Calorie Restriction Modulates Inactivity-Induced Changes in the Inflammatory Markers C-Reactive Protein and Pentraxin-3

Alessandra Bosutti, Grazia Malaponte, Michela Zanetti, Pietro Castellino, Martina Heer, Gianfranco Guarnieri, and Gianni Biolo

Department of Clinical, Technological, and Morphological Sciences (A.B., M.Z., G.G., G.B.), Division of Internal Medicine, University of Trieste, Trieste 34149, Italy; Departments of Biomedical Sciences (G.M.) and Internal Medicine (P.C.), University of Catania, 95121 Catania, Italy; and Deutsches Zentrum für Luft-und Raumfahrt (DLR)-Institute of Aerospace Medicine (M.H.), 51147 Cologne, Germany

Context: Energy balance and physical activity potentially influence systemic inflammation.

Objective: Our objective was to test the hypothesis that moderate energy restriction may prevent activation of inactivity-induced inflammatory response.

Design: Participants were studied four times at the end of 14-d periods of experimental bed rest or controlled ambulation, after receiving eucaloric or hypocaloric diets.

Setting: The study was conducted at the clinical research center of the German Space Agency.

Subjects: Nine healthy young volunteers participated.

Interventions: Energy intake was calibrated to physical activity and decreased by about 20% in hypocaloric conditions.

Main Outcome Measures: Changes in body fat by dual-energy x-ray absorptiometry as well as plasma inflammatory markers and cytokine mRNA levels in blood cells were measured.

Results: Fat mass did not change significantly in eucaloric conditions and decreased in hypocaloric periods (-1.0 ± 0.3 and -1.0 ± 0.3 kg in ambulatory and bed rest, respectively). Bed rest in eucaloric conditions increased plasma C-reactive protein (CRP) ($+143 \pm 53\%$) and both the ratios between plasma IL-6 and IL-10 (4 ± 1 times) and white blood cell IL-6 and IL-10 mRNAs (5 ± 1 times). Energy restriction prevented bed-rest-mediated increases in CRP and the IL-6 to IL-10 ratio. Bed rest increased ($P = 0.03$) long pentraxin-3 (PTX3) plasma concentration, without significant activity-by-diet interaction. In all conditions ($n = 36$), CRP and PTX3 were inversely correlated ($r = -0.61$; $P < 0.001$). Changes in fat mass, leptin, and IL-6 directly correlated with CRP and inversely correlated with PTX3. IL-10 inversely correlated with CRP and directly correlated with PTX3 ($r = 0.52$; $P < 0.01$).

Conclusions: Calorie restriction prevents the inflammatory response induced by 14 d of bed rest. We suggest an inverse regulation of CRP and PTX3 in response to changes in energy balance. **(*J Clin Endocrinol Metab* 93: 3226–3229, 2008)**

Physical activity and energy balance potentially influence inflammatory response. Exercise training down-regulates C-reactive protein (CRP) and proinflammatory cytokines independently from changes in body composition (1). Sedentary lifestyle is associated with an inflammatory response in population-based studies (2, 3) leading to increased cardiovascular risk (3). Calorie restriction decreases inflammation, whereas energy excess and fat deposition activate the inflammatory response (4). Leptin is

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-1684 Received July 30, 2007. Accepted May 14, 2008.

First Published Online May 20, 2008

Abbreviations: CRP, C-Reactive protein; IFN, interferon; PTX3, pentraxin-3; TGF, transforming growth factor.

a proinflammatory mediator secreted by adipose tissue in response to energy availability and level of physical activity (5, 6). Physical activity and energy balance can modulate inflammation, either directly through cytokine production in skeletal muscle and adipose tissue (1, 5–7) or indirectly through changes in endothelial function and free radical production (8).

In the attempt to discriminate the effect of physical inactivity from that of changes in energy balance, healthy volunteers were studied four times at the end of 14-d periods of experimental bed rest or controlled ambulation while receiving eucaloric or hypocaloric diets (9). Energy balance was defined by fat-mass changes throughout the intervention periods (9). We hypothesized that bed-rest-mediated activation of inflammation would be prevented by moderate energy restriction. Markers of inflammation included pro- and antiinflammatory cytokines as well as two representative acute-phase proteins as CRP and long pentraxin-3 (PTX3). These acute-phase reactants are structurally related (10), but whereas CRP is mainly derived from liver synthesis, circulating PTX3 is released from a variety of tissues, including skeletal muscle, heart, fat, and endothelium (11, 12).

