Clinical Nutrition 31 (2012) 1018–1021

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Short communication

The energy balance positively regulates the levels of circulating TNF-related apoptosis inducing ligand in humans

Gianni Biolo [a,d], Paola Secchiero [b,d], Sara De Giorgi [a], Veronica Tisato [b], Giorgio Zauli [c,*]

[a] Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy
[b] Department of Morphology and Embryology and LTTA Centre, University of Ferrara, Ferrara, Italy
[c] Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Via dell'Istria 65/1, 34137 Trieste, Italy

ARTICLE INFO

Article history:
Received 3 February 2012
Accepted 27 April 2012

Keywords:
TRAIL
Energy balance
Bed rest

SUMMARY

Background & aims: Although decreased levels of circulating TRAIL have been associated to cardiovascular risk and overall mortality, the mechanisms controlling TRAIL levels in physiopathological conditions are currently unknown. The aim of the present study was to investigate whether changes in the energy intake and insulin sensitivity may influence circulating TRAIL, and to analyze potential relationships between circulating TRAIL and changes in fat mass in healthy subjects receiving hypocaloric or hypercaloric diets.

Methods: Three distinct groups of participants were studied, at the end of a 14-day ($n = 9$), 35-day ($n = 30$) or 60-day ($n = 16$) period of experimental bed rest to induce insulin resistance and during controlled ambulation, after receiving eucaloric, hypocaloric or hypercaloric diets.

Results: After bed rest conditions, energy restriction significantly decreased circulating TRAIL, while overfeeding significantly increased TRAIL levels with respect to eucaloric control subjects. Moreover, a positive correlation was found between levels of circulating TRAIL and energy intake as well as between circulating TRAIL and energy balance, as determined by changes in fat mass in these subjects.

Conclusions: Circulating levels of TRAIL exhibit a clear-cut positive correlation with the energy intake and balance in healthy subjects during experimental physical inactivity.

1. Introduction

TNF-related apoptosis inducing ligand (TRAIL) is a TNF family member either expressed by several cell types as a type II transmembrane protein or, similarly to other membrane-bound ligands of the TNF superfamily, as a soluble protein, which is detectable in the plasma/serum under physiological conditions.[1] Although the best characterized biological activity of TRAIL, also known as Apo2 ligand, is represented by a potent induction of apoptosis in a variety of cancer cell types, accumulating evidence has shown that TRAIL plays multiple non-apoptotic functions in a variety of tissues, including the vascular system.[1] It is also noteworthy that while the induction of apoptosis in cancer cells usually requires high concentrations of recombinant soluble TRAIL (>10 ng/ml), TRAIL activates intracellular signal transduction pathways involved in cell survival, migration and proliferation in a variety of normal cells at lower concentrations (10–100 pg/ml),[1] comparable to those found physiologically in serum or plasma.[2–5] Although it has been shown that circulating TRAIL levels are inversely related to the risk of mortality in patients affected by cardiovascular disease,[2,3] only few studies have attempted to correlate circulating TRAIL with body adiposity and serum lipid levels.[4,5] In one of these studies,[4] increased levels of TRAIL were associated with greater body fat and LDL cholesterol as well as with diminished lean body mass. It is presently unknown, however, whether changes in energy balance, adipose tissue and skeletal muscle mass may affect the levels of circulating TRAIL.

On these bases, the aim of the present study was to evaluate whether the levels of soluble TRAIL in humans can be modulated in response to specific interventions that affect the energy balance in healthy volunteers, kept for different time periods under overfeeding or energy restriction conditions combined with experimental bed rest to induce changes in muscle mass.

Abbreviations: TRAIL, TNF-related apoptosis inducing ligand.

* Corresponding author. Tel.: +39 0 40 378478.
E-mail address: giorgio.zauli@unife.it (G. Zauli).

[d] The first two authors equally contributed to this work.

0261-5614/$ – see front matter © 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2012.04.016

2. Materials and methods

2.1. Subjects

The study populations comprise three different groups of healthy volunteers who have been previously characterized in studies aimed to investigate the effect of different periods of either calorie restriction or overfeeding coupled to experimental bed rest on the lean and fatty body mass.[6–9] All subjects had a normal body mass index and underwent a series of clinical and biochemical evaluations, which have been previously described.[6–9] Briefly, group 1 was composed by 16 healthy females aged 31 ± 4 years, body mass index 21 ± 2 kg/m^2, who were enrolled at MEDES Clinical Research Facility of the Rangueil University Hospital (Toulouse, France).[6] Group 2 was composed by 30 healthy males aged 23 ± 0.4 years, body mass index 24 ± 0.4 kg/m^2, who were enrolled for the present study at the Valdoltra Hospital, University of Primorska (Ankaran-Capodistria, Slovenia).[7,8] Group 3 was composed by 9 healthy males aged 24 ± 1 years, body mass index 23 ± 1 kg/m^2, who were enrolled for the present study at the Clinical Research Center of the German Aerospace Institute (Cologne, Germany).[9] All subjects were physically active before the study. Each subject signed an informed consent form upon admission. The study was performed in accordance with the Declaration of Helsinki for human studies and relative amendments.

