Veterinary Research Forum
Vol: 1, No: 2, September, 2010, 91 - 97

Correlation of Serum Lipids and Lipoproteins with Trace Elements in Water Buffalo *(Bubalus bubalis)*

Javad Tajik [1]*
Saeed Nazifi[1]

[1]*Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

Received: 13 July 2010, Accepted: 28 August 2010

Abstract

To evaluate the correlations of the serum concentrations of trace elements with lipids and lipoproteins in water buffalo *(Bubalus bubalis)*, the serum concentrations of cholesterol, triglyceride, total lipids, very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol) and high density lipoproteins (HDL-cholesterol) and their correlations with serum concentrations of copper, zinc, selenium, iron and manganese were measured in 100 clinically healthy water buffaloes. Serum concentration of copper had a significant correlation with HDL-cholesterol ($r = 0.467$, $P < 0.01$). Serum total lipids had a significant correlation with iron ($r = 0.25$, $P = 0.019$) and the correlation between total lipids and manganese was marginally significant ($r = -0.197$, $P = 0.07$). Additionally, separate evaluation of age groups showed that serum cholesterol had a significant correlation with copper ($r = 0.643$, $P = 0.007$) in buffaloes up to 2 years of age and with zinc in animals between 2-5 years old ($r = -0.382$, $P = 0.049$). Serum selenium had a significant correlation with VLDL- cholesterol ($r = 0.345$, $P = 0.049$) in buffaloes more than 5 years old.

Key words: Trace elements; Serum lipids and lipoproteins; *Bubalus bubalis*

***Corresponding author:**
Javad Tajik, DVM, DVSc
Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
E-mail address: tajik@shirazu.ac.ir

Introduction

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.[1] Trace elements also affect many aspects of lipid metabolism through enzymes and have modulatory effects on the synthesis and metabolism of lipids and it is clear that deficiencies of some of trace elements cause marked alterations in lipid and lipoprotein metabolism.[1] The mechanisms of their effects are not completely obvious and in spite of intense research, the role of this microelement needs further elucidation. Additionally, there are some contradictory findings regarding the relation between serum trace elements and cholesterol and triglycerides and the correlations between the serum concentrations of trace elements with lipids and lipoproteins in the physiological concentration may be different from the changes observed during their deficiencies.[2,3]

To the best of our knowledge, there is little information about the serum lipids in water buffaloes and there is no information about the relation of serum trace elements with lipids and lipoproteins. Therefore, this study was undertaken to investigate the serum profiles and the relationship between these parameters.

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 2009. Overall, 26 male buffaloes and 74 female buffaloes were sampled. The average ages (mean ± SEM) of the male and female buffaloes were 3.135 ± 0.348 and 6.81 ± 0.414 years, respectively.

After clinical examination, jugular blood samples in plane tubes, free from anticoagulant, were collected from 100 clinically healthy water buffaloes. Buffaloes were of both sexes, with different ages and 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 blood serum was separated after centrifugation at 750 *g* for 15 min and the serum samples stored at -20 ˚C until analysis. The samples with haemolysis were thrown away. The serum was analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method[4] (ZiestChem Diagnostics Tehran, Iran), triglyceride by the enzymatic procedure (ZiestChem Diagnostics Tehran, Iran) of McGowan *et al.* (1983),[5] and total lipids by the method described by Zollner and Kirsch (1962)[6] (Merck, Germany). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. HDL-cholesterol was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins, which were sedimented by centrifugation (10000 *g* for 5 min). The residual cholesterol was then measured by the enzymatic method.[4] LDL-cholesterol was calculated as the difference between the cholesterol measured in the precipitate and in the HDL fraction.[6] VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides.[7] 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).

Statistical analysis was performed using SPSS12 (Illinois, Chicago). Correlations

of each of the serum lipids and lipoproteins with measured trace elements were analyzed by Pearson's correlation tests, and differences were considered significant at $P < 0.05$.

Results

The average age of the female buffaloes was significantly higher than the male buffaloes ($P < 0.01$). The results of the measurement of serum concentrations of the trace elements, lipids and lipoproteins are shown in Table 1. There were no significant differences between the male and female buffaloes in the serum concentrations of the measured trace elements, lipids and lipoproteins, but the difference of the serum concentration of HDL-cholesterol between the sexes was marginally significant ($P = 0.061$). The results of the measurement of the correlations between the serum concentrations of the measured trace elements, lipids and lipoproteins are shown in Table 2.