Subjects and Methods

The experimental design has been previously described (9). The study was conformed to the standards set by the Declaration of Helsinki (2002). Briefly, nine healthy male subjects [age, 24 ± 1 (SEM) yr; body mass index, 23 ± 1 kg/m^2] were studied four times during separate 14-d periods in ambulatory-eucaloric, bed-rest-eucaloric, ambulatory-hypocaloric and bed-rest-hypocaloric conditions. During the first phase (July to August 2001), four subjects in ambulatory-eucaloric conditions and five subjects in bed-rest-eucaloric conditions were studies for a period of 14 d. Five months later, during the second phase (February to March 2002), the subject group crossover was performed; the four patients previously studied in ambulatory-eucaloric conditions were studied in bed-rest-eucaloric conditions, and the five subjects studied in bed-rest-eucaloric conditions in the first phase were studied in ambulatory-eucaloric conditions. The third (July to August 2002) and fourth (February to March 2003) phases involved studying subjects in hypocaloric conditions according to the same ambulatory-bed-rest crossover described for the first and second phases. The crossover design randomized potential seasonal effects on inflammatory markers. In bed rest conditions participants were continuously exposed to 6° head-down-tilt bed rest for 14 d. In ambulatory conditions, participants were exposed to a controlled level of physical activity for 14 d (9). In eucaloric conditions, energy intake was 1.4 (ambulatory) or 1.1 (bed rest) times the calculated resting energy expenditure (9). In hypocaloric conditions, energy intake was 1.1 (ambulatory) or 0.9 (bed rest) times the calculated resting energy expenditure. Ten percent of total kilocalories was added to account for dietary-induced thermogenesis. Mean total energy intake (kcal/kg·d) was as follows: ambulatory-eucaloric condition, 36 ± 0.4; bed-rest-eucaloric condition, 28.5 ± 0.3; ambulatory-hypocaloric condition, 28.8 ± 0.4; bed-rest-hypocaloric condition, 23.4 ± 0.4. Dietary fat content was 30% of energy during eucaloric periods. During hypocaloric periods, energy restriction was achieved by decreasing fat intake to a minimum of 60 g/d. Subjects received 1 g protein/kg body weight per day in all study phases. Body composition was measured by dual-energy x-ray absorptiometry at the end of the adaptation period and at the beginning of the recovery period with a Hologic QDR-2000 (Waltham, MA). Blood samples were taken in the fasting state in the morning at the end of each intervention period to determine markers of energy balance and inflammation.

Plasma CRP levels were determined using a high-sensitivity CRP ELISA kit (Diagnostics Biochem, Canada Inc., London, Ontario, Canada). Plasma leptin concentrations were determined by ELISA (catalog item DLP00; R&D Systems, Minneapolis, MN). Plasma insulin concentrations were determined by RIA (Insulin RIA; Adaltis, Montreal, Canada). Plasma levels of IL-6, interferon (IFN)-γ, TNF-α, IL-10, and transforming growth factor (TGF)-β1 were determined using Quantikine ELISA kits (R&D Systems). TGF-β1 was activated from the latent to immunoreactive form detectable by the Quantikine ELISA TGF-β1 immunoassay as follows: 0.1 ml 2.5 N acetic acid/10 M urea was added to 0.1 ml of platelet-poor plasma. After incubation at room temperature, sample solutions were neutralized by adding 0.1 ml 2.7 N NaOH/1 M HEPES. Plasma PTX3 concentrations were quantified by a sandwich ELISA detection system (Alexis Biochemical Italia, Vinci, Italy) as previously described (13). TNF-α, IL-6, IFN-γ, TGF-β, and IL-10 mRNA levels were determined in blood cells by real-time PCR (7900 Sequence Detection System; Applied Biosystems, Foster City, CA) as previously described (14). Cytokine mRNA amounts were expressed as fraction of GAPDH mRNA levels.

