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Original Article

Gender-Based Differences in Leptinemia in Healthy Aging, Non-obese Individuals Associate with Increased Marker of Oxidative Stress

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Abstract: Aging associates with an increased pro-inflammatory activity that is commonly associated with oxidative tissue damage. One pro-inflammatory molecule that facilitates oxidative stress is leptin, a hormone that regulates metabolism, endocrine and immune functions. To address whether leptin levels correlated with oxidative stress in aging, we performed cross-sectional measurements of circulating plasma leptin in groups of healthy individuals that were divided by age and gender. It was found that leptin levels were comparable between young (N=54) and elderly humans (N=56) in each gender, and women had higher levels than men, irrespectively of age. Interestingly, oxidative stress measured as total glutathione levels correlated positively with elevated leptin levels in elderly women but not in men or in the groups of younger individuals. The data suggest that a gender bias for leptin that is maintained with age can associate with increased propensity to oxidative stress in the elderly.

Key Words: Leptin, aging, gender, oxidative stress

Introduction

Aging is associated with an increased oxidative stress and a subsequent predisposition to chronic inflammation [1]. These processes concur in the elderly and are interrelated through several factors and mediators that have a pro-inflammatory activity [2-3].

Oxidative stress is a complex phenomenon that involves the contribution of several factors and is caused by an insufficient ability of the host to neutralize reactive intermediates that derive from the production of reactive oxygen species. Oxidative stress is often manifested by a decreased reducing capacity of the cellular redox couples such as glutathione - a peptide present in reduced (GSH) and oxidized (GSSG) states [4]. The anti-oxidant capacity of glutathione against free radicals is due to its activity of electron donor. In the reduced GSH state, the thiol group of its cysteine donates a reducing equivalent to reactive oxygen species, becoming reactive with another glutathione to form glutathione disulfide (GSSG). More than 90% of the total glutathione pool is present in its reduced form, since the enzyme that reverts it from its oxidized form (glutathione reductase) is constitutively active and inducible upon oxidative stress [5].

One molecule that can promote the secretion of oxygen radicals (such as hydrogen peroxide, H_2O_2, and superoxide, O_2^-) through direct and indirect mechanisms is leptin [6]. Leptin is a hormone that controls metabolism and endocrine functions but also has strong pro-inflammatory effects on many cell and tissue targets [7]. Since in the elderly the caloric intake is often reduced and a pro-inflammatory background is present, one would predict that the levels of leptin might increase with age.

We report here that the concentration of plasma leptin is not influenced by aging in healthy, non obese individuals, and a gender bias is observed for women, irrespectively of

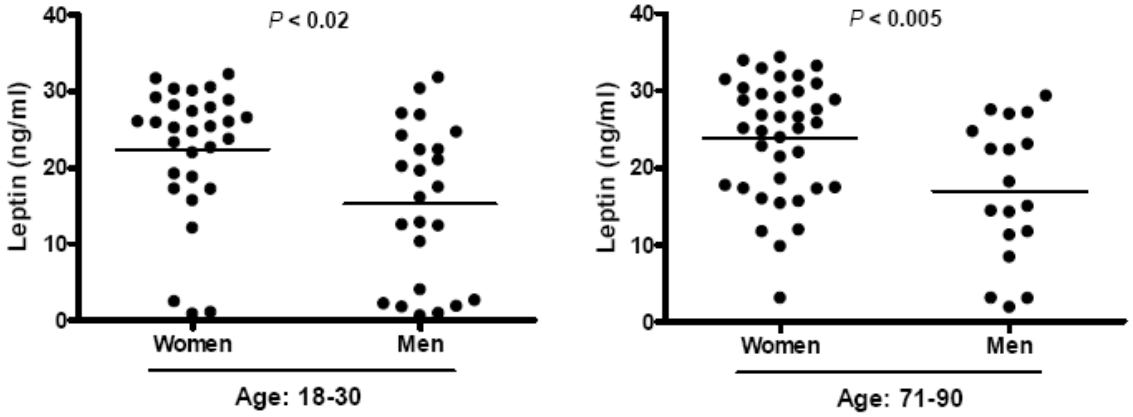

Figure 1. Circulating levels of leptin in young and elderly healthy, non-obese humans. Means and P are shown. Leptin measurements were done by ELISA, as described in the Materials ad Methods.

age. More important, plasma leptin and total glutathione positively correlate in elderly women. Altogether, the data show a novel correlation between leptin and oxidative stress in relation to aging.