There was a significant correlation between the serum concentration of copper and HDL-cholesterol ($r = 0.467$, $P < 0.01$). The serum total lipids had a significant correlation with iron ($r = 0.25$, $P = 0.019$) and the correlation between the total lipids and manganese was marginally significant ($r = -0.197$, $P = 0.07$).

Both sexes were evaluated separately. In male buffaloes, serum HDL-cholesterol had significant correlations with copper ($r = 0.778$, $P < 0.001$) and selenium ($r = -0.671$, $P = 0.002$), while LDL-cholesterol had marginally significant correlations with manganese ($r = -0.409$, $P = 0.066$) and selenium ($r = 0.442$, $P = 0.066$). There was a significant correlation between the serum VLDL-cholesterol and selenium ($r = 0.56$, $P = 0.016$) and the correlation between the serum VLDL-cholesterol and copper was marginally significant ($r = 0.418$, $P = 0.06$). Significant correlations were also found between the total cholesterol and copper ($r = 0.715$, $P < 0.001$), between triglyceride and selenium ($r = 0.56$, $P = 0.016$), and between total lipids and manganese ($r = -0.523$, $P = 0.015$). In female buffaloes, only serum total lipids had a significant correlation with iron ($r = 0.261$, $P = 0.034$).

The buffaloes were divided into three groups, according to their age as $G_1 \leq 2$ years, 2 years $< G_2 \leq 5$ years and, $G_3 > 5$ years. The correlations of serum trace elements with lipids and lipoproteins were measured in each of the age groups separately. In the G_1 group, consisting of 20 buffaloes, serum copper had significant correlations with the total cholesterol ($r = 0.643$, $P = 0.007$) and HDL-cholesterol ($r = 0.768$, $P = 0.001$). In the G_2 group, which consisted of 30 buffaloes, the serum total lipids had a significant correlation with manganese ($r = -0.384$, $P = 0.05$) and had a marginally significant correlation with iron ($r = 0.355$, $P = 0.07$). Cholesterol and Zinc had a significant correlation ($r = -0.382$, $P = 0.049$). In the G_3 group, which consisted of 50 buffaloes, a significant correlation was found between the serum VLDL-cholesterol and selenium ($r = 0.345$, $P = 0.049$).

Discussion

Although it is clear that deficiencies of some of trace elements, such as Cu, Mn and Zn, can result in marked alterations in lipid and lipoprotein metabolism.[1] To the best of our knowledge, there has been no previous research regarding the correlations of the serum trace elements with lipids and lipoproteins (cholesterol, triglyceride, total lipids, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol) 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.[1] A reduction in glucose utilization in Zn deficiency has been linked to changes in lipid metabolism. A Zn deficiency-induced hypercholesterolemia has been

Table 1. The concentrations (mean ± SEM) of measured serum trace elements, lipids and lipoproteins in water buffaloes *(Bubalus bubalis)*

	All sampled buffaloes	**Male buffaloes**	**Female buffaloes**
Number of buffaloes	100	26	74
Manganese (µmol L^{-1})	1.04 ± 0.09	0.095 ± 0.11	1.09 ± 0.11
Copper (µmol L^{-1})	9.06± 0.79	11.02 ± 2.36	8.185 ± 0.63
Iron (µmol L^{-1})	5.5 ± 0.34	5.05 ± 0.64	5.68 ± 0.37
Selenium (µmol L^{-1})	1.44 ± 0.13	1.27 ± 0.25	1.52 ± 0.25
Zinc (µmol L^{-1})	46.78 ±3.82	4.17 ± .05	4.13 ± 0.03
Cholesterol (mmol L^{-1})	4.15 ± 0.028	0.22 ± 0.01	0.21 ± 0.006
Triglyceride (mmol L^{-1})	0.215 ± 0.005	2.88 ± 0.03	2.9 ± 0.02
total lipids (g L^{-1})	2.9 ± 0.016	2.1 ± 0.05	2.04 ± 0.01
HDL-cholesterol (mmol L^{-1})	2.06 ± 0.015	2.05 ± 0.05	2.08 ±0.03
LDL-cholesterol (mmol L^{-1})	2.08 ± 0.025	0.044± 0.002	0.04 ± 0.001
VLDL-cholesterol (mmol L^{-1})	0.043 ±0.001	38.99 ±7.03	48.9 ± 4.59

