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Original article

# Anabolic resistance assessed by oral stable isotope ingestion following bed rest in young and older adult volunteers: Relationships with changes in muscle mass

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SUMMARY

*Background & aims:* Aging and experimental bed rest are associated with muscle atrophy and resistance to post-prandial stimulation of protein synthesis or anabolic resistance (AR). We have used in young and older adult volunteers, during short-term bed rest, a quick and non-invasive method, based on a single oral bolus of the stable isotope L[ring-$^2H_5$]phenylalanine ($D_5$Phe), to determine post-prandial AR, defined as ratio between irreversible hydroxylation and incorporation into body protein of ingested phenylalanine.

*Methods:* We compared in older (O, 59 ± 1 y) and young (Y, 23 ± 1 y) healthy male volunteers the effects of two-week bed rest on post-prandial protein kinetics, assessed during absorption of a standard ready-to-use oral nutritional supplement, through stable-labeled isotope amino acid $D_5$Phe, diluted in water, given as single oral load. The metabolic fate of $D_5$Phe is either utilization for protein synthesis or irreversible hydroxylation to L[ring-$^2H_4$]tyrosine ($D_4$Tyr). AR was defined as ratio between the areas under the curves of $D_4$Tyr-to-$D_5$Phe plasma concentrations over 6 h meal absorption. To determine the relationships between AR and muscle changes following bed rest, quadriceps muscle volume (QMV) was determined by magnetic resonance imaging (MRI).

*Results:* At baseline, in pooled Y and O subjects, values of AR were inversely correlated with QMV ($R = -0.75$; $p < 0.03$). Following 2-weeks of inactivity, there were significant bed rest effects on AR ($p < 0.01$) and QMV ($p < 0.03$), as well as significant bed rest × group interaction for AR ($p < 0.03$; +9.2% in Y; +21.9% in O) and QMV ($p < 0.05$; −5.7% in Y; −%7.3 in O). In pooled subjects, the percentage delta changes in AR and QMV, induced by bed rest, were inversely correlated ($R = -0.57$; $p < 0.05$).

*Conclusion:* Bed rest-induced AR is much greater in the older than in younger adults. We have developed a new, simple, non-invasive method for the assessment of AR. The results indicate that this metabolic abnormality is a key mechanism for sarcopenia of aging and inactivity.



## 1. Introduction

Several physiological and pathological conditions are characterized by a reduced ability of meal proteins to stimulate post-prandial protein synthesis. This metabolic alteration, defined as

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**Abbreviations**

| | |
|---|---|
| O | older |
| Y | young |
| AR | anabolic resistance |
| $D_5$Phe | L[ring-$^2H_5$]phenylalanine |
| $D_4$Tyr | L[ring-$^2H_4$]tyrosine |
| QMV | quadriceps muscle volume |
| MRI | magnetic resonance imaging |
| AUC | areas under the curves |
| BMI | body mass index |
| FM | fat mass |
| Phe | phenylalanine |
| Tyr | tyrosine |

anabolic resistance, is evaluated through complex and invasive methodology based on intravenous infusion of stable isotopes of amino acids [1–4]. We, and others, have found that anabolic resistance (AR) is the key mechanism leading to muscle atrophy following prolonged physical inactivity [2,3,5]. These observations were obtained using the experimental bed rest model in young volunteers [5].

Evidence in healthy, active older subjects indicates that physiological aging is associated with AR [4]. Furthermore, aging is also characterized by sarcopenia [6]. These results support the latest recommendation for a higher protein requirement in older subjects in order to counteract AR and delay sarcopenia [7]. Nonetheless, the effects of experimental bed rest on the onset of AR in an aged population compared to young controls have never been investigated.

We recently completed a European funded project (PANGeA 2014, ClinicalTrials.gov Identifier: NCT02694471) to compare the response of skeletal muscle mass and function to a two-week period of bed rest in older adults and young men [8]. The results showed that the impact of inactivity on muscle was greater in the older than in the younger subjects. In the present study we propose a rapid and non-invasive method, tested within the frame of the two-weeks of bed-rest PANGeA 2014 study, based on a single oral bolus of the stable isotope L[ring-$^2H_5$]phenylalanine ($D_5$Phe) to determine the AR to dietary protein stimuli for whole body protein synthesis. The metabolic fate of $D_5$Phe is either utilization for protein synthesis or irreversible hydroxylation to L[ring-$^2H_4$]tyrosine ($D_4$Tyr) [9]. In contrast to previous studies, which defined AR through assessment of post-prandial changes in endogenous protein kinetics, our new method defines AR as ratio between irreversible hydroxylation and incorporation into body proteins of ingested phenylalanine. Therefore, we defined AR as ratio between the areas under the curves (AUC) of $D_4$Tyr-to-$D_5$Phe plasma concentrations over 6-h absorption of a standard ready-to-use oral nutritional supplement. To determine the relationships between AR and muscle changes following bed rest, quadriceps muscle volume (QMV) was determined by magnetic resonance imaging (MRI) in all subjects [8].

