Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

Journal of Nutritional Biochemistry 15 (2004) 757–760

Journal of Nutritional Biochemistry

Relationship between plasma leptin and zinc levels and the effect of insulin and oxidative stress on leptin levels in obese diabetic patients

Dildar Konukoglu[a,*], Mehtap Sultan Turhan[a], Meltem Ercan[b], Ozden Serin[c]

[a]Cerrahpasa Medical Faculty, Department of Biochemistry, Istanbul University, Istanbul, Turkey

[b]Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul University, Istanbul, Turkey

[c]Department of Biochemistry, Taksim Education Hospital, Taksim, Turkey

Received 2 September 2003; received in revised form 25 June 2004; accepted 15 July 2004

Abstract

Leptin is thought to be a lipostatic signal that contributes to body weight regulation. Zinc plays an important role in appetite regulation also. Our aim is to evaluate the relationship between leptin and zinc in obese and nonobese type 2 diabetic patients and its relationship with oxidative stress and insulin. We studied 25 nonobese nondiabetic women (controls); 35 nonobese diabetic women; and 45 obese diabetic women. Plasma leptin concentration was determined by immunoradiometric assay. Thiobarbituric acid reactive substances (TBARS), markers of oxidative stress, were assayed by the spectrofotometric method. Plasma levels of zinc and insulin were measured by atomic absorption spectrophotometer and electrochemiluminescence methods, respectively. We found that nonobese diabetic patients had significantly lower zinc and higher TBARS levels than control subjects ($P<0.01$). There was no difference in plasma leptin levels between nonobese diabetic subjects and controls. Obese diabetic subjects had significantly higher plasma leptin, TBARS, and insulin levels and significantly lower plasma zinc levels than nonobese diabetic subjects (for each comparison; $P<0.01$). The univariate and multivariate analyses demonstrated a significant positive correlation between leptin and body mass index ($P<0.01$) and insulin ($P<0.01$), and a significant negative correlation between leptin and zinc in obese subjects. Additionally, TBARS levels was positive correlated with insulin and negative correlated with zinc in obese diabetic subjects. We conclude that zinc may be a mediator of the effects of leptin, although the detailed mechanism is still unknown and requires further investigation. Free radical induced mechanism(s) may be involved in this process.

Keywords: Leptin; Obesity; Zinc; Diabetes

1. Introduction

Obesity and associated diabetes are epidemic throughout the world. Obese individuals characteristically manifest insulin resistance and hyperinsulinemia, which predispose to glucose intolerance, diabetes, and cardiovascular disease [1]. A major advance in understanding the hormonal causation of morbid obesity is the discovery of the hormone leptin. Leptin, an *ob* gene product, is thought to be a lipostatic signal that contributes to body weight regulation through modulation feeding behavior or energy expenditure or both [2]. It has been suggested that administration of leptin increases energy expenditure and decreases appetite by decreasing hypothalamic levels of orexigenic neurotransmitters [3]. Although leptin secreted in proportion to the amount of body mass, a variety of hormonal factors such as insulin can also influence circulating leptin levels [4].

Zinc plays an important role in appetite regulation also [5]. The most widely accepted mechanism for zinc-induced changes in appetite is alteration in hypothalamic neurotransmitter metabolism by influencing the leptin system [6]. According to experimental data, zinc deficiency decreases leptin levels whereas zinc supplementation increases it [7]; however, the relationship between zinc status and the leptin in humans is unclear. Additionally, zinc is known to have insulinomimetric action of increasing peripheral glucose disposal [8].

To assess the possible relationship between leptin and zinc levels we studied obese and nonobese individuals with type 2 diabetes. To assess oxidative stress, plasma thiobarbituric acid reactive substances (TBARS) as a marker of

* Corresponding author. Fatih Sitesi, B-4 Blok, Daire 5, Silivrikapı Fatih, Istanbul, Turkey.

E-mail address: dkonuk@yahoo.com (D. Konukoglu).

0955-2863/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2004.07.007

lipid peroxidation were also determined. Since previous studies have shown that leptin levels of subjects were affected by sex and menopausal status [9], only postmenopausal women were included in the study.

2. Methods and materials

2.1. Subjects and procedures

We studied the following groups of women: 25 nondiabetic nonobese women (control subjects); 35 nonobese women with type 2 diabetes; and 45 obese women with type 2 diabetes. All subjects provided written informed consent before the study, and the study approved by our local ethic committee.