Materials and methods

Subjects

One hundred and ten healthy, non obese volunteer subjects between 18 and 90 years were enrolled in the study. Subjects with health disorders known or suspected to influence the level of inflammatory parameters in the blood were excluded. Informed consent was obtained from each subject included in the study, which was conducted according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board. The subjects were divided into groups according to age (N=54 between 18 and 30 years of age; N=56 between 71 and 90 years) and gender (N=68 women; N=42 men). Blood samples were drawn between 7 and 10 a.m. after an overnight fast. None of the included individuals suffered from acute illness prior to the collection of blood samples.

Dosage of leptin and glutathion

Plasma from blood centrifuged for 15 minutes at 1,000 x g within 30 minutes of collection was stored at -80°C until analysis. Concentration of leptin was measured using the commercially available enzyme-linked immunosorbent assay (ELISA) Human Leptin Quantikine kit (R&D Systems, Minneapolis, USA), following the procedures described elsewhere [8]. The minimum detectable dose of leptin was 7.8 pg/ml, and the experimental values were expressed in ng/ml. Total glutathione was measured by a fluorescence method using the commercially available Glutathione Assay kit from BioVision (Mountain View, CA, USA), according to the manufacturer's instructions. All samples and standards were run as duplicates and the mean of duplicates was used in the statistical analyses.

Statistics

Statistical calculations using the Student's *t* test were performed with the program Prizm 5 from GraphPad® software (La Jolla, CA, USA). Data are presented as means + SD. P values less than 0.05 were considered significant.

Results and Discussion

To address possible age-related changes in leptin concentration in physiological conditions, we compared healthy, non obese subjects of different age (18 to 30 versus 71 to 90 years) that had been separated into groups by gender. We found that women had higher levels of plasma leptin levels than men, and this difference was present in both the group of 18-to-30 and 71-to-90 year old

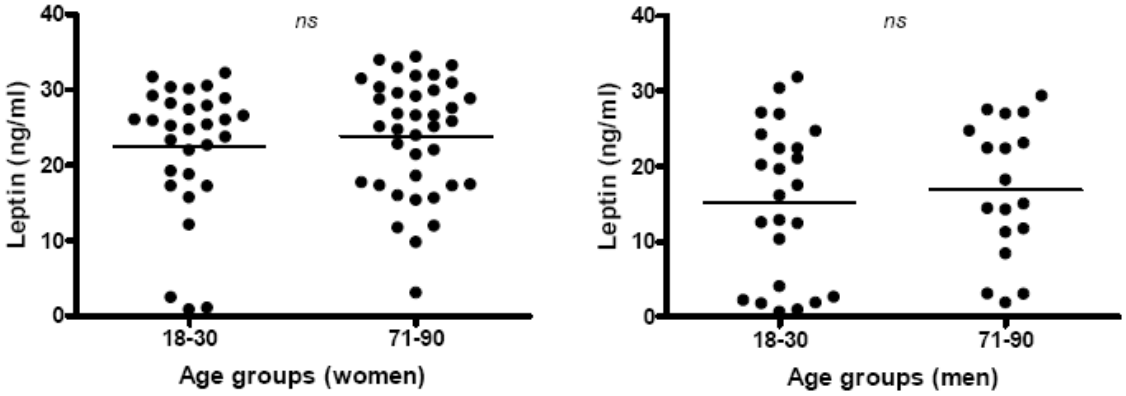

Figure 2. Levels of plasma leptin in same-gender individuals of different age groups. ns = not significant.

individuals (**Figure 1**). Moreover, the gender-based difference was slightly more pronounced in the elderly ($P<0.02$ in the younger group versus $P<0.005$ in the elderly, **Figure 1**).

