Neuroendocrinology Letters No.6 December Vol.26, 2005

Circulating leptın, zinc, and copper levels after extracorporeal circulation

Zulfikare Isık Gormus[1], Niyazi Gormus[2], Abdulkerim Kasim Baltaci[1*], Ihsan Halifeoglu[3] & Rasim Mogulkoc[1]

[1] Department of Physiology, Meram Medical School, Selcuk University, Konya, Turkey.
[2] Department of Cardiovascular Surgery, Meram Medical School, Selcuk University, Konya, Turkey.
[3] Department of Biochemistry, Meram Medical School, Fýrat University, Elazýg, Turkey.

Correspondence to: Dr. Abdulkerim Kasim Baltaci
Selcuk University, Meram Medical School
Department of Physiology
42080, Konya, TURKEY
FAX: +90 332 2236181
EMAIL: baltaci61@yahoo.com

Submitted: August 18, 2004 *Accepted: October 1, 2004*

Key words: **extracorporeal circulation; leptin; zinc; copper**

Neuroendocrinol Lett 2005; **26**(6):839–842 **PMID:** 16380701 NEL260605A11

This study has been presented in the Second International Symposium on Trace Elements and Minerals in Medicine and Biology in Munich between 13th and 15th May, 2004.

Abstract

OBJECTIVE: The role of leptin in the acute stress response to extracorporeal circulation has been well documented, however, the relationship between leptin and zinc has not been investigated previously. We aimed to research the circulating leptin, zinc, and copper levels before, during, and after the extracorporeal circulation, and effect of preoperative zinc administration to these.
METHODS: Twenty patients who were taken to elective coronary artery bypass grafting operations using extracorporeal circulation were taken to this research and divided into two equal groups (n_1, n_2). In both groups blood samples were taken just before the operation (T0), at the end of operation (T1), and at the first postoperative day (T2). In the second group (n_2) oral zinc (50 mg, once a day) was administered to patients for 5 days, preoperatively. The serum leptin, zinc, and copper levels were studied.
RESULTS: In group n_1 circulating leptin levels were significantly increased at T2 when compared to T0 and T1 ($p<0.05$); zinc levels were decreased at T2 when compared to T0 and T1 ($p<0.05$); copper levels were decreased at T2 when compared toT0 ($p<0.05$), and decreased at T1 when compared to T0 ($p<0.05$). In group n_2 circulating leptin levels were significantly increased at T2 when compared toT0 and T1 ($p<0.05$); zinc levels were decreased at T2 when compared toT0 and T1 ($p<0.05$); copper levels were increased at T2 when compared toT1 ($p<0.05$).
CONCLUSIONS: These results indicate that circulating leptin levels increase after the extracorporeal circulation as an acute response, while zinc and copper levels decrease at the same period. Preoperative zinc administration does not prevent the leptin response after extracorporeal circulation.

ORIGINAL ARTICLE

Introduction

Cardiopulmonary bypass (CPB) circuits initiate a severe systemic inflammatory response syndrome which is clinically characterized by pathological hypotension, fever of unknown origin, disseminated intravascular coagulation (DIC), diffuse tissue edema, injury, and multiple organ failure [1]. This syndrome is associated with the activation and accumulation of polymorphonuclear leukocytes (PMNL). The PMNLs adhere along the endothelium and migrate into the tissue by transendothelial way. Soluble adhesion molecules, such as tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), IL-8, and IL-10, that are placed on the surface of these activated leukocytes, platelets, and the endothelium mediate these interactions [2].

Leptin, is the hormone produced by transcription of the obese gene, primarily of the human adipocyte [3]. Recent evidence supports leptin's role in more complex physiologic systems such as immunity and inflammation. Although leptin increases in human stress response, it is unclear either leptin plays simply an adaptive marker or an active mediator in this response. In this clinical prospective study we aimed to bring up the systemic inflammatory response syndrome occurred after CPB by measuring the serum leptin levels. On the other hand, in this study we tried to search whether preoperative zinc administration prevents the SIRS and effects the leptin levels after CPB.

Materials and Methods

Patient Selection

After local ethics committee approval and informed consent, 20 patients were selected for operation according to American College of Cardiology guidelines for coronary artery bypass grafting (CABG) [4]. There was not any significant difference between the groups when they were compared according to ages (Table 1). Patients who had associated valve disease, patients using corticosteroid drugs, pentoxyfilline or allopurinol for a long-period (for gout disease) were excluded from the study. The patients were divided into two equal groups (n_1, n_2). In the second group (n_2) oral zinc (50 mg, once a day) was administered to patients for 5 days, preoperatively.