Diabetes was diagnosed according to the National Diabetes Data Group criteria [10]. All diabetic patients were treated with dietary therapy (1400–1600 kcal/day, 50% carbohydrates, 30% lipids, 20% proteins and <300 mg/day cholesterol) and oral hypoglycemic agents. Subjects were considered nonobese when body mass index (BMI) was <27 kg/m^2 [11]. Retinopathy in the all subjects was examined by fundus examination, soft exudates and intra-retinal hemorrhages, and photography. Nephropathy was evaluated by microalbuminuria defined as albumin excretion rates >20 μg/min but <200 μg/min (at least two overnight collections). Peripheral neuropathy examined by the absence of foot pulses, ankle and brachial Doppler pressures, and symptoms. None of the subjects had retinopathy, nephropathy, or neuropathy.

Menopausal status was confirmed by the absence of menstruation for at least 6 months and a serum concentration of FSH of >40IU/mL (FSH IRMA, Bioclone, Australia) and estradiol of <20 IU/mL (EIA gene Estradiol, Biochem Immune Systems, France). None had ever received hormone replacement therapy.

Exclusion criteria included cardiovascular disease or hypertension, renal or hormonal disease, smoking habits, alcohol abuse, or use of any drug therapy such as lipid lowering therapy, vitamins, or antioxidants. None of the patients met any clinical criteria for deficiency of vitamins.

After 12 hours of overnight fasting, venous blood samples of the subjects were drawn into Li-heparin containing tubes. Blood samples of 1 mL were used for the determination of glycated hemoglobin. The remaining blood was then centrifuged at 1500×g for 10 minutes in a refrigerated centrifuge, and plasma was obtained.

Plasma leptin concentration was determined by immunoradiometric assay using a human RIA kit (DSL Inc., USA). The assay used purified recombinant human leptin as calibrators (0.25–120 ng/mL), and a polyclonal antibody that was made to recombinant human leptin. Analytical performance of the assay was determined by running it in duplicate. The intra-assay analytical coefficient of variation ranged from 4.2% to 5.6%, and the inter-assay coefficient of variation ranged from 4.5% to 8.1%.

TBARS levels were determined according to the methods of Buege and Aust [12]. TBARS concentration was calculated using a molar absorption coefficient of 1.56×10^5 M^{-1} cm^{-1}. The intra and inter assay coefficients of variation for TBARS were 4.5% and 4.7% respectively.

The homeostasis model assessment–insulin resistance (HOMA-IR) score was estimated to show the insulin resistance by using the following formula: (fasting insulin [μU/mL]×fasting glucose [mmol/L])/22.5 [13]. Higher HOMA-IR scores indicate lower insulin sensivity.

Plasma glucose, total, HDL and LDL cholesterol, triglycerides, and creatinine levels were determined by enzymatic methods using commercial kits (Sigma Chemical, St. Louis, MO). Glycated hemoglobin levels were assayed by colon chromatographic methods (Sigma Chemical). Plasma insulin levels were measured by electrochemiuminescense immunoassay on Roche Elecsys 2010 analyzer (Roche, USA).

2.2. Statistical analysis

Values are expressed as means±SD. Comparisons were performed with an unpaired Student t test or repeated-measures of analysis of variance. The Tukey test was used for multiple comparisons. Linear regression or Pearson correlation or both were used to evaluate the relations among different variables. Log-converted values of leptin were used in the analyses. Differences were considered to be significant at the level of $P<0.05$.

3. Results

Table 1 lists the general plasma characteristics of groups studied. Subjects with diabetes had significantly higher plasma glucose, cholesterol, triglycerides, and glycated hemoglobin than control subjects ($P<0.01$). There was no significant difference in plasma biochemical and general characteristics between the diabetic groups. Control subjects had similar plasma leptin and insulin levels to the levels of nonobese diabetic subjects. Plasma zinc levels were significantly higher in control subjects than in either obese or nonobese diabetic subjects ($P<0.01$ and $P<0.001$). Obese diabetic subjects had significantly higher plasma TBARS, leptin and insulin levels than nonobese diabetic subjects ($P<0.001$, $P<0.001$ and $P<0.001$). HOMA-IR scores were significantly higher in obese diabetic subjects when compared with nonobese diabetic subjects ($P<0.01$). This finding suggested that obese diabetic subjects have an insulin resistance. Plasma zinc levels were significantly lower in obese diabetic subjects than in nonobese diabetic subjects ($P<0.01$).