Next, to address whether leptin concentration was affected by aging, we compared same-gender individuals of 18-30 years of age versus individuals of 71-90 years of age. No statistically significant difference was observed, indicating a lack of age-related changes in plasma levels of leptin (**Figure 2**). While this finding was confirmatory of similar observations by others [9-10], it was at the same time of interest for the fact that aging associates with an increase of insulin resistance, a decrease of glucose uptake, and impaired fatty acid re-esterification [11] - and these aspects are all influenced by leptin [12]. However, although the similar levels of expression of leptin in young and elderly suggested a similar production of this molecule in the two groups, it remains open the possibility that differences in sensitivity to leptin may occur between young and adults [13-14]. Under similar considerations, we cannot exclude that at a tissue level (e.g. lymph nodes and bone marrow) the concentration of local leptin could differ between young and adults, due to changes in mass and distribution of fat that occur during the aging process in the bone marrow and peri-lymph node areas [15]. If so, this aspect could influence locally the oxidative stress and immune function [16].

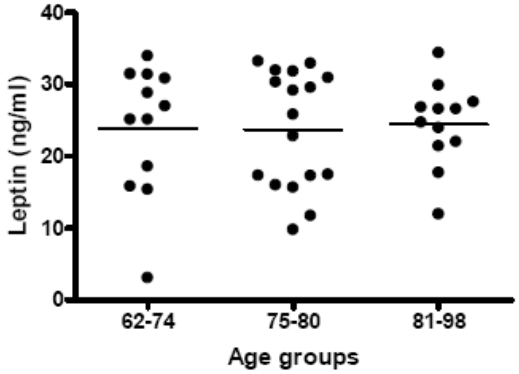

Figure 3. Circulating levels of leptin in elderly women of different age groups. Not significant for all comparisons.

Finally, we acknowledge that the elderly population in our study was biased due to the fact that biological changes associated with aging had not been associated with concurring pathological processes. This choice was dictated by a selection on biological criteria representing as much as possible a "physiological" aging process (in the absence of medical disorders). While our approach reproduced the continuum of changes that occur in aging in the absence of pathology, it selected at the meantime individuals that were somehow exceptional in that their health status in the elderly might or might not be achieved by the people included in the younger group – which was therefore more heterogeneous in that respect. Moreover, we reasoned that in the elderly the onset of

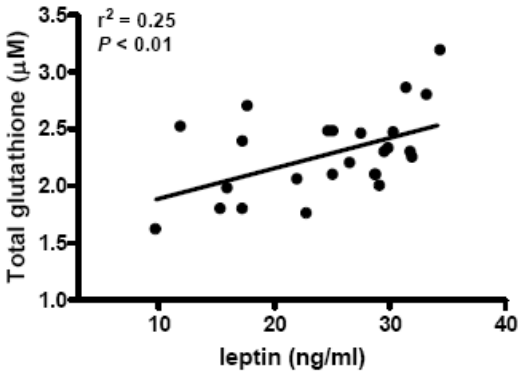

Figure 4. Correlation between leptin and glutathione in the elderly women. High plasma levels of leptin positively correlated in univariate analyses with total glutathione levels in the elderly women.

clinical manifestations of disease may become apparent after years of subclinical development over decades. Therefore, to address whether the changes in the levels of leptin would be present at certain ages and/or would be masked following an aggregation into larger groups, we fractionated the elderly group into three smaller groups of similar ages. **Figure 3** shows that similar leptin concentrations were present in elderly women of 64-to-74, 75-to-80, and 81-to-90 years. Analogous findings were obtained in the elderly men, where again no differences of plasma leptin levels were found upon subdivision into smaller groups of 64-to-74, 75-to-80, and 81-to-90 years of age (not shown).

The above findings suggested the maintenance of a gender bias in leptinemia in aging, and a lack of significant changes in the levels of expression within the same gender (although elderly women had significantly higher levels of leptin compared to elderly men, **Figure 1**).