All data were expressed as means ± SEM. All data were log-transformed when appropriate and analyzed by repeated-measures ANOVA with activity (ambulatory or bed rest) and diet (eucaloric or hypocaloric) as the two factors. *Post hoc* analysis was performed, when appropriate, by using a *t* test with Bonferroni's adjustment. Regression analysis was performed in individual data obtained from the four experimental periods (n = 36). Statistical analysis was performed with SPSS software (version 12; SPSS Inc., Chicago, IL).

Results

Baseline and postintervention values of lean and fat mass during the ambulatory and bed rest periods with eucaloric and hypocaloric diets have been previously reported (9). During the 14-d intervention periods, fat mass did not change significantly in eucaloric conditions (ambulatory, 0.1 ± 0.3 kg; bed rest, −0.3 ± 0.2 kg), although it was significantly decreased from baseline ($P < 0.001$) in hypocaloric conditions (ambulatory, −1.0 ± 0.3 kg; bed rest, −1.0 ± 0.3 kg). Table 1 shows the effects of bed rest at different energy intake on selected hormones, acute-phase proteins, and cytokines. Bed rest did not change insulin and leptin plasma concentrations during either eucaloric or hypocaloric diets. There was a significant bed rest effect in increasing PTX3 plasma concentration, without significant activity-by-diet interaction. There was a significant effect of hypocaloric diet in decreasing plasma CRP concentration by 143 ± 53%, with significant activity-by-diet interaction. Bed rest significantly increased CRP concentrations in eucaloric conditions. Bed-rest-mediated changes in CRP concentrations were significantly greater ($P = 0.04$) in eucaloric conditions than during hypocaloric diet. There was significant activity-by-diet interaction for plasma concentration of IL-6. Bed-rest-mediated changes in IL-6 concentrations were significantly greater ($P < 0.01$) in eucaloric conditions (299 ± 74%) than during the hypocaloric diet. Significant changes in plasma concentrations of the other cytokines, mediated either by bed rest or hypocaloric nutrition, were not observed. mRNA levels of the antiinflammatory cytokine IL-10 decreased by −51 ± 23% in bed rest and eucaloric diet, although it did not significantly change during bed rest in hypocaloric conditions. There was significant activity-by-diet interaction for IL-10 mRNA levels. Bed-rest-mediated changes in IL-10 mRNA levels were significantly lower ($P = 0.03$) in eucaloric conditions than during hypocaloric diet. Significant changes in mRNA lev-

TABLE 1. Effects of bed rest at different energy intake levels on selected hormones, acute-phase proteins, and cytokines

	Eucaloric diet		Hypocaloric diet		*P*		
	Ambulatory	Bed rest	Ambulatory	Bed rest	Activity effect	Diet effect	Interaction
Plasma concentrations							
Insulin (μU/ml)	6.8 ± 0.6	6.1 ± 0.4	7.3 ± 1.1	6.4 ± 0.8	0.29	0.45	0.76
Leptin (ng/ml)	5.2 ± 1.1	4.1 ± 0.8	5.2 ± 1.4	4.2 ± 1.0	0.17	0.27	0.86
PTX3 (pg/ml)	808 ± 126	934 ± 177	901 ± 178	1139 ± 205	0.03	0.28	0.72
CRP (μg/ml)	1.2 ± 0.4	2.3 ± 0.6[a]	1.1 ± 0.4	0.8 ± 0.2	0.20	0.02	0.03
IL-6 (pg/ml)	3.4 ± 1.0	11.4 ± 3.4[a]	3.4 ± 0.7	1.7 ± 0.3	0.23	0.08	0.01
IFN-γ (pg/ml)	5.1 ± 0.5	6.2 ± 0.5	6.6 ± 0.6	6.8 ± 0.7	0.21	0.19	0.35
TNF-α (pg/ml)	16 ± 2	22 ± 4					
IL-10 (pg/ml)	9.2 ± 0.5	9.5 ± 0.9	9.0 ± 0.5	9.9 ± 1.1	0.75	0.99	0.74
TGF-β (pg/ml)	1143 ± 190	1055 ± 164					
IL-6/IL-10	0.4 ± 0.1	1.4 ± 0.5[a]	0.4 ± 0.1	0.2 ± 0.1	0.33	0.08	0.01
White blood cell mRNA							
IL-6	4.9 ± 0.8	5.2 ± 1.3	6.3 ± 1.3	6.8 ± 1.8	0.63	0.59	0.81
IFN-γ	143 ± 28	188 ± 52	75 ± 21	173 ± 49	0.21	0.09	0.12
TNF-α	0.6 ± 0.1	0.9 ± 0.4	1.0 ± 0.2	0.8 ± 0.2	0.37	0.08	0.47
IL-10	0.72 ± 0.20	0.24 ± 0.08[a]	0.47 ± 0.09	0.50 ± 0.13	0.02	0.45	0.01
TGF-β	111 ± 22	67 ± 9	66 ± 31	51 ± 24	0.16	0.03	0.84
IL-6/IL-10	9 ± 2	44 ± 15[a]	16 ± 3	20 ± 5	0.06	0.64	0.02