Anesthesia Procedure

The intraoperative anesthesia procedure was standardized in both groups and consisted of intravenous fentanyl (20 µg/kg), midazolam (10 mg total) and vecuronium (0.1 mg/kg). Patients were ventilated with oxygen/air (inspired oxygen fraction 0.5) with a tidal volume of %5–7.5 ml/kg at normocapnia. All of the fentanyl was administered before sternotomy. Regarding midazolam, 6 mg was administered before sternotomy, 2 mg during rewarming and 2 mg during sternal closure. If required, inhaled sevoflurane was used before initiation of CPB.

Operation Procedure

A standard cardiopulmonary bypass procedure was undertaken with moderate hypothermia (32 °C), and a nonpulsatile flow from membrane oxygenator (Dideco 708, Italy). Priming volume consisted of 2.0 l of Ringer's solution. Non-pulsatile flows were maintained between 2.4 and 2.8 l/min/m^2, and mean arterial pressure was kept between 50–70 mmHg. Cold-blood antegrade cardioplegia and hot-shot blood cardioplegia before removing the aortic cross-clemp were used in all patients. The single aortic cross-clemp technique was preferred in all operations. Left internal thoracic artery and one or two peripheral vein grafts taken from the great saphenous veins were used.

Sample Collection and Measurements

In both groups blood samples were taken just before the operation (T0), at the end of operation (T1), and at the first postoperative day (T2). In the second group (n_2) oral zinc (50 mg, once a day) was administered to patients for 5 days, preoperatively. The serum of blood samples were prepared and serum leptin, zinc, and copper levels were studied.

The analysis of serum copper and zinc levels were made with atomic absorption spectrophotometer (Shimadzu, AA-6701F). The serum level of leptin was estimated with ELISA (DRG Instruments GMBH, Germany) by using a ELx-800 (BIO-TEK Instruments Inc. Highland, USA).

Statistical Analysis

The results were transferred to computer and 13.0 Minitab was used for statistical comparison of these results. One sample t test was used for the statistical analysis of difference of serum leptin, zinc, and copper levels in T0, T1, and T2 times in each group. The analysis of variances for leptin, zinc, and copper were made by using a paired t test between the groups. ANOVA was used for statistical analysis of demographic data between the groups. A p value less than 0.05 was considered to be statistically significant in all tests.

Results

There were no deaths in either group. Comparison of total pump (cardiopulmonary bypass) and aortic occlusion times showed no significant difference between the groups (Table 1). In both groups the patients were extubated within 24 hours after arrival to ICU.

Statistical analysis of serum leptin levels showed statistically significant increase in group 1 between the T0 and T2 ($p<0.05$), and between the T1 and T2 ($p<0.05$). However, there was not any significant difference between the T0 and T1 ($p>0.05$) (Table 2). In group 2 the results were the same as in group 1; there were significant differences between the T0 and T2 ($p<0.05$), and between the T1 and T2 ($p<0.05$), while no difference was found between the T0 and T1. Comparison of each leptin results between the groups showed no significant differences ($p>0.05$). Therefore, we decided that preoperative zinc administration does not effect the leptin response after CPB.

Observing the serum zinc levels of group 1; there was a significant decrease between the periods of T2 and T0 ($p<0.05$), and between T2 and T1 ($p<0.05$) (Table 3). This means that CPB causes a significant decrease in serum zinc levels especially 24 hours after the open heart surgery. When we compared the serum zinc levels in group 2 same results were observed; significant decrease between the periods of T2 and T0 ($p<0.05$), and between the T2 and T1 ($p<0.05$). When the serum zinc levels were compared between the groups there were not any significant differences between the periods of T0-T1 and T0-T2 ($p<0.05$). However, comparison of the changes of serum zinc levels between T1-T2 periods showed a borderline p value ($p=0.057$). Therefore, we decided that preoperative zinc administration (50 mg, once a day, for 5 days, oral) is not sufficient in order to replace the serum zinc levels that is used during the CPB.

Finally, serum copper levels were measured in both groups (Table 4). The statistical analysis of copper levels considered different results from those of leptin and zinc levels. Copper levels of group 1 were significantly decreased in T1 when compared toT0 ($p<0.05$), while they were significantly decreased in T2 when compared toT0 ($p<0.05$). In group 2 the serum copper levels were significantly increased in T2 when compared toT1 ($p<0.05$). When the serum copper levels were compared between the groups the only significant difference was between the periods of T0-T2 ($p=0.045$).