Correlation analyses were performed between leptin and the studied parameters in obese and nonobese subjects. In univariate and multivariate analyses, there was no significant relationship between leptin and any of the variables in control subjects and nonobese diabetic subjects. In univariate and multivariate analyses, a significant relationship between leptin was observed only for plasma zinc

Table 1
General and plasma characteristics of nonobese and obese subjects, with or without type 2 diabetes, and control subjects

Characteristic	Control subjects (n=25)	Nonobese diabetic subjects (n=35)	Obese diabetic subjects (n=45)
Age (y)	54.5±3.5	53.7±4.6	56.3±5.5
BMI (kg/m^2)	21.5±2.5	22.5±3.5	34.4±3.5[b]
Waist-hip ratio	0.77±0.05	0.79±0.05	0.94±0.07
SBP (mm Hg)	95.5±6.5	98.5±5.5	98.7±5.3
DBP (mm Hg)	75.4±5.5	76.5±4.5	76.5±5.5
Plasma glucose (mmol/L)	4.72±0.83	7.98±0.50[a]	8.80±0.73[b]
Plasma total Ch (mmol/L)	4.95±0.72	6.72±0.85[a]	6.85±0.75
Plasma LDL CH (mmol/L)	3.07±0.54	4.59±0.55[a]	4.61±0.70
Plasma creatinine (mmol/L)	73±10	74±9	74±10
Glycated hemoglobin	4.3±0.9	7.6±1.7	8.3±1.5
Duration of menopause (y)	5.5±4.5	8.5±1.7	8.8±1.5
Leptin (μg/L)	4.27±1.25	4.55±2.41	23.4±12.4
Zinc (μmol/L)	22.3±3.2	16.2±3.9[a]	11.6±2.9[b]
Fasting insulin (pmol/L)	48±28	52±31	141±36
TBARS (μmol/L)	3.15±0.95	5.75±1.25[a]	7.32±1.33[b]
HOMA-IR	1.36±0.16	2.66±0.15[a]	7.93±0.20[b]

Values are means±SD. $P<0.05$ statistically significant.
BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; Ch=cholesterol; HOMA-IR=Homeostasis Model Assessment–Insulin Resistance; TBARS=thiobarbituric acid reactive substances.
[a] Statistical comparison with control subjects.
[b] Statistical comparison with nonobese diabetic subjects.

(coefficient±standard error [SE], −0.425±0.135; −0.486±0.140; for all, $P<0.01$) insulin (coefficient±SE, 0.415±0.075; 0.455±0.08; for all, $P<0.01$), and BMI (coefficient±SE, 0.455±0.055; 0.480±0.155; for all, $P<0.01$) in obese diabetic subjects. In Pearson correlation analyses, plasma zinc was negatively correlated with insulin and TBARS levels ($r=-0.412$ $P<0.01$ and $r=-0.520$, $P<0.01$, respectively) in obese diabetic subjects. Plasma TBARS levels were also positively correlated with insulin ($r=0.410$, $P<0.01$) in obese diabetic subjects. Plasma zinc levels were negatively correlated with TBARS in nonobese diabetic subjects ($r=-0.430$, $P<001$).

4. Discussion

This study investigated possible interrelationships between two molecules that are involved in the physiologic regulation of energy homeostasis, namely, leptin and zinc, in obese and nonobese subjects with and without diabetes.

It has been shown that *ob* gene expression and leptin production are increased in animal models of obesity. It has been also demonstrated that elevated leptin levels in obese women result from accelerated secretion rates of leptin from adipose tissue because of increased *ob* gene expression [14]. It has been recently demonstrated that there is resistance to leptin in obese individuals, and this resistance results in elevated plasma leptin levels [15]. It is generally accepted that serum leptin concentration is positively associated with obesity and hyperinsulinism irrespective of the degree of obesity, and that the presence of chronic hyperglycemia increases adipose tissue leptin synthesis and secretion in insulin resistant subjects [16]. Obesity is a particularly common problem among individuals with type 2 diabetes mellitus. One of the potential factors implicated in the pathogenesis of diabetes mellitus in obese patients include insulin resistance. Obesity can intensify the situation by increasing adipose tissue mass and insulin resistance [17]. Plasma leptin levels were found to be elevated, decreased, or unchanged in diabetic patients [18–20]. Previous studies reporting on lean and obese nondiabetic individuals have shown elevated leptin levels to be associated with insulin resistance, independent of BMI [21]. Our results suggested that although plasma leptin levels did not change in nonobese diabetic subjects when compared with control subjects, in the presence of obesity, leptin levels elevated and insulin resistance were determined. It has been reported that insulin resistance may indirectly contribute to hyperleptinemia by increased insulin levels [22]. The exact sequence of this regulation is not understood.

