjects showing an AA/EPA ratio higher than the median value (i.e. AA/EPA = 44) were identified as High AA/EPA group, while subjects with lower values were assigned to the Low AA/EPA group. The differences between the two groups (High-AA/EPA and Low-AA/EPA) were evaluated through the Mann–Whitney non-parametric test for independent samples.

Table 2

Body composition changes induced by bed rest in low AA/EPA and in high AA/EPA groups.

	Low AA/EPA group		High AA/EPA group		<i>p</i>	
	Baseline	Day 35	Baseline	Day 35	Bed rest effect	Group × bed rest interaction
LMB (kg)	61 (59–64)	59 (58–62)	64 (61–67)	61 (56–64)	<0.001	0.03
FM (kg)	10 (9–12)	11 (8–13)	12 (11–14)	13 (11–15)	0.001	0.16
HOMA index	1.2 (0.8–1.5)	1.3 (1.1–1.7)	1.1 (0.9–1.6)	2.1 (1.3–3.3)	<0.001	0.25
TBARS (μmol/L)	1.7 (1.4–2.4)	1.5 (0.7–5.4)	1.5 (1.0–2.5)	2.0 (1.3–3.2)	0.11	0.65
High-CRP (mg/L)	0.04 (0.02–0.07)	0.03 (0.01–0.08)	0.07 (0.02–0.17)	0.05 (0.01–0.14)	0.60	0.30

LMB, lean body mass; FM, fat mass; HOMA, homeostatic model assessment of insulin resistance index; TBARS, thiobarbituric acid reactive substances; high-CRP, high-C-reactive protein. The table shows values at baseline and after bed rest (Day 33). Data are expressed as median values and in parenthesis interquartile range. Data were log-transformed where appropriate. Volunteers were stratified accordingly to AA/EPA ratio assessed at baseline. Subjects showing an AA/EPA ratio higher than the median value (i.e. AA/EPA = 44) were identified as High AA/EPA group, while subjects with lower values were assigned to the Low AA/EPA group. Statistical analysis was performed by analysis of variance (ANOVA) with activity (baseline and bed rest) as within-subject factor and group (Low and High AA/EPA groups) as between-subject factor.

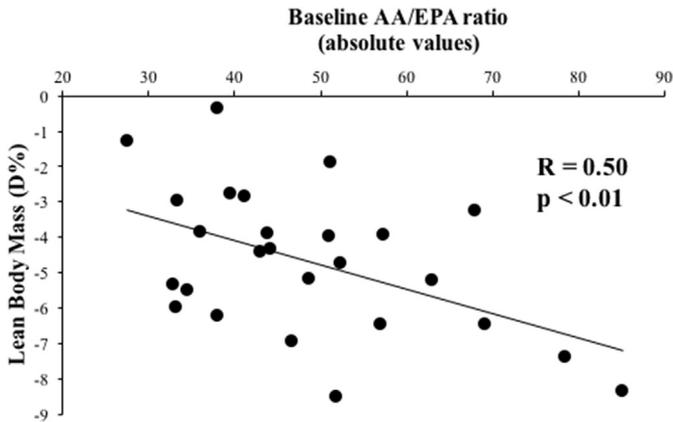


Fig. 1. Correlation between percent lean body mass changes mediated by inactivity and baseline AA/EPA in cell membranes. It shows the significant linear inverse correlation between percent changes of lean body mass induced by 5 weeks of bed rest and absolute values of arachidonic to eicosapentaenoic acid ratio measured at baseline in erythrocyte membranes (baseline AA/EPA). Statistical significance was assessed by Spearman's test for analysis of correlations.

that dietary supplementation with EPA prevented muscle atrophy by inhibition of the ubiquitin-dependent proteolytic pathways [19], while higher AA availability accelerates protein turnover [20]. In conclusion, we demonstrated in humans that baseline AA/EPA can predict the extent in lean body mass wasting after a long term immobilization. We suggest that, lowering AA/EPA by a higher EPA dietary intake, before and during a period of inactivity could blunt lean body mass wasting induced by unloading.

Sources of financial support

The present work was supported by grants from the Italian Space Agency (OSMA project), the Slovenian Minister of Defense and the Regione Friuli Venezia Giulia (LR11).

Contribution of authors

FGDG data analysis and interpretation, drafting the article and revising it critically for important intellectual content, final approval of the version to be submitted; FA data acquisition, analysis and interpretation, drafting the article; SM data acquisition, analysis and interpretation, drafting the

article; RS revising the article critically for important intellectual content, final approval of the version to be submitted; FM final approval of the version to be submitted; PV final approval of the version to be submitted; NF data analysis and interpretation, revising the article critically for important intellectual content, final approval of the version to be submitted; GB conception and design of the study, data analysis and interpretation, revising the article critically for important intellectual content, final approval of the version to be submitted.

Conflict of interest

The authors have nothing to disclose.

Acknowledgments

We thank all the volunteers that participated to the Valdoltra 2006–2007 and 2008 bed rest studies and the excellent assistance of the staff members of the Valdoltra Orthopaedic Hospital (Koper, SLO). We particularly acknowledge prof. Rado Pišot, prof. Igor B. Mekjavic and prof. Mihaela Jurdana for excellent scientific collaboration and precious help in the studies organization. We acknowledge the valuable dietetic assistance of Mrs L. Vouk-Grbac and a special thank should be addressed to the excellent nursing of Mrs Olivera Rakovic Bošnjak. We wish to acknowledge the skilful technical assistance of Mrs Mariella Sturma for sample analyses performance and useful help during metabolic studies. This study was supported by grants from Italian Space Agency (ASI) OSMA 2006–2009.

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