Discussion

The systemic inflammatory response syndrome following CPB is a well known and well documented disorder in open heart surgery operations. TNF-alpha, IL-1, IL-6, IL-8, and IL-10 have been used in many clinical and experimental studies in order to project the inflammatory response seen after cardiopulmonary bypass. TNF-alpha and IL-1 induce anorexia and loss of body mass, which are common manifestations of acute and chronic inflammatory conditions. These effects seem to be related to a prompt increase in serum leptin levels and mRNA expression in the adipose tissue [5]. Anorexia and tissue catabolism could be beneficial to the organism during acute inflammation [6], and it has recently been postulated that leptin might play a role in the anorexia associated with acute infection [7]. However, the mechanism of the induction of leptin secretion in acute inflammation remains still unclear. It has been observed that in vivo administration of proinflammatory cytokines, such as IL-1 alpha or beta or TNF-alpha, increases the expression of leptin [8–10].

Stryjewski G and Dalton HJ reported that it is unclear what the overall role for leptin in stress is. It appears that this interaction may be an adaptive response to physiological stimulus. On the other hand, as with other cytokines, it may act as a proinflammatory cytokine [11].

Table 1: Demographic data of both groups including total pump time (TPT), aortic-occlusion time (AOT), and age of patients.

	TPT	AOT	Age
Group 1	83.90 ± 24.79	64.10 ± 22.92	67.3 ± 9.87
Group 2	103.60 ± 24.73	77.30 ± 19.39	62 ± 11.51

Table 2: Leptin levels (mean ± SD) in both groups.

	T0-Leptin	T1-Leptin	T2-Leptin
Group 1	5,88 ± 7,53	5.42 ± 10.80	20.30 ± 22,38*#
Group 2	8,22 ± 6,88	5.80 ± 7.22	28.04 ± 26,86&+

T0: Sample taken before the CPB; T1: Sample taken at the end of CPB; T2: Sample taken 24 hours after the operation.
*: Leptin levels of group 1 were significantly increased in T2 when compared toT0 ($p=0.017$).
#: Leptin levels of group 1 were significantly increased in T2 when compared toT1 ($p=0.005$).
&: Leptin levels of group 2 were significantly increased in T2 when compared toT0 ($p=0.024$).
+: Leptin levels of group 2 were significantly increased in T2 when compared toT1 ($p=0.008$).

Table 3: Serum zinc levels (mean ± SD) in both groups.

	T0-Zn	T1-Zn	T2-Zn
Group 1	72.46 ± 11.2	61.37 ± 6.14	44.89 ± 6.86*+
Group 2	96.03 ± 22.13	91.24 ± 17.20	61.51 ± 11.34&#

Zn: Zinc
*: Zinc levels of group 1 were significantly decreased in T2 when compared toT0 ($p=0.000$).
+: Zinc levels of group 1 were significantly decreased in T2 when compared toT1 ($p=0.001$).
&: Zinc levels of group 2 were significantly decreased in T2 when compared toT0 ($p=0.003$).
#: Zinc levels of group 2 were significantly decreased in T2 when compared toT1 ($p=0.001$).

Table 4: Serum copper levels (mean ± SD) in both groups.

	T0-Cu	T1-Cu	T2-Cu
Group 1	120.68 ± 25.8	69.14 ± 15.65*	88.20 ± 21,31&
Group 2	102.29 ± 31.73	81.11 ± 30.20	100.73 ± 26.20#

Cu: Copper
*: Copper levels of group 1 were significantly decreased in T1 when compared toT0 ($p=0.000$).
&: Copper levels of group 1 were significantly decreased in T2 when compared toT0 ($p=0.004$).
#: Copper levels of group 2 were significantly increased in T2 when compared toT1 ($p=0.015$).

Recently, it has been shown that leptin has an anti-inflammatory effect in acute injury [12–14]. Cakır and colleagues [15] reported an experimental colitis created in Sprague-Dowley rats. They decided that the anti-inflammatory effect of leptin involves a tissue neutrophil-dependent mechanism and is dependent on the release of glucocorticoids.

The role of leptin in immunity is also an astonishing subject. The immune response is divided into two arms: natural immunity (phagocytic cells) and the adaptive (specific) immunity (T and B lymphocytes). It has been shown that leptin affects both of the natural and adaptive immune response and stimulates the T helper 1 proinflammatory adaptive immune response [16].

Quasim T and colleagues [17] examined the relationships between the circulating leptin concentrations, mediators (IL-6, C-reactive protein, albumin, cortisol, and growth hormone) of the systemic inflammatory response in critically ill surgical patients admitted to the intensive care unit. They noted that leptin concentrations appeared to be low on admission which is thought to be related to the magnitude of the systemic inflammatory response.

Recently, Zivna and colleagues [18] reported a successful experimental study in young rats. In this experimental study 36 young rats were divided into 6 equal groups. Groups were divided as partial hepatectomy, splenectomy, unilateral nephrectomy, laparotomy, laparotomy+ibuprofen, and partial hepatectomy+ibuprofen. They noted statistically significant decrease in serum leptin concentrations in laparotomy, laparotomy+ibuprofen, and partial hepatectomy+ibuprofen treatment performed groups. As a result they concluded that surgical interventions and ibuprofen treatment are accompanied by decrease in serum leptin concentrations.