On the other hand, it has been suggested that zinc supplementation of zinc-deficient subjects, in addition to its well known effects on appetite and body composition, may increase circulating leptin levels [23]. Ott et al. [24] reported that zinc deficiency may reduce leptin gene expression and leptin secretion from adipose tissue in rats. They also found that insulin-stimulated leptin secretion is augmented by a low rather than incubation with zinc at high concentrations. It has been described that although plasma zinc levels were decreased in obesity [25] and diabetes [26], Raz et al. [27] suggested unchanged plasma zinc levels in type 2 diabetes. Our results have shown that obese diabetic subjects had lower zinc levels than nonobese subjects with or without diabetes, and that plasma zinc levels were higher in nonobese diabetic subjects than in nonobese diabetic subjects. In contrast, in previous studies that suggested that there have been a positive correlation between zinc and leptin in humans [28], in our study plasma zinc levels were negatively correlated with plasma leptin and insulin levels in obese diabetic subjects. Chen et al. [23] suggested that there was an inverse correlation between zinc and leptin levels in obese individuals. They have also shown that zinc treatment significantly increased leptin production; however, this increment did not surpass that by insulin in vitro. Tallman et al. [8] reported a negative correlation between serum leptin and adipose zinc concentration in rats given high-fat diets. Gaetke et al. [29] indicated that low serum leptin concentrations, slowed metabolic rate, and decreased physical activity were more likely the result of the reduced food intake in zinc deficiency. On the other hand, zinc has been linked to insulin resistance [30]. Several potential mechanisms have been suggested for zinc affecting insulin action, including a role for zinc in modulation of insulin receptor tyrosine kinase activity, and zinc has been shown to enhance tyrosine kinase phosphorylation compared with other cations

[31]. In persons with type 2 diabetes mellitus, decreased insulin-stimulated kinase activity has been reported. [32]. However, there is a lack of information on the effect of zinc status on leptin resistance. Our results have also shown that oxidative stress was elevated in both obese and nonobese diabetic subjects, and that TBARS levels were negatively correlated with plasma zinc levels. Decreased plasma zinc levels have been observed in response to enhanced oxidative stress in diabetes [33]. TBARS levels were also significantly correlated with insulin levels in obese diabetic subjects. This finding made us think that the oxidative stress–induced leptin or insulin resistance may be a factor in obesity as well.

Although our data did not support a suggestion that zinc may contributions influence the effects of leptin by leptin receptor resistance, we thought that low zinc status may be involved in leptin resistance directly or an oxidative stress–mediated process in obese diabetic subjects. Zinc may be a mediator of leptin effects, although the precise mechanism is still unknown and requires further investigation.

References

[1] Smith SR. The endocrinology of obesity. Endocrinol Metab Clin North Am 1996;25:921–42.

[2] Wilding JP. Leptin and the control of obesity. Curr Opion Pharmacol 2001;1:656–61.

[3] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.

[4] Widjaja A, Stratton IM, Horn R, Holman RR, Turner R, Brabant G. UKPDS 20: plasma leptin, obesity, and plasma insulin in type 2 diabetic subjects. J Clin Endocrinol Metab 1997;82:654–7.

[5] Lee RG, Rains TM, Tovar-Palacio C, Beverly JL, Shay NF. Zinc deficiency increases hypothalamic neuropeptide Y and neuropeptide Y mRNA levels and does not block neuropeptide Y-induced feeding in rats. J Nutr 1998;128:1218–23.

[6] Mantzoros CS, Prasad AS, Beck FW, Grabowski S, Kaplan J, Adair C, et al. Zinc may regulate serum leptin concentrations in humans. Am Coll Nutr 1998;17:270–5.

[7] Mangian H, Lee R, Paul G, Emmert, Shay N. Zinc deficiency suppresses plasma leptin concentrations in rats. Nutr Biochem 1998; 9:47–51.