For *P* values, data were analyzed with two-factor (activity × diet) ANOVA with interaction.

[a] Significantly different from ambulatory condition at $P < 0.025$ based on Bonferroni's *post hoc* analysis to assess the effects of bed rest either in eucaloric or hypocaloric diets.

els of the other three cytokines, mediated by either bed rest or hypocaloric nutrition, were not observed. There was significant activity-by-diet interaction for the ratios between IL-6 and IL-10. Bed rest in eucaloric conditions increased both the ratios between IL-6 and IL-10 plasma concentrations by 4 ± 1 times ($P < 0.01$) and between IL-6 and IL-10 white blood cell mRNAs by 5 ± 1 times ($P < 0.001$). Energy restriction prevented bed-rest-mediated increases in IL-6/IL-10 concentrations and mRNAs.

Regression analysis was performed in individual data obtained from the four experimental periods (n = 36) to define determinants of hormone and inflammatory marker variability (Table 2). Plasma insulin and leptin concentrations were directly correlated with fat mass, energy intake, and CRP concentration. In addition, leptin correlated directly with energy balance, as expressed by changes in fat mass over the intervention periods, and indirectly with PTX3 and IL-10. The best regression model predicting plasma leptin variability (multiple r = 0.86; $P < 0.0001$) included fat mass (coefficient ± SEM, 0.00046 ± 0.00006) and energy intake (coefficient ± SEM, 0.00328 ± 0.00149). CRP and PTX3 were inversely correlated. CRP directly correlated with fat mass and energy balance as well as with insulin, leptin, and IL-6 levels, whereas PTX3 inversely correlated with the same parameters (statistical significance was not achieved with energy intake and insulin). Plasma IL-10 concentrations directly correlated with PTX3 and inversely correlated with CRP. Multiple regression analysis indicated that only leptin concentrations independently predicted CRP concentrations ($P < 0.001$). In contrast, the best model predicting variability of PTX3 (multiple r = 0.59; $P = 0.001$) included changes in fat mass (coefficient ± SEM, −0.19 ± 0.08) and IL-10 (coefficient ± SEM, 95 ± 33).

TABLE 2. Correlations between selected variables at different levels of activity and energy intake

Variable	Insulin	Leptin	CRP	PTX3
Fat mass (g)	0.59[c]	0.78[c]	0.62[c]	−0.35[a]
Changes in fat mass (g/14 d)	0.31	0.40[a]	0.51[b]	−0.43[b]
Energy intake (kcal/d)	0.51[b]	0.60[c]	0.40[a]	−0.24
Insulin (μU/ml)		0.51[b]	0.45[b]	−0.14
Leptin (ng/ml)	0.51[b]		0.63[c]	−0.53[c]
CRP (μg/ml)	0.45[b]	0.63[c]		−0.61[c]
PTX3 (pg/ml)	−0.14	−0.53[c]	−0.61[c]	
IL-6 (pg/ml)	0.19	0.31	0.49[b]	−0.38[a]
IFN-γ (pg/ml)	0.11	0.12	0.06	0.22
IL-10 (pg/ml)	−0.17	−0.35[a]	−0.37[a]	0.52[b]