## 2. Subjects and methods

In the framework of the PANGeA project, eight healthy older adults (age, 59 ± 1 y; body mass index, BMI, 27 ± 1 kg/m$^2$) and seven young healthy adults (age, 23 ± 1 y; BMI 24 ± 1 kg/m$^2$) participated to 14-day experimental bed rest. The study was performed at the Orthopaedic Valdoltra Hospital, University of Primorska (Ankaran-Capodistria, Slovenia). The protocol was approved by the Ethic Committee of the University of Ljubljana (Slovenia), and conformed to the standards set by the Declaration of Helsinki (2002) and its amendments. A written informed consent was obtained from each subject upon enrollment. Subjects were admitted at the hospital 3 days before bed rest in order to undergo basal measurements and get acquainted with the hospital environment (ambulatory period) and remained in the hospital for another 3 days (recovery phase) after the bed rest period. The whole study design is reported elsewhere [8]. Daily dietary intake was planned and monitored by an expert dietitian to maintain each subject in near-neutral eucaloric balance [10] and to provide a standard macronutrient intake, of around 60% carbohydrate, 25% fat, and 15% protein (about 1.0–1.1 g/kg/day). In spite of the careful monitoring of food intake by the dietitian, one of the older subjects was excluded from the data analysis because of a severe negative energy balance (body mass loss of 4.5 kg) developed during the bed rest period, since we have previously shown that a negative energy balance accelerates muscle loss during bed rest [3].

### 2.1. Metabolic test

Before the start (baseline) and at the last day of the bed rest protocol, we performed in each subject a metabolic test aimed at assessing the ratio between irreversible hydroxylation and incorporation into body protein of ingested phenylalanine, applying a new method based on bolus meal and oral stable isotope administration. The day of the study, at 7 AM, after an overnight fast, a polyethylene catheter was inserted into a forearm vein for blood collection. After a blood sampling, at time 0, for baseline data, a liquid oral meal and an oral bolus of $D_5$Phe (0.3 g), dissolved in 150–200 mL of water, were administered to each subject to be consumed in 10 min. The meal was a ready-to-use standard product (Nutricomp®, B.Braun, 500 mL, 500 Kcal, vanilla flavor) composed by 15% protein (i.e., 18.75 g of proteins mainly derived from casein and soy), 30% fat and 55% carbohydrate. We have assessed in post-prandial conditions, changes in plasma $D_5$Phe concentration, which is the net result of $D_5$Phe absorption, utilization for protein synthesis and hydroxylation into $D_4$Tyr (Fig. 1). The ratio between the AUC of both amino acids (i.e., AUC $D_4$Tyr-to-AUC $D_5$Phe ratio) expresses the amount of $D_5$Phe, hydroxilated into $D_4$Tyr and not incorporated into body protein (Fig. 2A and B). Blood was collected in EDTA tubes, immediately centrifuged at 3000 g at 4 °C for 10 min and plasma was immediately stored at −80 °C. In the same days, before the metabolic test, body fat mass has been assessed by bioimpedance analysis (BIA101, Akern, Florence, Italy), following manufacturer instructions, while muscle mass changes were assessed by continuous magnetic resonance images (MRI) (Magnetom Avanto; Siemens Medical Solution, Erlangen, Germany).

#### 2.1.1. Anabolic resistance: analysis and calculations

Isotopic enrichments of plasma $D_5$Phe and $D_4$Tyr, derived by phenylalanine hydroxylation, were determined by gas chromatography–mass spectrometry (GC–MS) (HP 5890; Agilent Technologies, Santa Clara, CA) as t-butyldimethylsilyl derivatives [11]. Plasma concentrations of phenylalanine and tyrosine were assessed in all samples by GC–MS, using the internal standard technique, as previously described [11]. Known amounts of $^{13}$C-phenylalanine and $^2H_2$-tyrosine (Cambridge Isotope Laboratories, Andover, MA) were added as internal standards. Isotopic enrichments were assessed considering the following mass-to-charge ratios (m × z$^{-1}$): phenylalanine m × z$^{-1}$ 234–239; tyrosine m × z$^{-1}$ 466–470. Amino acid concentrations were assessed considering the following mass-to-charge ratios (m × z$^{-1}$): phenylalanine m × z$^{-1}$ 336–337 and tyrosine m × z$^{-1}$ 466–468.

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