[8] Chen MD, Lin PY, Tsou CT, Wang JJ, Lin WH. Selected metal status with noninsulin –dependent diabetes mellitus. Biol Trace Elem Res 1995;50:119–24.

[9] Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, et al. The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin resistance, and energy expenditure. J Clin Endocrinol Metab 1997;82:1293–300.

[10] National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039–57.

[11] World Health Organization. WHO document. Measuring obesity-classification and description of anthropometrics data. Copenhagen: WHO Regional Office for Europe; 1998 [Nutrition Unit EUR/ICP/NUT 125].

[12] Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302–10.

[13] Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20:1087–92.

[14] Lonnqvist F, Nordfors L, Jansson M, Thorne A, Schalling M, Arner P. Leptin secretion from adipose tissue in women. J Clin Invest 1997;599:2398–404.

[15] Auverx J, Staels B. Leptin. Lancet 1998;351:757–842.

[16] Fischer S, Hanefeld M, Haffner SM, Fusch C, Schwanebeck U, Kohler C, et al. Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels indepently of body fat mass. Acta Diabetol 2002;39:105–10.

[17] Wauters M, Considine RV, Yudkin JS, Peiffer F, De Leeuw I, Van Gaal LF. Leptin levels in type 2 diabetes: associations with measures of insulin resistance and insulin secretion. Horm Metab Res 2003;35: 92–6.

[18] Haque Z, Rahman MA. Serum leptin levels in female patients with NIDDM. J Coll Phys Surg Pak 2003;13:130–4.

[19] Passaro A, Calzoni F, Zamboni PF, Manservigi D, Alberti L, Dalla Nora E, et al. Role of diabetes in influencing leptin concentration in elderly overweight patients. Eur J Endocrinol 2001;145:173–9.

[20] Tatti P, Masselli L, Buonanno A, Di Mauro P, Strollo F. Leptin levels in diabetic and nondiabetic subjects. Endocrine 2001;15:305–8.

[21] Donahue RP, Prineas RJ, Donahue RD, Zimmet P, Bean JA, De Courten M, et al. Is fasting leptin associated with insulin resistance among nondiabetic individuals? The Miami Community Health Study. Diabetes Care 1999;22:1092–6.

[22] Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, et al. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. J Clin Endocr Metab 1999;84:670–6.

[23] Chen MD, Song YM, Lin PY. Zinc may be a mediator of leptin production in humans. Life Sci 2000;21:2143–9.

[24] Ott ES, Shay NF. Zinc deficiency reduces leptin gene expression and leptin secretion in rat adipocytes. Exp Biol Med 2001;226:841–6.

[25] Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, et al. Increased oxidative stress and hypozincemia in male obesity. Clin Biochem 2002;35:627–31.

[26] Terres-Martos C, Navarro-Alarcon M, Martin-Lagos F, Lopez G, de la Serrana H, Perez-Valero V, et al. Serum zinc and copper concentrations and Cu/Zn ratios in patients with hepatopathies or diabetes. J Trace Elem Med Biol 1998;12:44–9.

[27] Raz I, Havivi E. Trace elements in blood cells of diabetic subjects. Diabetes Res 1989;10:21–4.

[28] Mantzoros CS, Prasad AS, Beck FW, Grabowski S, Kaplan J, Adair C, et al. Zinc may regulate serum leptin concentrations in humans. J Am Coll Nutr 1998;17:270–5.

[29] Gaetke LM, Frederich RC, Oz HS, McClain CJ. Decreased food intake rather than zinc deficiency is associated with changes in plasma leptin, metabolic rate, and activity levels in zinc deficient rats. J Nutr Biochem 2002;13:237–44.

[30] Faure P, Roussel A, Coudray C, Richard MJ, Halimi S, Favier A. Zinc and insulin sensitivity. Biol Trace Elem Res 1992;32:305–10.

[31] Findik D, Presek P. Zn^{2+} enhances protein tyrosine kinase activity of human platelet membranes. FEBS Lett 1988;235:51–6.

[32] Sinha MK, Pories WJ, Flickinger EG, Meelheim D, Caro JF. Insulin-receptor kinase activity of adipose tissue from morbidly obese humans with and without NIDDM. Diabetes 1987;36:620–5.

[33] DiSilvestro RA. Zinc in relation to diabetes and oxidative disease. J Nutr 2000;130:1509S–11S.