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RESEARCH ARTICLE

A Study of Correlation of Serum Leptin with Trace Elements in Water Buffalo (*Bubalus bubalis*)

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ABSTRACT

The present study was designed to evaluate the correlations of the serum leptin with trace elements in water buffalo (*Bubalus bubalis*). The serum concentration of leptin and its correlations with serum concentrations of copper, zinc, selenium, iron and manganese were measured in 80 clinically healthy water buffaloes. The serum concentration of leptin had no significant correlation with the measured trace elements (P>0.05). Separate evaluation of the age groups also showed no significant difference between age groups and no significant correlation between serum leptin and the measured trace elements. In male buffaloes, the serum concentration of leptin had a significant correlation with the zinc/copper ratio (r= 0.53, P=0.014). These relationships were not measured in water buffalo previously and were partially different from those of other species.

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INTRODUCTION

Leptin is a 16-kDa plasma protein that is primarily produced by adipocytes and has a variety of important central and peripheral actions to regulate energy balance and metabolism (Soliman *et al*., 2002; Koh *et al*., 2008). It has been proved that leptin is initially included in the regulation of food intake, energy balance and adiposity in several mammalian. Leptin also is associated with other biological processes such as reproduction, hematopoiesis, immune response and bone formation (Olusi *et al*., 2003).

Trace elements such as manganese (Mn), copper (Cu), iron (Fe), selenium (Se) and zinc (Zn) are essential nutrients for humans and animals and are needed in very small amounts for many physiological functions, including immune and antioxidant function, growth and reproduction (Cunnane, 1998). Their deficiencies are often associated with alterations in many metabolic processes and cause various kinds of diseases. The mechanisms of their effects are not completely obvious and in spite of intense research, the role of this microelement needs further elucidation.

Due to having the same biological effects, it has been hypothesized that serum leptin may be associated with the trace elements (Olusi *et al*., 2003). However, there is very little published information available about the association of serum leptin with trace elements in healthy populations of domestic animals. The role of leptin in buffalo is still unknown (Di Palo *et al*., 2005). To the best of our knowledge, little information about the serum leptin in water buffaloes exists, and there is no information about the probable relationships between the serum profiles of trace elements and the leptin status in water buffalo. Therefore, this study was designed to investigate the relationship between these parameters in water buffalo.

MATERIALS AND METHODS

The investigation was carried out on Iranian water buffaloes (*Bubalus bubalis*) which were slaughtered in a slaughter house reserved only for buffaloes in Ahvaz City, southwestern Iran, from July to September 2010. After clinical examination, jugular blood samples in plane tubes, free from anticoagulant, were collected from 80 clinically healthy water buffaloes. Buffaloes of both sexes with different age groups were selected randomly. The age of the animals was estimated using dental characteristics. All animals had grazed the previous summer on ranges around the city.

The serum was separated after centrifugation at 1800 *g* for 10 min and the serum samples were stored at -20 ^{o}C until analysis. The samples with hemolysis were thrown away. Serum leptin was measured (Kirk *et al*., 2009) using commercial ELISA detection kits (RD291001200 sandwich ELISA kit, Biovendor, Czech Republic). For this kit the reported intra and inter- assays variabilities are 2.2 and 3.4%, respectively. Digestion of the serum was performed by a mixture of perchloric and nitric acid (3:7

ratios respectively). Manganese, copper, iron, selenium and zinc were measured using an atomic absorption spectrophotometer (Shimadzo AA-670, Kyoto, Japan) (Badiei *et al*., 2006).

Statistical analysis was performed using SPSS12 (Illinois, Chicago). Correlations of the serum leptin with serum trace elements were analyzed by Pearson's correlation tests. Two sample t-tests were used to detect differences in the parameters between the two sexes. Analysis of variance (ANOVA) tests were used to compare the measured factors between the different age groups of water buffaloes. Differences were considered significant at $P<0.05$.

RESULTS

Overall, 21 male buffaloes and 59 female buffaloes were sampled. The average ages (mean±SEM) of the male and female buffaloes were 3.5±0.395 and 7.11±0.488 years, respectively. The average age of the female buffaloes was significantly more than the male buffaloes ($P<0.01$). The serum concentration of leptin was 7.26±0.16 ng/ml. There were no significant differences between the male and female buffaloes in the serum concentrations of leptin and the measured trace elements. The results of the measurement of the serum concentrations of the leptin and the measured trace elements are shown in Table 1.

Serum concentration of leptin had no significant correlation with manganese, copper, iron, selenium, zinc, and zinc to copper ratio, and there was no significant correlation with age. The results of the measurement of the correlations between the serum concentrations of the measured trace elements and leptin are shown in Table 2.