2.2. Experimental design

In order to investigate the potential contribution of body fat, energy balance, muscle mass and insulin sensitivity in regulating the levels of circulating TRAIL, we have used a well-characterized bed rest protocol as a model of experimental muscle atrophy and insulin resistance in humans. In particular, we took advantage of previous studies performed on healthy volunteers, who underwent experimental bed rest for 60 days (group 1, $n = 16$), 35 days (group 2, $n = 30$) and 14 days (group 3, $n = 9$) at different levels of energy intake (calorie restriction, near-neutral energy balance, overfeeding) according to different experimental protocols.[6–9] Energy balance was calculated according to changes in fat mass during the experimental periods. Fat mass was determined by dual-emission X-ray absorptiometry (DXA) in group 1 and 3 and by bioimpedance in group 2. Subjects of group 1 were randomized to complete 60 days of strict bed rest ($n = 8$) or to combine bed rest with daily resistance or aerobic exercise routines in supine position ($n = 8$), energy balance resulted near-neutral and slightly negative in the two sub-groups, respectively.[6] Subjects of group 2 completed 35 days of strict bed rest at different levels of energy intakes.[7] Nineteen out of the 30 subjects of group 2 were randomized to combine strict bed rest with overfeeding ($n = 10$) or with near-adequate energy intake ($n = 9$) according to a parallel design.[8] Subjects of group 3 have been studied 4 times for 14-day periods over 2 years in bed rest or in ambulatory condition in combination with eucaloric or hypocaloric diets using a crossover experimental design. Energy intakes were individually tailored to account for the decrease in requirement during bed rest and then decreased by about 20% during the hypocaloric periods.[9]

2.3. Analyses

Insulin, glucose and leptin concentrations were measured by laboratory analyses as referenced.[6–9] Body fat mass was determined by DEXA or bioimpedance, as previously described.[6–9] Insulin sensitivity was calculated according to the formula of the homeostasis model assessment (HOMA-IR index) method: insulin resistance = fasting plasma insulin (μUI/ml) × fasting plasma glucose (mmol/l)/22.5. The index is highly correlated with the insulin resistance index assessed by the euglycemic-hyperinsulinemic clamp, which is the gold standard of insulin resistance measurement and is widely adopted in clinical studies for individuals with various degree of insulin sensitivity.

Plasma TRAIL was measured in duplicate by using a specific, commercially available ELISA kit, which detect total TRAIL levels (both free and bound to other proteins, such as osteoprotegerin) in accordance with the manufacturer's instructions (R&D Systems, Minneapolis, MN) in frozen (−80 °C) aliquots of plasma, obtained at the time of the original studies.[6–9] It is important to point out that the present study was possible thanks to the good stability of circulating TRAIL in plasma samples correctly frozen −80 °C at over periods of many years.[1] Sensitivity of the assay was 2.86 pg/ml and the intra- and inter-assay coefficients of variation were 3.9% and 6%, respectively.

2.4. Statistics

The study populations comprise 3 different groups who were kept for different time periods at different energy levels under bed rest or controlled physical activity conditions, as previously described.[6–9] In the first analysis, the results obtained from the 3 groups of subjects were pooled together. One observation was obtained from each subject of group 1 and 2 while four observations were obtained from each subjects of group 3. The levels of circulating TRAIL were cumulatively considered in relationship with the data relative to change of fat mass over experimental periods of different durations. Pearson's tests were used to investigate association between variables.

To evaluate the effect of bed rest at different energy intake levels, results were analyzed in group 1 by repeated-measures ANOVA with interactions.[6] In the parallel group design study performed in group 2,[7,8] activity (ambulatory or bed rest) and treatment (higher or lower energy intake) were the within-subject and between-subject factors, respectively. In the crossover design study performed in group 3,[9] activity (ambulatory or bed rest) and treatment (adequate or lower energy intake) were the within-subject factors. Since there was no significant gender or bed rest effects on TRAIL circulating levels, results obtained during the different studies, in ambulatory or bed rest conditions, were pooled together, expressed as means ± SD and analyzed by paired Student *t*-test or Mann Withney test where appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The circulating levels of TRAIL exhibit a significant correlation with changes of fat mass, but not of insulin sensitivity or lean mass, in healthy subjects kept in experimental physical inactivity

In the first analysis, the levels of circulating TRAIL of subjects from group 1, 2 and 3 were cumulatively considered in relation with the data relative to change of fat mass over experimental periods of different durations.[6–9] Pre-bed rest values of TRAIL concentrations were not significantly different in male (104 ± 21 pg/ml) and female (94 ± 19 pg/ml) subjects. As shown in Supplemental Fig. 1, a significant ($n = 82$; $R = 0.34$; $p < 0.001$) positive correlation was noticed between levels of circulating TRAIL at the end of the experimental period and energy balance, as determined by individual changes of fat mass (kg) over the experimental period. Insulin sensitivity significantly decreased (groups 1 and 2) after 35 and 60 days of bed rest (HOMA-IR index: from 1.37 ± 0.61 to