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Original Article

Inactivity-mediated insulin resistance is associated with upregulated pro-inflammatory fatty acids in human cell membranes

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SUMMARY

Background & aims: Low-grade systemic inflammation and pro-inflammatory pattern of cell membrane fatty acid composition characterize patients affected by type 2 diabetes and metabolic syndrome. We hypothesize that inactivity-induced insulin resistance could affect levels of pro-inflammatory fatty acids in cell membranes.

Methods: Thirty healthy, male, young volunteers were investigated before and after 35-day experimental bed rest. Diet composition was adapted to previous dietary habits. Fatty acid composition of erythrocyte membranes was analyzed by gas-chromatography using flame ionization detector.

Results: Following bed rest, the HOMA index of insulin resistance significantly increased by $+51 \pm 11\%$ ($P < 0.01$). Bed rest was associated with increased n-6 polyunsaturated ($+4.7 \pm 2.2\%$; $P < 0.01$) and decreased monounsaturated ($-4.8 \pm 1.5\%$; $P < 0.01$) fatty acid content in erythrocyte membranes. Fractional content of arachidonic acid increased by $+14 \pm 12\%$ ($P = 0.01$) following inactivity. Δ5 and Δ9 desaturase indexes, as estimated from product-to-precursor ratios, significantly diminished following bed rest from 9.6 ± 0.4 to 8.4 ± 0.3 ($P < 0.001$) and from 0.72 ± 0.02 to 0.69 ± 0.01 ($P < 0.05$), respectively. The n-3 fatty acids, α-linolenic and eicosapentaenoic, were decreased ($P = 0.05$) following inactivity by $4.7 \pm 13.2\%$ and $3.8 \pm 5.2\%$, respectively.

Conclusions: Inactivity-mediated insulin resistance was associated with altered Δ5 and Δ9 desaturase indexes and with pro-inflammatory fatty acid pattern in erythrocyte membranes. These abnormalities could contribute to the low-grade inflammation associated to inactivity.

1. Introduction

Physical inactivity is a key risk factor for metabolic syndrome and type 2 diabetes.[1] Inactivity leads to insulin resistance[2] and low-grade systemic inflammation[3] which contribute to cardiovascular risk associated with inactive life-style.[4,5] Cross-sectional studies identified strong relationships between insulin resistance, systemic inflammatory response and alteration in fatty acid (FA) composition of cell membrane phospholipids.[6–8] The polyunsaturated FAs (PUFA) of the n-6 and n-3 series, are involved in up-regulation and down-regulation of the inflammatory response, respectively. Their precursors, linoleic and α-linolenic acid respectively, are essential FA since they are not synthesized in humans. The n-9 and n-7 series are derived from the non-essential oleic acid and palmitoleic acid, respectively. FA biosynthesis is promoted by desaturases and elongases.[9] Metabolic pathways of FAs are schematically reported in Fig. 1. In clinical conditions characterized by insulin resistance, such as metabolic syndrome and type 2 diabetes, typical alterations in membrane FAs are observed. The ratios of saturated to polyunsaturated FAs and of n-6 to n-3 PUFAs are increased.[10] Insulin resistance is also associated with decreased activity of selected FA desaturase enzymes. Δ5 desaturase activity was found consistently decreased[11] while Δ6 and Δ9 desaturase activities were decreased in most of the studies.[12] These abnormalities contribute to the activation of systemic inflammatory response associated with insulin resistance.

The physiological effects of inactivity are currently investigated using the experimental model of bed rest in healthy volunteers.[13] The aim of the present study was to determine the effects of 35 days of bed rest on insulin sensitivity and erythrocyte membrane FAs.

Abbreviations: FA, Fatty acid; PUFA, Polyunsaturated fatty acid; FAME, Fatty acid methyl ester.