The buffaloes were divided into three groups according to their age as G1 ≤2 years, 2 years<G2 ≤5 years and, G3> 5 years. The correlations of serum leptin with measured trace elements were measured in each of the age groups separately. No significant difference between either sex nor any significant correlation between serum leptin and the measured trace elements was found ($P>0.05$). The serum concentrations of leptin and the measured trace elements had no significant differences between age groups. Because of the unequal variances, the Kruskal-Wallis test was used to compare Cu between the three age groups. This showed that the differences were marginally significant ($P=0.055$).

Both sexes were evaluated separately. In male buffaloes, serum concentration of leptin had a significant correlation with zinc/copper ratio ($r= 0.53$, $P=0.014$). Serum leptin also had a significant correlation with age ($r= 0.545$, $P=0.011$). The serum concentration of leptin had a significant difference between age groups ($P=0.045$). A post hoc Bonferroni test showed that the serum leptin of the G3 group was significantly more than G1 group ($P=0.06$).

DISCUSSION

Although the relationship of serum leptin with some of the measured trace elements has been investigated in some species, to the best of our knowledge, there has been no previous research regarding these correlations in water buffalo.

Zinc is essential for the function of more than 200 enzymes, and Zn-containing enzymes are found in metabolic pathways involved in lipid metabolism (Cunnane, 1988). It has been demonstrated that in different species, the administration of leptin increases energy expenditure and decreases appetite. Zinc also affects the appetite regulation and zinc deficiency decreases the appetite, while zinc supplementation increases the appetite (Mantozoros *et al*., 1998). It is postulated that zinc status influences the regulation of the appetite and metabolism by influencing the leptin system of mice (Tallman and Taylor, 2003). Zinc acts as a cofactor and regulates the expression of several genes and may affect leptin production directly by regulating gene expression, and/or indirectly by affecting circulating concentrations of IL-2 and TNF-α (Mantozoros *et al*., 1998; Marreiro *et al*., 2006). There are some controversies regarding the relationship of the serum concentration of leptin with serum zinc (Taghdir *et al*., 2010). A negative correlation between plasma leptin and Zn levels has been found in some studies on healthy humans (Canatan *et al*., 2004; Chen *et al*., 2000). However, similar to our results, no significant relationship between these was reported in some experiments on healthy humans (Olusi *et al*., 2003; Taghdir *et al*., 2010). The same controversies were reported about the change in serum leptin following Zn supplementation in humans (Chen *et al*., 2000; Marreiro *et al*., 2006; Taghdir *et al*., 2010). In rats, serum leptin and leptin mRNA levels in inguinal adipocytes showed a tendency to increase during the Zn-depletion period and decreased back during the Zn-repletion period. However, change in leptin mRNA in abdominal adipocytes during Zn-depletion and Zn-repletion periods had a different pattern (Lee *et al*., 2003). It is proposed that the relationship of serum concentrations of leptin and Zn may be different between healthy individuals, and individuals that are affected by different diseases (Taghdir *et al*., 2010). On the other hand, the relationships may be different among species.

Table 1: The concentration (Mean±SEM) of serum leptin, copper, zinc, selenium, iron and manganese in water buffaloes (*Bubalus bubalis*).

	Number of buffaloes	Leptin (ng/ml)	Copper (µmol/L)	Zinc (µmol/L)	Selenium (µmol/L)	Iron (µmol/L)	Manganese (µmol/L)	Zinc/ copper
All sampled buffaloes	80	7.26±0.16	0.59±0.05	3.13±0.26	0.18±0.016	3.06±0.2	0.057±0.005	7.99±1.26
Male buffaloes	21	7.14±0.3	0.74±0.17	2.82±0.49	0.097±0.019	2.68±0.38	0.05±0.006	6.13±1.45
Female buffaloes	59	7.3±0.19	0.53±0.04	3.24±0.032	0.126±0.02	3.2±0.28	0.059±0.007	8.67±1.64
G_1 (<2 years)	16	7.07±0.31*	0.85±0.22	2.91±0.62	0.08±0.024	3.68±0.63	0.052±0.007	4.54±0.81
G_2(2years<and <5years)	24	7.33±0.3	0.6±0.065	2.9±0.44	0.0106±0.023	2.71±0.24	0.071±0.016	8.42±3.38
G_3 (5 years<)	40	7.3±0.23*	0.47±0.043	3.37±0.4	0.13±0.026	3.02±0.27	0.051±0.005	9.16±1.41

* The difference is significant ($P<0.05$)

Table 2: Correlations of serum concentration of leptin with measured trace elements in water buffaloes (*Bubalus bubalis*)