Table 2. Correlations of serum concentrations of measured trace elements (copper, zinc, selenium, iron and manganese) with cholesterol, triglyceride, total lipids, very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol) and high density lipoproteins (HDL-cholesterol) in water buffaloes *(Bubalus bubalis)*

	cholesterol (mmol L^{-1})	**triglyceride (mmol L^{-1})**	**total lipids (g L^{-1})**	**HDL-cholesterol (mmol L^{-1})**	**LDL-cholesterol (mmol L^{-1})**	**VLDL-cholesterol (mmol L^{-1})**
Copper (µmol L^{-1})	r = 0.18 $P > 0.05$	r = 0.174 $P > 0.05$	r = 0.119 $P > 0.05$	r = 0.467 $P < 0.001$	r = 0.134 $P > 0.05$	r = 0.174 $P > 0.05$
Zinc (µmol L^{-1})	r = -0.06 $P > 0.05$	r = -0.07 $P > 0.05$	r = 0.055 $P > 0.05$	r = -0.02 $P > 0.05$	r = -0.32 $P > 0.05$	r = -0.076 $P > 0.05$
Selenium (µmol L^{-1})	r = -0.048 $P > 0.05$	r = 0.177 $P > 0.05$	r = -0.04 $P > 0.05$	r = -0.06 $P > 0.05$	r = -0.37 $P > 0.05$	r = 0.177 $P > 0.05$
Iron (µmol L^{-1})	r = -0.18 $P > 0.05$	r = -0.03 $P > 0.05$	r = 0.25 $P < 0.05$	r = -0.1 $P > 0.05$	r = -0.115 $P > 0.05$	r = -0.031 $P > 0.05$
Manganese (µmol L^{-1})	r = -0.057 $P > 0.05$	r = -0.02 $P > 0.05$	r = 0.194 $P = 0.07$	r = 0.059 $P > 0.05$	r = 0.174 $P > 0.05$	r = -0.022 $P > 0.05$

demonstrated in rat and dog models.[1] Similar to our results, Sulibursk*a et al.* (2010) [3] found a negative relationship between serum Zn and cholesterol concentrations in human, and El Hendy *et al.* (2001) showed that Zn deficiency increases serum cholesterol in a dose-dependent manner.[8]

According to our results serum Cu had significant positive correlations with cholesterol, and HDL-, LDL- and VLDL-cholesterol. The elevation of the serum total cholesterol level during Cu deficiency and in association with high concentrations of HDL- and LDL-cholesterol has been proved in rats. The cause of this elevation is the change in the metabolism of lipoproteins.[1] Copper alters adipose tissue metabolism and decreases cholesterol synthesis, which is the cause of hypercholesterolemia during Cu deficiency.[9] Several studies reported an inverse relation between serum copper and cholesterol in rats during Cu deficiency[10,11] while Koo and Williams (1981) found no significant correlation between the serum copper and cholesterol levels in non copper deficient rats.[12] These findings suggest that the correlations between the serum concentration of Cu with lipids and lipoproteins in the physiological concentration may be different from the changes observed during Cu deficiency. Engle *et al.* (2000) found that Cu supplementation did not affect serum cholesterol concentrations in Cu deficient steers during the growing phase, but it does decrease serum cholesterol in the finishing period.[9] Limited researches in ruminants suggest that dietary Cu at physiological concentration may affect lipid metabolism in ruminants,[9] and it seems that more research is needed to clarify the correlation of the serum Cu with lipid and lipoproteins in non copper deficient ruminants during different physiological status.

Some authors 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 cholesterol metabolism.[13] As mentioned, Koo and Williams (1981) found no significant correlation between the serum copper and cholesterol levels in non Cu deficient rats, however, a significant correlation between the serum cholesterol and serum Zn/Cu was found.[12] In contrast, other studies did not show such relationships between the Zn/Cu ratios and serum cholesterol levels in experimental animals and humans.[12] Statistical analysis of our data showed no significant correlation between the serum Zn/Cu ratio and the cholesterol level.

In our study, there was a significant correlation between the serum total lipids and Fe. Some researchers found that iron deficiency is linked to low serum lipid level and Fe has been shown as a potential risk factor for hyperlipidemia in humans.[3] Also, Fields and Lewis (1999) found that plasma triglyceride concentrations were elevated by the consumption of a high iron diet.[14] However, Sulibursk*a et al.* (2010) found no significant correlation between the serum or diet concentration of iron and the serum cholesterol or triglycerides level.[3] Choi *et al.* (2001) believes that different observations about the correlation between serum or dietary Fe and serum lipid and lipoproteins suggest that changes in serum lipid parameters depend on the age, sex, strain of the animal model and the composition of the diets used in the experiments.[15]