The possibility remained that the levels of pro-inflammatory leptin could correlate with oxidative stress in aging. To address this, the levels of plasma leptin against total glutathione were analyzed in univariate regression analysis. It was found that the levels of plasma leptin significantly associated with total glutathione in the group of elderly women (**Figure 4**), although they did not in the other three groups (young men and women, and elderly men). One consequence of these results would be that increased glutathione levels in elderly women could favor inflammation, and this could in turn lead to accelerated tissue senescence [17] - which is a general characteristic feature of human aging. On the other hand, while these events would take place, it is likely that other inflammatory as well as anti-inflammatory mediators resulting from the activation of the inflammatory cascade might enter into action to adjust the system towards an internal equilibrium. Alternatively, the association between oxidative stress might just as well go the other way around, e.g. the increased inflammatory activity in aging could subsequently lead to or sustain oxidative stress and, additionally, other parameters and factors could become involved (diet, environment, genetic background etc.). Thus, while suggestive of an association of pro-inflammatory leptin with the presence of oxidative stress in aging, these studies prompt at further testing, e.g. in animal models, to address the nature and mechanistic implications of the relationship between a key molecule in the control of metabolism, leptin, and the development/maintenance of chronic inflammation in the elderly.

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References

[1] Ji LL. Exercise at old age: does it increase or alleviate oxidative stress? Ann N Y Acad Sci 2001; 928: 236-47.

[2] Labinskyy N, Csiszar A, Veress G, Stef G, Pacher P, Oroszi G, Wu J, and Ungvari Z. Vascular dysfunction in aging: potential effects of resveratrol, an anti-inflammatory phytoestrogen. Curr Med Chem 2006; 13: 989-996.

[3] Dinarello CA. Histone deacetylase inhibitors as

a treatment option for inflammatory disease. Int J Clin Exp Med 2008; In press
[4] Schafer FQ and Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001; 30: 1191–1212.
[5] Stadtman ER. Role of oxidant species in aging. Curr Med Chem 2004; 11: 1105-1112.
[6] La Cava A and Matarese G. The weight of leptin in immunity. Nat Rev Immunol 2004; 4: 371-379.
[7] Iikuni N, Lam QL, Lu L, Matarese G, and La Cava A. Leptin and inflammation. Curr Immunol Rev. In press
[8] De De Rosa V, Procaccini C, Calì G, Pirozzi G, Fontana S, Zappacosta S, La Cava A, and Matarese G. A key role of leptin in the control of regulatory T cell proliferation. Immunity 2007; 26: 241-255.
[9] Bruunsgaard H, Pedersen AN, Schroll M, Skinhøj, and Pedersen BK. TNF-α, leptin and lymphocyte function in human aging. Life Sci. 2000; 67: 2721-2731.
[10] Carraro R and Ruiz-Torres A. Relationship of serum leptin concentration with age, gender, and biomedical parameters in healthy, non-obese subjects. Arch Gerontol Geriatr 2006; 43: 301-312.
[11] Yu YH and Zhu H. Chronological changes in metabolism and functions of cultured adipocytes: a hypothesis for cell aging in mature adipocytes. Am J Physiol Endocrinol Metab. 2004; 286: E402-410.
[12] Matarese G, Mantzoros C, and La Cava A. Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. Curr Pharm Des 2007; 13: 3676-3680.
[13] Wang ZW, Pan WT, Lee Y, Kakuma T, Zhou YT, Unger RH. The role of leptin resistance in the lipid abnormalities of aging. FASEB J 2001; 15: 108-114.
[14] Ma XH, Muzumdar R, Yang XM, Gabriely I, Berger R, Barzilai N. Aging is associated with resistance to effects of leptin on fat distribution and insulin action. J Gerontol A Biol Sci Med Sci. 2002; 57: B225-231.
[15] Qiu J, Ogus S, Lu R, Chehab FF. Transgenic mice overexpressing leptin accumulate adipose mass at an older, but not younger, age. Endocrinology 2001; 142: 348-358.
[16] Atzmon G, Yang XM, Muzumdar R, Ma XH, Gabriely I, Barzilai N. Differential gene expression between visceral and subcutaneous fat depots. Horm Metab Res 2002; 34: 622-628.
[17] Ashfaq S, Abramson JL, Jones DP, Rhodes SD, Weintraub WS, Hooper WC, Vaccarino V, Alexander RW, Harrison DG, and Quyyumi AA. Endothelial function and aminothiol biomarkers of oxidative stress in healthy adults. Hypertension 2008; 52: 80-85.