Correlation analysis of individual data from the four experimental periods (n = 36): ambulatory-eucaloric diet, bed-rest-eucaloric diet, ambulatory-hypocaloric diet, and bed-rest-hypocaloric diet. Data are Pearson's r correlation coefficients. Significant correlation between variables is indicated by superscript letters.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Discussion

The most important finding in this study was that 2-wk bed rest at neutral energy balance activated proinflammatory cascade, as shown by increases in plasma CRP and IL-6 and decreases in mRNA transcript of antiinflammatory cytokine IL-10. The IL-6 to IL-10 ratios in plasma and mRNA transcripts increased to a similar extent after bed rest, suggesting an imbalance in the expression of pro- and antiinflammatory cytokines. The proinflammatory effect of bed rest was completely abolished by moderate energy restriction leading to about 5% loss of fat mass. This is in agreement with previous evidence showing antiinflammatory effects of negative energy balance (4). Leptin variability throughout the four experimental phases (in response to changes in activity, fat mass, and energy intake and balance) indepen-

dently predicted individual CRP levels, supporting the concept that this adipose tissue hormone is a link between energy metabolism and inflammation (6, 15). We have studied a selected population of healthy, young male volunteers, but bed rest effects on inflammatory response could also be enhanced or downregulated by genetic or environmental factors, such as cytokine polymorphisms, gender, age, or disease states (14, 16, 17). Future studies are needed to determine whether less extreme reductions in physical activity, such as reducing daily step numbers (18), would also lead to increases in inflammatory markers as shown here in the more extreme condition of bed rest.

PTX3 is an acute-phase reactant structurally related but distinct from CRP (10). PTX3 is either associated with increased systemic inflammation or involved in termination of acute inflammatory response (10, 19). In the present study, bed rest significantly increased PTX3 plasma concentration, without significant activity-by-diet interaction. Furthermore, PTX3 inversely correlated with fat mass, energy balance, and leptin, suggesting that, in addition to inactivity, nutritional factors may play a role in regulation of this acute-phase reactant. Our data show that nutrient intake and decreases in body fat affect plasma levels of CRP and PTX3 in opposite directions. An inverse relationship between PTX3 and body fat decreases represents a novel finding. PTX3 is highly expressed in adipose tissue and is modulated by cytokines with paracrine mechanisms (11, 12). In our study, IL-10 levels were independent determinants of PTX3 variability. We may therefore speculate that the association between negative energy balance and increases in PTX3 levels could be mediated by increases in expression of antiinflammatory cytokines, like IL-10, in circulating cells or in adipose tissue (20).

In conclusion, the results of our study are relevant in understanding the interactions between physical inactivity and energy balance in the pathogenesis and progression of diseases characterized by low-grade inflammation, such as diabetes and cardiovascular diseases (3). Novel insight was also provided into the inverse regulation of the acute-phase reactants PTX3 and CRP by energy balance.

Acknowledgments

We thank the volunteers who gave time and effort to ensure the success of this project. We acknowledge the excellent technical assistance of Mrs. Anna De Santis and Mrs. Mariella Sturma. We also thank all scientific and technical staff of the Clinical Research Center at the DLR-German Aerospace Institute, Cologne, Germany. We acknowledge the invaluable support of Dr. B. Elmann-Larsen (Life Science Unit, European Space Research and Technology Centre (ESTEC).

Address all correspondence and requests for reprints to: Gianni Biolo, Department of Clinical, Technological, and Morphological Sciences, University of Trieste, Clinica Medica, Ospedale di Cattinara, Strada di Fiume 447, Trieste 34149, Italy. E-mail: biolo@units.it.

This work was supported by grants from the Italian Space Agency (ASI) and the Italian Ministry of Education, University, and Research (MIUR) (PRIN 2001). The general experimental design was supported by grants from the European Space Agency (ESA) and from the German Space Agency (DLR).