	Number of buffaloes	Relationship of leptin with trace elements					
		Copper	Zinc	Selenium	Iron	Manganese	Zinc/ copper
All sampled buffaloes	80	r=-0.08 P>0.05	r=0.06 P>0.05	r=0.04 P>0.05	r=-0.03 P>0.05	r=0.1 P>0.05	r=0.1 P>0.05
Male buffaloes	21	r=-0.17 P>0.05	r=0.17 P>0.05	r=0.17 P>0.05	r=0.02 P>0.05	r=0.08 P>0.05	r=0.53* P=0.014
Female buffaloes	59	r=0.03 P>0.05	r=0.025 P>0.05	r=-0.004 P>0.05	r=-0.06 P>0.05	r=0.1 P>0.05	r=0.018 P>0.05
G1 (<2 years)	16	r=-0.14 P>0.05	r=-0.07 P>0.05	r=-0.08 P>0.05	r=0.22 P>0.05	r=0.07 P>0.05	r=0.277 P>0.05
G2(2years<and <5years)	24	r=0.21 P>0.05	r=0.07 P>0.05	r=0.31 P>0.05	r=-0.29 P>0.05	r=0.21 P>0.05	r=-0.05 P>0.05
G3 (5 years<)	40	r=-0.21 P>0.05	r=0.1 P>0.05	r=-0.08 P>0.05	r=-0.03 P>0.05	r=0.025 P>0.05	r=0.255 P>0.05

* The correlation is significant (P<0.05)

According to our results, the serum concentration of leptin had no significant correlation with serum Cu. Olusi *et al*. (2003) reported a positive correlation between the serum leptin level and serum copper, but not the serum zinc level in healthy adult humans. They proposed that copper may be involved in leptin metabolism or leptin may be a regulator of copper metabolism. They believe that because copper is a component of many enzymes, it is likely that it will be involved in the synthesis of leptin, and copper deficiency may result in decreased leptin production. However, this relationship was not reported in other studies on humans (Canatan *et al*., 2004).

We found a significant correlation between serum concentration of leptin and zinc/copper ratio without any significant correlation of leptin with Zn or Cu. Copper and Zn have been proposed as the probable mediators of leptin regulation in humans and rodents, although results are controversial (Casimiro-Lopes *et al*., 2009). Significant correlation between the serum concentration of leptin and the zinc/copper ratio in human has been reported by other authors (Olusi *et al*., 2003; Canatan *et al*., 2004) and some researchers presented a hypothesis that a relative or an absolute deficiency of copper characterized by a high ratio of zinc to copper may be a major etiological factor in leptin metabolism (Olusi *et al*., 2003). However, similar to our results, lack of a significant correlation between the serum concentration of leptin with zinc or copper has been reported in different studies and it seems that the zinc/copper ratio may be more important in leptin metabolism than these separately. On the other hand, only in male buffaloes was the correlation between serum leptin and Zn/Cu ratio significant. Ban-Tokuda *et al*. (2007) proposed the difference in the body fat accumulation due to sex hormones and different sex maturity time as the probable cause of the difference between sexes of crossbred water buffaloes and Brahman cattle in the leptin metabolism.

The probable roles of Fe, Se and Mn in the leptin metabolism are less well-defined. It was suggested that leptin may be involved in erythropoiesis (Nasri, 2006). On the other hand, lack of association between plasma leptin levels and the degree of appetite was reported in iron deficient children (Topaloglu *et al*., 2001). However, we found no significant correlation between the serum concentrations of leptin and Fe. The same result was reported by Nasri (2006) in human hemodialysis patients. Recognition of the probable relationship between leptin and iron metabolism needs more research in different species.

Our results showed no significant correlations of serum leptin with Se and Mn. Although the mechanisms are not completely clear, the roles of both Se and Mn in lipid and lipoprotein metabolism are known in different species (Tajik and Nazifi, 2010). Leptin also affects the metabolism of lipids and lipoproteins. Therefore, the relationships of the serum leptin with serum Mn and Se were evaluated in the current study. There is no data about the probable correlation of serum leptin with these trace elements in other species, and although no significant correlation was found in the current study, more research in other species is recommended.

Conclusions

The cause of these findings and some contradictory findings regarding the relations between the serum leptin with the measured trace elements are not clear and may be due to the effect of some factors such as species, age, sex, health status, breed, pregnancy, body fat composition, geographic and dietary factors on the serum leptin or trace element profiles in domestic animals. Also, the correlations between the serum concentrations of leptin with the measured trace elements in physiological concentrations may not be the same as the changes observed during deficiencies of the leptin or measured trace elements. However, only a few researches have been done regarding the association of serum leptin with trace elements in normal and healthy populations of humans and rodents, and it seems that more work is required on a larger number of animals from different species before the importance of these findings can be assessed.

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