According to our results, the serum concentration of selenium had positive correlations with triglyceride, LDL- and VLDL-cholesterol but a negative correlation with the serum HDL-cholesterol. The decrease in the serum LDL-cholesterol and HDL-cholesterol increment due to the addition of a selenium supplement to the ration of rats has been reported.[16] Observational studies in human populations showed positive associations of serum selenium with total cholesterol and LDL-cholesterol concentrations in high selenium-replete

population. In populations with low serum selenium concentrations the associations were inconsistent. The association of the serum selenium with the HDL-cholesterol was not consistent.[17]

Manganese is critical for lipid and lipoprotein metabolism, and Mn deficiency in domestic animals causes defects. It has been demonstrated that Mn enhance cholesterol synthesis in the liver and hypocholesterolemia has been reported in a human case of Mn deficiency. Manganese -deficient rats also have low plasma cholesterol and low HDL-cholesterol. High levels of dietary Mn affect lipid metabolism when animals are fed high-fat diets.[1] However, our results showed negative correlations of the serum manganese with the total lipids and LDL-cholesterol. We found no explanation for these different relations.

The cause of these findings and some contradictory findings regarding the relation between the serum trace elements, and lipids and lipoproteins are not clear and may be due to the effect of some factors such as age, sex, health status, breed, pregnancy, geographic and dietary factors on the serum lipids and lipoproteins profile in domestic animals. These findings also suggest that the correlations between the serum concentrations of trace elements with lipids and lipoproteins in physiological concentrations may not be the same as the changes observed during deficiencies of the trace elements. However, it seems that more work is required on a larger number of animals before the importance of these findings can be assessed.

References

1. Cunnane SC. Role of zinc in lipid and fatty acid metabolism and in membranes. Prog Food Nutr Sci 1988; 12: 151-188
2. Suliburska J, Bogdański P, Pupek-Musialik D, et al. Dietary intake and serum and hair concentrations of minerals and their relationship with serum lipids and glucose levels in hypertensive and obese patients with insulin resistance. Biol Trace Elem Res 2010; 20195917 (In press)
3. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry. WB Saunders, Philadelphia 1994: 1002–1093
4. McGowan MW, Artiss JD, Strandbergh DR. A peroxidase coupled method for the colorimetric determination of serum triglycerides. Clin Chem 1983; 29(3): 538–542
5. Zollner N, Kirsch K. Determination of the total lipid concentration in serum. Zentralbl Ges Exp Med 1962; 135: 545
6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499
7. Rucker R, Fascetti AJ, Keen CL. Trace minerals. In: Clinical Biochemistry of Domestic Animals, 6th edn, ed. J. J. Kaneko, Academic Press Inc. 2008: 660−693
8. El Hendy HA, Yousef MI, Abo El-Naga NI. Effect of dietary zinc deficiency on hematological and biochemical parameters and concentrations of zinc, copper, and iron in growing rats. Toxicol 2001; 167: 163-170
9. Engle TE, Spears JW, Xi L, et al. Concentrations in finishing steers Dietary copper effects on lipid metabolism and circulating catecholamine. J Anim Sci 2000; 78: 2737-2744
10. Petening HG. The effect of cadmium and lead on copper and zinc metabolism. In: Trace elements metabolism in animals-2, eds. Hoekstra WG, Suttie JW, Ganther HE, Merz W, Baltimore: University Park Press 197: 4311-4325
11. Petening HG, Murthy U, O'Flaherty E. Influence of dietary copper and zinc on

rat lipid metabolism. J Agnic Food Chem 1977; 25: 1105-1109
12. Koo I, Williams DA. Relationship between the nutritional status of zinc and cholesterol concentration of serum lipoproteins in adult male rats. Am J Clin Nutr 1981; 34: 2376- 2381
13. Klevay LM. Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Am J Clin Nutr 1973; 26: 1060-1068
14. Fields M, Lewis CG. Level of dietary iron, not type of dietary fat, is hyperlipidemic in copper deficient rats. J Am Coll Nutr 1999;18(4): 353–357
15. Choi JW, Kim SK, Pai SH. Changes in serum lipid concentrations during Iron depletion and after iron supplementation. Ann Clin Lab Sci 2001, 31(2): 151-156
16. Mysliwiec Z, Machoy-Mokrzynska A, Juzyszyn Z, et al. Effects of selenium on serum lipids and enzyme activities in fluoride- intoxicated rats. Fluoride 2002; 35(3): 168-175
17. Yang KC, Lee LT, Lee YS, et al. Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study. Nutr Metab 2010; 7: 38-44