Investigations searching the leptin levels in cardiac surgery performed patients are very few in the literature. Most of them are reported by Modan-Moses and colleagues [19, 20]. They performed their studies in pediatric cardiac surgery performed children. They noted that CPB is associated with acute changes in circulating leptin levels, and there is an inverse relationship between leptin and cortisol in these cases. They concluded that leptin may play a role in the inflammation occurred during CPB.

In the present study we found that leptin increases after CPB protocols as an inflammatory cytokine. However, preoperative zinc administration, as an anti-oxidant medication, did not affect the leptin concentrations and in zinc administered group postoperative zinc levels were also diminished when compared to preoperative concentrations.

In conclusion, these results indicate that circulating leptin levels increase after the extracorporeal circulation as an acute response, while zinc and copper levels decrease at the same period. Preoperative zinc administration (50 mg, once a day, for 5 days, oral) does not prevent the leptin response and decrease circulating zinc levels after extracorporeal circulation.

REFERENCES

1 Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. Chest 1997; **112:**676–692.
2 Wei M, Kuukasjarvi P, Laurikka J, Kaukinen S, Iisalo P, Laine S, et al. Soluble adhesion molecules and myocardial injury during coronary artery bypass grafting. World J Surg 2003; **27:**140–144.
3 Frühbeck G, Jebb S, Prentice A. Leptin: Physiology and pathophysiology. Clin Physiol 1998; **18:**399–419.
4 Eagle KA, Guyton RA. ACC/AHA guidelines for coronary artery bypass graft surgery. J Am Coll Cardiol 1999; **34:**1262–1346.
5 Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, et al. Multiple cytokines and inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 1997; **185:**171–175.
6 Plata-Salaman CR. Leptin, anorexia nervosa, and anorexia of acute and chronic disease. Nutrition 1999; **15:**943–945.
7 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its homologue. Nature 1994; **372:**425–432.
8 Janik JE, Curti BD, Considine RV, Rager HC, Powers GC, Alvord WG, et al. Interleukin 1 alpha increases serum leptin concentrations in humans. J Clin Endocrinol Metab 1997; **82:**3084–3086.
9 Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR, Grunfeld C. IL-1 beta mediates leptin induction during inflammation. Am J Physiol 1998; **274:**R204–208.
10 Mantzoros CS, Moschos S, Avrampoulos I, Kaklamani V, Liolios A, Doulgerakis DE, et al. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. J Clin Endocrinol Metab 1997; **82:**3408–3413.
11 Stryjewski G, Dalton HJ. Circulating leptin: mediator or marker of the neuroendocrinological stress response?. Crit Care Med 2001; **29:**2397–2398.
12 Gualillo O, Eiras S, Lago F, Dieguez C, Casanueva FF. Elevated serum leptin concentrations induced by experimental acute inflammation. Life Sciences 2000; **67:**2433–2441.
13 Bozkurt A, Cakir B, Ercan F, Yegen BC. Anti-inflammatory effects of leptin and cholecystokinin on acetic acid-induced colitis in rats: role of capsa icin-sensitive vagal afferent fibers. Regul Pept 2003; **116:**109–118.
14 Voegeling S, Fantuzzi G. Regulation of free and bound leptin and soluble leptin receptors during inflammation in mice. Cytokine 2001; **14:**97–103.
15 Cakir B, Bozkurt A, Ercan F, Yegen BC. The anti-inflammatory effect of leptin on experimental colitis: involvement of endogenous glucocorticoids. Peptides 2004; **25:**95–104.
16 La Cava A, Alviggi C, Matarese G. Unraveling the multiple roles of leptin in inflammation and autoimmunity. J Mol Med 2004; **82:**4–11.
17 Quasim T, McMillan DC, Wallace AM, Kinsella J. The relationship between leptin concentrations, the systemic inflammatory response and illness severity in surgical patients admitted to ITU. Clin Nutr 2004; **23:**233–238.
18 Zivna H, Zivny P, Palicka V. Serum leptin concentrations after surgery in young rats. Nutrition 2002; **18:**643–646.
19 Modan-Moses D, Ehrlich S, Kanety H, Dagan O, Pariente C, Esrahi N, et al. Circulating leptin and the perioperative neuroendocrinological stress response after pediatric cardiac surgery. Crit Care Med. 2001; **29:**2377–2382.
20 Modan-Moses D, Kanety H, Dagan O, Pariente C, Ben-Abraham R, Freedman L, et al. Circulating leptin levels after cardiopulmonary bypass in children. J Cardiothorac Vasc Anesth 2001; **15:**740–744.