References

1. **Petersen AM, Pedersen BK** 2005 The anti-inflammatory effect of exercise. J Appl Physiol 98:1154–1162
2. **Hjelstuen A, Anderssen SA, Holme I, Seljeflot I, Klemsdal TO** 2006 Markers of inflammation are inversely related to physical activity and fitness in sedentary men with treated hypertension. Am J Hypertens 19:669–675
3. **Mora S, Lee IM, Buring JE, Ridker PM** 2006 Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. JAMA 295:1412–1419
4. **Holloszy JO, Fontana L** 2007 Caloric restriction in humans. Exp Gerontol 42:709–712
5. **Blanc S, Normand S, Pachiaudi C, Duvareille M, Gharib C** 2000 Leptin responses to physical inactivity induced by simulated weightlessness. Am J Physiol Regul Integr Comp Physiol 279:R891–R898
6. **Kershaw EE, Flier JS** 2004 Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556
7. **Pedersen BK, Fischer CP** 2007 Physiological roles of muscle-derived interleukin-6 in response to exercise. Curr Opin Clin Nutr Metab Care 10:265–271
8. **Taddei S, Galetta F, Virdis A, Ghiadoni L, Salvetti G, Franzoni F, Giusti C, Salvetti A** 2000 Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. Circulation 101:2896–2901
9. **Biolo G, Ciocchi B, Stulle M, Bosutti A, Barazzoni R, Zanetti M, Antonione R, Lebenstedt M, Platen P, Heer M, Guarnieri G** 2007 Calorie restriction accelerates the catabolism of lean body mass during 2 wk of bed rest. Am J Clin Nutr 86:366–372
10. **Mantovani A, Garlanda C, Doni A, Bottazzi B** 2008 Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. J Clin Immunol 28:1–13
11. **Abderrahim-Ferkoune A, Bezy O, Chiellini C, Maffei M, Grimaldi P, Bonino F, Moustaid-Moussa N, Pasqualini F, Mantovani A, Ailhaud G, Amri EZ** 2003 Characterization of the long pentraxin PTX3 as a TNFα-induced secreted protein of adipose cells. J Lipid Res 44:994–1000
12. **Introna M, Alles VV, Castellano M, Picardi G, De Gioia L, Bottazzai B, Peri G, Breviario F, Salmona M, De Gregorio L, Dragani TA, Srinivasan N, Blundell TL, Hamilton TA, Mantovani A** 1996 Cloning of mouse PTX3, a new member of the pentraxin gene family expressed at extrahepatic sites. Blood 87:1862–1872
13. **Bevelacqua V, Libra M, Mazzarino MC, Gangemi P, Nicotra G, Curatolo S, Massimino D, Plumari A, Merito P, Valente G, Stivala F, La Greca S, Malaponte G** 2006 Long pentraxin 3: a marker of inflammation in untreated psoriatic patients. Int J Mol Med 18:415–423
14. **Biolo G, Amoroso A, Savoldi S, Bosutti A, Martone M, Pirulli D, Bianco F, Ulivi S, Bertok S, Artero M, Barazzoni R, Zanetti M, Grassi G, Guarnieri G, Panzetta G** 2006 Association of interferon-γ +874A polymorphism with reduced long-term inflammatory response in haemodialysis patients. Nephrol Dial Transplant 21:1317–1322
15. **Somech R, Reif S, Golander A, Spirer Z** 2007 Leptin and C-reactive protein levels correlate during minor infection in children. Isr Med Assoc J 9:76–78
16. **El Yousfi M, Mercier S, Breuillé D, Denis P, Papet I, Mirand PP, Obled C** 2005 The inflammatory response to vaccination is altered in the elderly. Mech Ageing Dev 126:874–881
17. **van Eijk LT, Dorresteijn MJ, Smits P, van der Hoeven JG, Netea MG, Pickkers P** 2007 Gender differences in the innate immune response and vascular reactivity following the administration of endotoxin to human volunteers. Crit Care Med 35:1610–1612
18. **Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK** 2008 Metabolic responses to reduced daily steps in healthy nonexercising men. JAMA 299:1261–1263
19. **He X, Han B, Liu M** 2007 Long pentraxin PTX3 in pulmonary infection and acute lung injury. Am J Physiol Lung Cell Mol Physiol 292:L1039–L1049
20. **Skurk T, Alberti-Huber C, Herder C, Hauner H** 2007 Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92:1023–1033