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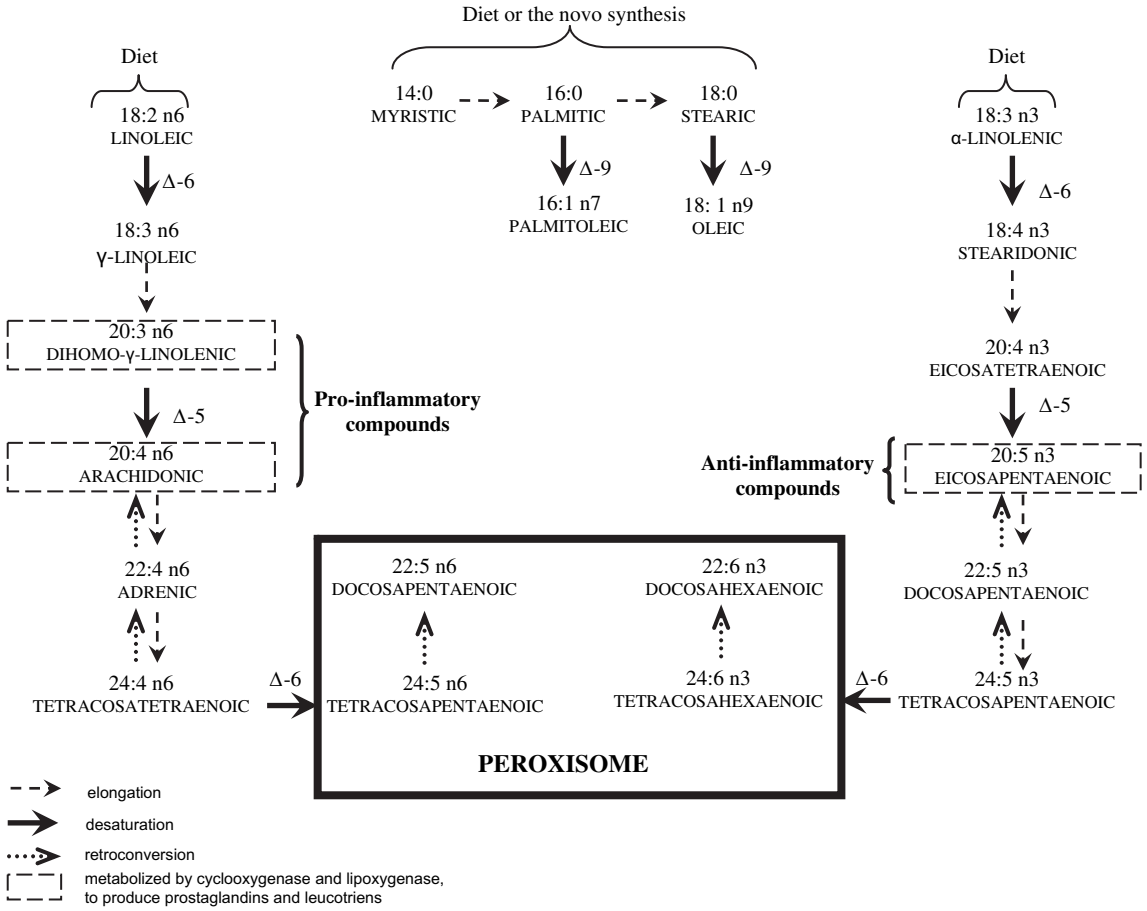

Fig. 1. Fatty acid biosynthesis. Fatty acids are metabolized by desaturases and elongases in the endoplasmic reticulum; only 24:5 n-6 and 24:6 n-3 are β-oxidized into the peroxisome.

2. Material and methods

2.1. Subjects and experimental protocol

Thirty healthy young male subjects (mean ± S.E.M.; age 23.3 ± 0.4 years; body mass index 23.6 ± 0.4 kg m^{-2}) were selected for three separated 35-day bed rest studies performed at the Valdoltra Hospital, University of Primorska, Ankaran-Capodistria, Slovenia, in July–August 2006, 2007 and 2008. The experimental design of the 2006 and 2007 studies have been previously described.[13] The 2008 study followed the 2007 standards. The project was approved by the ethical committee of the University of Ljubljana and the experimental protocol was in accordance to the Declaration of Helsinki (2002). A written informed consent was obtained by each subject upon enrolment. All subjects were physically active before the admission to the Valdoltra Hospital (Slovenia). None of the subjects was under medication and their body weight was stable during the previous month. Preliminary standard anthropometric measures and routine medical screening were performed. Volunteers were admitted at the hospital one week before the bed rest period for dietary and environmental adaptation period (Ambulatory period). At the end of the ambulatory period, each subject underwent 35 days of strict bed rest in which all daily activities were performed in clinostatic conditions. Subjects were under periodical medical control and constant nursing assistance. During the ambulatory and bed rest periods, diet composition and energy intake were daily monitored by a dietician. Individual diet composition reflected previous dietary habits. Individual dietary preferences were collected by appropriate questionnaires received by each subject upon study enrolment and implemented during the ambulatory and bed rest periods in order to maintain individual dietary habits. Subjects received daily 3 main meals (breakfast, lunch and dinner) and 3 snacks. Fat mass and fat-free mass were monitored by multifrequency bioelectrical impedance (Human IM Plus; DS Dietosystem, Milan, Italy). Several research groups were involved in these 35-days bed rest studies and our experiments were scheduled on day 33 of bed rest; other groups worked on days 34 and 35. Blood samples (5 mL) were collected in the postabsorptive state at the end of the ambulatory and bed rest (day 33) periods (EDTA Tubes, BD Vacutainer, NJ, USA). Whole blood was immediately centrifuged and plasma and erythrocyte aliquots were stored at −80 °C until analysis. Plasma glucose was analyzed by standard colorimetric enzymatic assay. Plasma insulin was assayed by standard chemiluminescence approach. FA membrane compositions of red blood cells were analyzed as referenced.[14,15] Organic solvents and buffering salts were purchased from Sigma–Aldrich, Inc, MO, US. Erythrocytes (200 μL) were washed six times with decreasing concentrations (10 mmol/L, 2.5 mmol/L; 1.25 mmol/L; 0.625 mmol/L; 0.312 mmol/L) of phosphate buffered saline (PBS). Total lipid extraction was performed in 5 mL of a chloroform–methanol (2:1) solution, containing 50 mg/L of butylhydroxytoluene (Sigma–Aldrich, Inc, MO, US) as antioxidant, and 1 mL of 1 M NaCl solution. After centrifugation, the lower lipid phase was collected and dried under nitrogen flux at 40 °C. Pellets were redissolved in toluene (500 μL) and, after the addition of 1 mL methanol solution containing 2% of H_2SO_4, samples were